spin/ligation states on the equilibrium disorder and heme reorientation rate are in progress.

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Registry No. Hemin, 16009-13-5; deuterohemin, 21007-21-6; mesohemin, 21007-37-4.

Alkyldinitrogen Species Implicated in the Carcinogenic, Mutagenic, and Anticancer Activities of *N*-Nitroso Compounds: Characterization by ^{15}N NMR of ^{15}N-Enriched Compounds and Analysis of DNA Base Situselectivity by ab Initio Calculations†

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Abstract: The syntheses of certain specifically ^{15}N-labeled (*E*)- and (*Z*)-alkanediazoates and alkylnitrosamines (which have been implicated in the carcinogenic, mutagenic, or anticancer activities of *N*-nitroso compounds) together with related dinitrogen species including diazoalkanes are reported. Study of their conformational and configurational equilibria by ^{15}N and ^{13}C NMR revealed that corresponding pairs of (*E*)- and (*Z*)-alkanediazoates do not interconvert at ambient temperature in aprotic solvents. A fast interchange of metal counterion occurs between oxygen and nitrogen in the *Z* diazoates, whereas in contrast there is a slower interchange of metal ion between oxygen and nitrogen in the corresponding *E* diazotates. Interconversion of (*Z*)-arenediazoates occurs via detectable *Z* and *E* diazohydroxides to the *E* diazoates. Rapid stereoelectronically assisted conversion of (*Z*)-alkanediazoates to diazoalkane contrasts with the properties of the more stable *E* diazoates. The ^{1}H and ^{15}N NMR spectra in aprotic solvents at −50 °C of the unstable methyl nitrosamine showed averaged line positions in accord with an equilibrium between *Z* and *E* forms. SCF ab initio calculations show that the *Z*-OH (syn) conformation of the methanediazohydroxide is the highest in energy and the most reactive form with a larger LUMO coefficient on carbon than for the *E* isomer. In contrast, the *E*-OH (syn) conformation is more stable (by ~19.8 kcal M^{-1}) and with a zero LUMO carbon coefficient. SCF calculations predict a somewhat lower energy of activation for rotational inversion of *Z* and *E* configurations via the methanediazoates than by direct configurational inversion of the methanediazohydroxides. Complementary molecular orbital examinations of the main contributions of individual sites in the HOMO of guanine combined with a hard and soft acids and bases (HSAB) analysis are in accord with the view that the *Z*-I form (from methylnitrosamine) with a softer carbon may attack the softer O^6 position preferentially while *E*-I with a harder carbon would prefer to react at N_7. The results may provide a rationale for the in vivo reactions of the carcinogens such as dipropylnitrosamine with DNA. In this case propylation of G-N_7 occurs predominantly by an S_N2 process and without rearrangement and the concomitant propylation and isopropylation of G-O^6 occurs via a more S_N1-like process. The results may serve to explain the formation of the characteristic G-O^6 alkyl carcinogenic lesion by nitrosamines of DNA.

N-Nitroso compounds including nitrosamines, nitrosocarbamates, and clinically useful anticancer nitrosoureas are carcinogenic and/or mutagenic.[1-4] Several lines of evidence indicate that damage of cellular DNA by these agents may be responsible for these biological effects.[1,3,4] Certain DNA lesions, e.g., guanine O^6-alkylation,[5-7] appear to be more critical in giving rise to cell transformation than others, e.g., the relatively innocuous guanine N_7-alkylation.[8] The key reaction appears to be generation of electrophilic species, either by enzymatic action during metabolism in the case of nitrosamines[1,3] or by spontaneous decomposition in the case of (2-chloroethyl)nitrosoureas.[2,4] However, the nature of the electrophile that is ultimately responsible is by no means clear. Possible candidate electrophiles generated from nitrosamines include the (*Z*)- and (*E*)-alkanediazohydroxides, the alkanediazonium ion, diazoalkane, and the carbenium ion. Recent evidence in the cases of DNA base alkylation by N_1N_3-bis(2-chloroethyl)-*N*-nitrosourea[9,10] as well as by nitrosopropylamine[11-13] require largely S_N2 processes, which rules out a free carbenium ion.

High-Field ^{15}N NMR of specifically ^{15}N-enriched compounds has provided detailed information on the structure, conformation, and reactions of (2-chloroethyl)nitrosoureas.[14] Accordingly we

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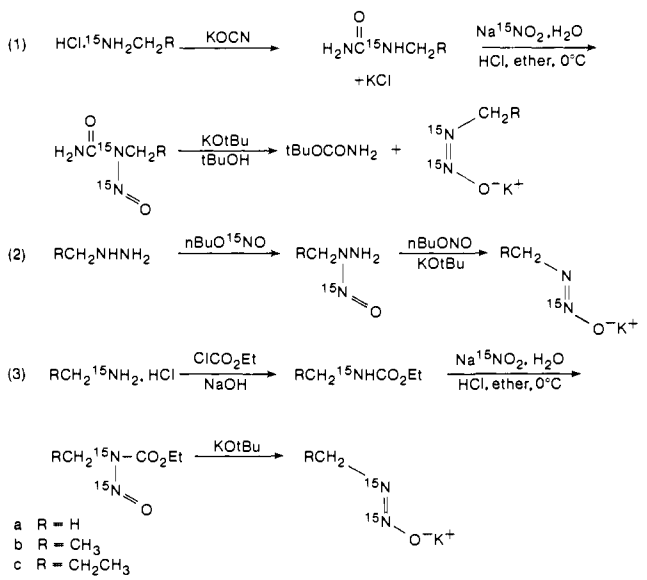
Scheme I. Reaction Schemes for Preparation of Singly and Doubly ^{15}N-Labeled (*Z*)- and (*E*)-Alkanediazoates



report the synthesis of specifically ^{15}N-labeled *N*-nitroso compounds and the characterization by ^{15}N NMR of the alkyldinitrogen species to which they give rise. We also report ab initio calculations on configurationally related electrophilic species and the derivation of geometric parameters, electronic distribution, and relative energies. These are pertinent to the dicussion of their situselectivity in reaction with DNA base nucleophilic site which may ultimately determine in vivo activity.

Synthesis of Specifically Labeled *N*-Nitroso Compounds and Alkyldinitrogen Species. The required specifically ^{15}N-labeled nitrosoureas were prepared from the (95%) ^{15}N-enriched primary amines and potassium isocyanate with subsequent nitrosation of the labeled urea with solid $Na^{15}NO_2$ (95–99% enriched) in formic acid at 0 °C.[14] The intermediate ureas and the N_1-nitrosoureas were examined by ^{15}N NMR, ^1H NMR, and mass spectrometry to confirm the position and extent of incorporation. The required doubly ^{15}N-labeled (*Z*)-alkanediazoates were prepared by treatment of the $^{15}N_2$nitrosoureas with an equivalent of potassium *tert*-butoxide in butanol [Scheme I, (1)] by analogy with the unlabeled compounds[15] and their ^{15}N and ^{13}C NMR examined in different solvents.

The singly $^{15}N_2$-enriched (*E*)-alkanediazoates were prepared as shown in Scheme I, (2), from reactions of the hydrazine with 95% enriched *n*-BuO^{15}NO with subsequent treatment of the nitrosohydrazine with *n*-BuONO and KO-*t*-Bu by analogy with the reported preparation of the unlabeled counterparts.[16] In one case monomethyl(^{15}N)nitrosohydrazine (**3a**)[17] has been isolated and treatment with 1 equiv of *n*-BuONO and KO-*t*-Bu yielded the (*E*)-($^{15}N_2$)methanediazoate (**4a**). The corresponding $^{15}N_2$-enriched (*E*)-alkanediazoates were prepared via base-catalyzed decomposition of the nitrosocarbamates as shown in Scheme I, (2). An authentic sample of ($^{15}N_2$)diazomethane was prepared by treating potassium (*Z*)-methanediazoate with KO-*t*-Bu in CD_3OD at −80 °C.[18]

Results

NMR Characterization of Alkyldinitrogen Intermediates. The methyl group ^1H NMR signal of (*Z*)-methanediazoate in Me_2SO-d_6 appeared at δ 3.16[19] with $^3J(^{15}N–H)$ = <0.8. These and other physical data are in accord with reported values.[20] The ^{13}C NMR signal appeared at δ 31.23 ($^2J^{15}N–^{13}C$ = <1.8 Hz). The assignment is supported by comparison with the syn methyl group signal in dimethylnitrosamine at δ 31.86 with $^2J(^{15}N–^{13}C)$ = 1.4 Hz.[21] The differential shieldings for the ^{13}C signals in the (*Z*)- and (*E*)-methanediazoates agree well with the observed differences of the methyl signals in dimethylnitrosamine (see below). The ^{15}N chemical shifts of the specifically ^{15}N-enriched (*Z*)-methanediazoate in Me_2SO-d_6 appeared at δ 339.50 (N_1) and 514.9 (N_2) with $^1J(^{15}N–^{15}N)$ = 17.4 Hz. The structure of potassium (*Z*)-methanediazoate can best be represented as **2C** (Scheme II) since similar structures have been confirmed by X-ray diffraction for (*Z*)-alkane-[22] and (*Z*)-arenediazoates.[23]

In contrast the ^1H NMR spectrum of the (*E*)-methanediazoate (**4a**) in Me_2SO-d_6 showed a broad partially resolved signal at δ 3.52 indicative of an equilibrium between two species. This was confirmed in the corresponding ^{13}C NMR spectra, which showed two doublets at δ 39.0 ($^2J^{15}N–^{13}C$) = 10.0 Hz) and 37.3 (^2J-($^{15}N–^{13}C$) = 6.0 Hz) in the ratio (3:1). The latter assignments to the CH_3 anti to the N=O moiety may be compared with the values of δ 39.75 and $^2J(^{15}N–^{13}C)$ = 7.5 Hz reported for the anti CH_3 group in dimethylnitrosamine.[21]

The proton-decoupled ^{15}N NMR spectrum of the doubly ^{15}N-labeled (*E*)-methanediazoate (**4a**) in Me_2SO-d_6 showed two sets of signals at δ 363.1 (N_1) and 564.7 (N_2) with $^1J(^{15}N–^{15}N)$ = 21.0 Hz and δ 321.6 (N_1) and 539.8 (N_2) with ^1J(In a CD_3OD solution of **4a** two closely placed sets of signals were observed δ 347.8 (N_1) and 568.7 (N_2) ($^1J(^{15}N–^{15}N)$ = 18.2 Hz) and δ 348.5 (N_1) and 569.05 (N_2) ($^1J(^{15}N–^{15}N)$ = 21 Hz).[24] It was not possible to estimate the signal intensity ratios accurately because of the presence of the relaxing agent necessary for the suppression of negative NOEs in the ^{15}N signals. In the aprotic solvent the lower field sets of ^{15}N signals are assigned to the structure with the potassium ion centered on nitrogen, **4aA**, since they are characteristic of an N=O nitrogen.[21] The higher field signal is assigned to the species with potassium centered on the oxygen, **4aB**, i.e., more diazene-like.[14]

In the protic medium a rapid exchange and proton transfer to the diazoate to produce the diazohydroxide predicted by Moss[25] suggests the assignment of the set of ^{15}N signals at δ 347.8 and 568.7 to the equilibrium form **4aC** and therefore the set of signals at δ 348.5 and 569.05 are tentatively ascribed to the (*E*)-methanediazohydroxide **6aB**.

The corresponding pairs of (*Z*)- and (*E*)-alkanediazoates (**2** and **4**) are quite stable at ambient temperature in MeSO-d_6 and do not interconvert. The (*Z*)-methanediazoate (**2a**) is rapidly converted into ($^{15}N_2$)diazomethane (**7a**) via **8a** in the protic solvent CD_3OD at room temperature.[26] The corresponding (*E*)-al-

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(15) Although the preparation of (*Z*)-alkanediazoates from alkylnitrosamides has been described (Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421. Muller, E.; Haiss, H.; Rundel, W. *Chem. Ber.* **1960**, *93*, 1541), for reasons of simplicity and cost of ^{15}N intermediates we have adapted the procedure of Hecht and Kozarich (Hecht, S. M.; Kozarich, J. W. *J. Org. Chem.* **1973**, *38*, 1821).
(16) Thiele, J. *Chem. Ber.* **1908**, *41*, 2806; *Justus Liebigs Ann. Chem.* **1910**, *376*, 239.
(17) Pederson, C. T. *Acta Chem. Scand.* **1964**, *18*, 2199. Wherein are reported these spectral data for nitroso(^{15}N)methylhydrazine: ^1H NMR (pyridine-d_5) 3.80 (d, 3 H, CH_3, $^3J(^{15}N–H)$ = 2.20 Hz), 7.60 (brm, 2 H, NH_2); ^{13}C NMR (pyridine-d_5) 39.72 (d, CH_3, $^2J(^{15}N–^{13}C_1)$ = 6.2 Hz); ^{15}N NMR (MeSO-d_6) 474.6 (NO); ^{15}N NMR (THF) 92.4 (NH_2), 25.20 (NCH_3), 487.0 (NO).
(18) Pechmann, H. v. *Chem. Ber.* **1894**, *27*, 1888; **1895**, *28*, 855.
(19) The ^1H chemical shift of the methyl group in (*Z*)-methanediazoate has been reported in MeSO-d_6 at δ 3.18 by: Suhr, H. *Chem. Ber.* **1963**, *96*, 1720.
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(24) Similar types of solvent effects were observed in the case of the (*E*)-arenediazoates, for example, in MeSO-d_6 (372.73 (N_1), 565.62 (N_2), $^1H(^{15}N–^{15}N)$ = 16.6 Hz and in CD_3OD 377.65 (N_1), 538.63 (N_2) $^1J(^{15}N–^{15}N)$ = 15.7 Hz.
(25) Moss, R. A. *J. Org. Chem.* **1966**, *31*, 1082.
(26) Authentic ($^{15}N_2$)diazomethane required for comparison could be prepared by reaction of (*Z*)methanediazoate (**2a**) with 2 equiv of KO-*t*-Bu in CD_3OD at −80 °C and was characterized by ^{15}N NMR: 289.3 (N_1), 395.9 (N_2) $^1J(^{15}N–^{15}N)$ = 7.5 Hz. See also: Duthaler, R. O.; Forster, H. G.; Roberts, J. D. *J. Am. Chem. Soc.* **1978**, *100*, 4974. Albright, T. A., Freeman, W. J. *Org. Magn. Reson.* **1977**, *9*, 75.

Table I. ^1H NMR Chemical Shifts and Coupling Constants for (*E*)- and (*Z*)-Alkanediazoates and Related Compounds[a]

compd	solvent	^1H NMR
2a	Me_2SO-d_6	3.16 (lit.[13] 3.18) (s, 3 H, CH_3, $^3J(^{15}N-H)$ = <0.8 Hz)
2b	Me_2SO-d_6	1.05 (t, 3 H, CH_2CH_3), 3.15 (q, 2 H, CH_2CH_3)
2c	Me_2SO-d_6	0.88 (t, 3 H, $CH_2CH_2CH_3$), 1.45 (m, 2 H, $CH_2CH_2CH_3$), 3.05 (t, 2 H, $CH_2CH_2CH_3$)
4aA	Me_2SO-d_6	3.25 (d, $^3J(^{15}N-H)$ = 5.0 Hz)[b]
4aB	Me_2SO-d_6	3.52 (d, $^3J(^{15}N-H)$ = 2.0 Hz)[b]
4aA	CD_3OD	3.35 (d, $^3J(^{15}N-H)$ = 5.0 Hz)[b]
4aB	CD_3OD	3.52 (d, $^3J(^{15}N-H)$ = 2.2 Hz)[b]
4bA	Me_2SO-d_6	1.05 (t, 3 H, CH_2CH_3), 3.45 (q, 2 H, CH_2CH_3)
4bB	Me_2SO-d_6	1.03 (t, 3 H, CH_2CH_3), 4.50 (q, 2 H, CH_2CH_3)
4bA	CD_3OD	1.20 (t, 3 H, CH_2CH_3), 3.45 (q, 2 H, CH_2CH_3)
4bB	CD_3OD	1.20 (t, 3 H, CH_2CH_3), 3.60 (q, 2 H, CH_2CH_3)
4c	Me_2SO-d_6	0.90 (t, 3 H, $CH_2CH_2CH_3$), 1.50 (m, 2 H, $CH_2CH_2CH_3$), 3.45 (t, 2 H, $CH_2CH_2CH_3$, $^3J(^{15}N-H)$ = 4.0 Hz)
4c	CD_3OD	0.90 (t, 3 H, $CH_2CH_2CH_3$), 1.60 (m, 2 H, $CH_2CH_2CH_3$, 3.50 (t, 2 H, $CH_2CH_2CH_3$, $^3J(^{15}N-H)$ = 4.0 Hz)
5aA (6aA)	$CH_3C_6H_5$	2.72 (s, CH_3)
7a	$Cl_2FCCFCl_2$	3.08 (s, 3 H, CH_3)[c]
7b	$Cl_2FCCFCl_2$	1.70 (d, 3 H, CH_2CH_3), 3.22 (q, 2 H, CH_2CH_3)[c]

[a] 400-MHz spectra on ca. 5% solutions with Me_4Si as internal standard. [b] Assigned on the basis that the negative charge on the adjacent nitrogen will shield the methyl group preferentially. [c] Ledwith, A.; Friedrick, E. C. *J. Chem. Soc.* **1964**, 504.

kanediazoates are stable at room temperature in CD_3OD for up to 12 h and required treatment of a suspension of **4a** in ether with CO_2 to afford (^{15}N)diazomethane (**7a**).[16]

(*Z*)-Methanediazohydroxide (**5aB**), which is a possible intermediate in the conversion of (*Z*)-methanediazoate (**2aC**) to diazomethane (**7a**), was difficult to detect under these conditions by ^{15}N NMR even with enriched intermediates. However treatment of benzene($^{15}N_2$)diazonium chloride with potassium hydroxide in Me_2SO-d_6 afforded potassium (*Z*)-benzene($^{15}N_2$)diazoate (**9**).[27] Addition of 10% /v/ water to this solution allowed the detection of the following species that were identified by ^{15}N NMR: (*Z*)-benzenediazohydroxide (*Z*-**10**), (*E*)-benzenediazohydroxide (*E*-**10**), and then (*E*)-benzenediazoate (*E*-**9**). The signals due to the more stable *E*-**9** increased over the course of 6 h at the expense of the *Z*-**9** signals.

Evidence was now sought directly on the possible equilibration between species **5A**, **5B**, **6A**, and **6B**. When methylamine was treated with $^{15}NOCl$ in anhydrous toluene at −50 °C the ^1H NMR showed a methyl group signal at δ 2.72. Dimethylnitrosamine (DMNA) under similar conditions shows signals at δ 2.95 and 2.41 corresponding to the anti and syn methyls, respectively, on the basis of the differential shieldings observed for DMNA in $CDCl_3$.[21] The observation of the ^1H resonance at δ 2.72 therefore suggests an averaging due to a rapid equilibration between *Z* and *E* forms of monomethylnitrosamine.

The ^{15}N NMR spectrum in MeSO-d_6 showed two signals at δ 553.62 and 395.34. The former signal is ascribed to monomethylnitrosamine (**5aA** ⇌ **6aA**) on the basis of ^{15}N chemical chemical shift considerations.[14,17,21] The smaller peak at δ 395.34 is ascribed to its decomposition product diazomethane (**7a**)[26,28] by comparison with an authentic sample. The ^1H, ^{15}N, and ^{13}C NMR spectral data are given in Tables I–III.

Ab Initio Calculations of Optimized Geometries, Charge Distributions and LUMO Energies and Atom Contributions of (*Z*)-and (*E*)-Methanediazohydroxides and HOMO Atom Contributions of Guanine. Self-consistent field (SCF) ab initio calculations were made, using full geometry optimization of the principal conformers, of the energies and net atomic charges of (*Z*)-methanediazohydroxide (**5aB**) and its *E* isomer **6aB**. Additional calculations of full geometry optimization and energies were performed on the corresponding methanediazoate anions **2aB** and **4aB** as well as certain intermediates (T and R, see Table IV) considered in the formal interconversion of the *Z* and *E* configurations. The calculations were performed with 6-31G*[29] basis set, which is a split valence set with hydrogens having 1s + 1s′ and other atoms having 1s, 2s, + 2s′, 2p + 2p′, and d functions added, as implemented by the GAUSSIAN 80 program.[30] The geometry optimization was

Table II. ^{13}C NMR Chemical Shifts and Coupling Constants for (*E*)- and (*Z*)-Alkanediazoates and Related Compounds[a]

		^{13}C NMR				
compd	solvent	C_1	C_2	C_3	^1J-($^{15}N-^{13}C$), Hz	^2J-($^{15}N-^{13}C$), Hz
2a	Me_2SO-d_6	31.23			6.9	<1.2
2b	Me_2SO-d_6	36.71	15.65			
2b	BuOH	36.66	16.45			
2c	Me_2SO-d_6	48.77	21.87	12.98	6.2	<1.5
2c	EtOH	44.20	24.54	11.69		
4aA	Me_2SO-d_6	37.25[b]				6.0
4aB	Me_2SO-d_6	39.0[b]				10.0
4a	CD_3OH	40.31				8.2
4b	Me_2SO-d_6	49.30	15.76			
4b	CD_3OH	48.61	14.75			
4c	Me_2SO-d_6	57.45	23.14	12.11		8.3
4c	CD_3OD	59.35	23.67	12.30		
7a	$CDCl_3$	21.1[c]				
7b	CD_3OD	35.71	15.18			
7c	CD_3OD	46.18	21.49	11.96	22.51	
8a	$HFSO_3$	43.78[d]				

[a] Measured at 100.6 MHz in ca. 5% solutions with ca. 1–2K scans. [b] Assigned on the basis that the negative charge on the neighboring nitrogen will shield the adjacent carbon in **4Aa** preferentially. [c] Albright, T. A.; Freeman, W. J. *Org. Magn. Reson.* **1977**, *9*, 75. [d] McGarrity, J. F. *J. Am. Chem. Soc.* **1979**, *79*, 3135. Spectrum taken on $CH_3N^+{\equiv}N\ FSO_3^-$ at −120 °C.

Table III. ^{15}N NMR Chemical Shifts and Coupling Constants for (*E*)- and (*Z*)-Alkanediazoates and Related Compounds[a]

		^{15}N NMR			
compd	solvent	N_1	N_2	$^1J(^{15}N-^{15}N)$, Hz	$^3J(^{15}N-H)$, Hz
2a	Me_2SO-d_6	339.5	514.9	17.4	*d*
2b	Me_2SO-d_6	345.0	510.8	17.5	*d*
2c	Me_2SO-d_6	356.6	509.3	17.6	*d*
4aA	Me_2SO-d_6	363.1	564.7	21.0	4.5
4aB	Me_2SO-d_6	321.6	539.8	19.0	2.2
4aC	CD_3OD	347.8	568.7	18.2	
4cA	Me_2SO-d_6		535.4		3.5
4cB	Me_2SO-d_6		517.3		*d*
5aA	ether[b]		553.62		
6aB	CD_3OD	348.15	569.05	21.0	
7a	CD_3OD	289.3	395.9	7.5	
7b	CD_3OD	305.4	427.3	9.5	
7c	CD_3OD	303.8	428.9	9.1	
E-**9**[c]	Me_2SO-d_6	371.92	540.58	16.1	
Z-**9**[c]	Me_2SO-d_6	351.8	501.8	19.5	
E-**10**[c]	Me_2SO-d_6	372.35	555.38	16.4	
Z-**10**[c]	Me_2SO-d_6	310.45	495.08	15.1	

[a] Proton-coupled spectra were obtained with 1–4K scans at 20.283 MHz and are reported by using ammonia as standard and dimethylformamide as external reference. [b] This value was obtained with singly labeled **5aA**. [c] **9** is potassium benzenediazoate; **10** is benzenediazohydroxide. [d] In these cases $^{15}N-^1H$ and other proton coupling constants were not sufficiently resolved owing to the presence of Cr(AcAc)$_3$ as relaxing agent.

(27) Moss, R. A. *Acc. Chem. Res.* **1974**, *7*, 421.
(28) Muller, E.; Haiss, H.; Rundel, W. *Chem. Ber.* **1960**, *93*, 1541.
(29) Hariharan, P. C.; Pople, J. A. *Theor. Chim. Acta* **1973**, *28*, 213.

Scheme II. Interrelationship of Electrophilic Alkyldinitrogen Species Generated from Alkylnitroso Compounds

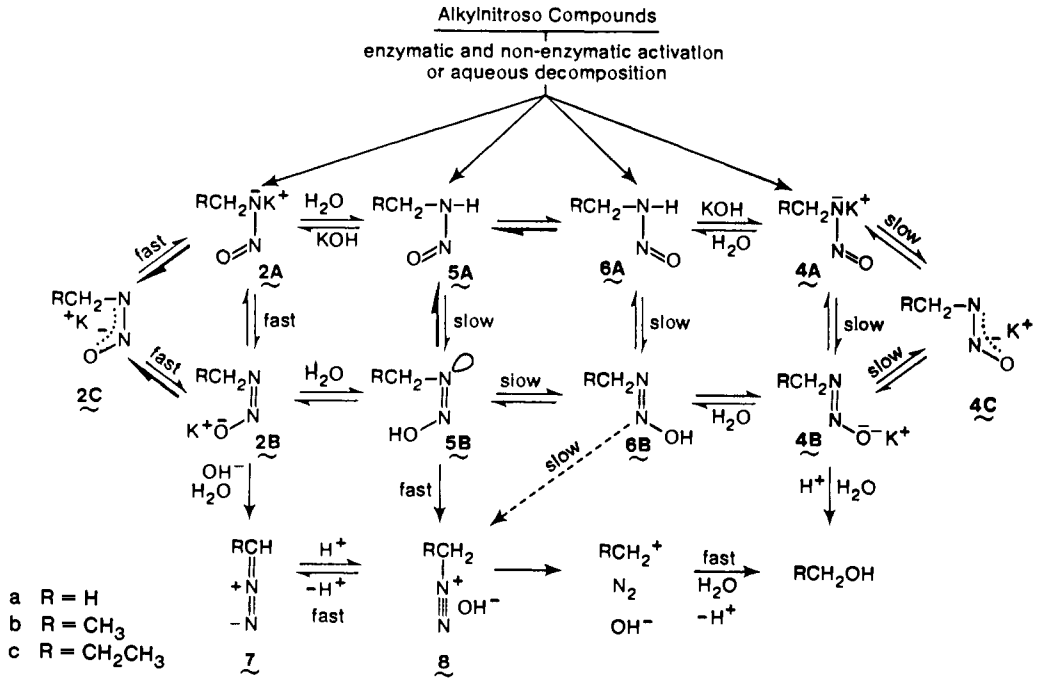

Table IV. Optimized Geometry Parameters and Energies for (*Z*)- and (*E*)-Methanediazohydroxides and -Diazoates Obtained by SCF 6-31G* Calculations

parameter, Å or deg	*Z*-I	*Z*-II	**2aB**	*E*-I	*E*-II	**4aB**	T[a,c]	R[b,c]
N–N	1.19	1.22	1.258	1.20	1.20	1.255	1.18	1.352
N–O	1.35	1.33	1.259	1.34	1.36	1.255	1.41	1.202
O–H	0.95	0.95		0.96	0.95		0.95	
N–C	1.45	1.47	1.463	1.45	1.46	1.455	1.42	1.451
∠H–O–C	111.5	108.7		107.2	104.2			
∠O–N–N	122.1	122.7	121.4	112.9	110.5	115.5	114.9	115.9
∠N–N–C	121.0	127.1	117.3	113.2	112.5	111.5	180	111.06
energy (au)	−223.8273	−223.8403	−223.2569	−223.8589	−223.8563	−223.2561	−223.8025	−223.1882

[a] Structure T has the carbon and the two nitrogens colinear. The optimized barrier for the interconversion *Z*-I ⇌ T ⇌ *E*-I is 0.0471 au ≡ 48.8 kcal mol^{-1}. [b] Structure R has the carbon in a plane orthogonal to the NNO plane. The optimized rotational barrier for the interconversion *Z*-I ⇌ *R* ⇌ *E*-I is 42.6 kcal mol^{-1}. [c] These geometries were locally optimized for the parameters indicated.

performed using the Berny optimization technique.[31] The only nonoptimized parameters are the C–H bond length (in the methyl), which was kept frozen at 1.1 Å, and the HCH angles kept at 109.5°.[32] The optimized geometry parameters are given in Table IV as well as the energies of the four structures in atomic units.

It may be seen from Table IV that the *E*-I (Figure 3) structure is predicted to be the most stable and the *Z*-I the least stable and that the energy difference is 0.0316 hartrees = 19.83 kcal mol^{-1}. The energy difference between *E*-I and *E*-II is small as expected at 1.6 kcal mol^{-1}.

The NMR evidence indicated that the *Z* and *E* configurations of the alkanediazohydroxides and -diazoates were not readily interconvertible in solution. This interconversion was examined by calculations on the idealized structure T (which has C–N–N atoms colinear), which is a plausible intermediate in the configurational inversion of *Z* to *E*. The geometry optimized parameters for T are given in Table IV, and its energy was computed to be −223.780 25 au. This corresponds to an optimized *Z* to *E* inversion barrier of 48.8 kcal mol^{-1}. The geometry optimized parameters of the (*Z*)- and (*E*)-methanediazoates (**2aB** and **4aB**) were calculated and the values are given in Talbe IV together with their energies. These species were examined in order to compute the energy barrier to interconversion of *Z* to *E* by the alternative rotational pathway.[33] This proceeds via the idealized intermediate

Table V. Net Atomic Charges for (*Z*)- and (*E*)-Methanediazohydroxides (au) Obtained by SCF 6-31G* Calculations

atom[a]	*Z*-I	*Z*-II	*E*-I	*E*-II
N_1	−0.232	−0.249	−0.277	−0.191
N_2	0.183	0.128	0.159	0.067
O	−0.612	−0.599	−0.568	−0.565
OH	0.462	0.477	0.445	0.459
C	−0.346	−0.346	−0.325	−0.326
CH_A	0.109	0.193	0.188	0.177
CH_B	0.217	0.198	0.190	0.190
CH_C	0.217	0.198	0.190	0.189

[a] Methyl proton H_A is in the plane of the molecule with H_B and H_C symmetrically disposed above and below the plane.

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(32) Hartree-Fock calculations are known to predict accurate geometries.[30,34] The 6-31G* basis set predicts particularly good bond lengths and angle for small molecules. For larger molecules 6-31G gives good correspondence in many cases with molecular parameters obtained from X-ray diffraction.

(33) The 6-31G* and 6-31G level calculations did not include configuration interaction. CI calculations are particularly necessary when, for example, reaction pathways are studied. However, this is not the case when isomers are compared where it is fairly safe to assume that the correlation energy will be similar for all structures and as such will not affect trends (see: Schaeffer, R. F. "The Electronic Structure of Atoms and Molecules"; Addison-Wesley Co.: Reading, MA, 1972). Similarly a CI calculation would show the participation of some excited levels to the LUMO but the inclusion of CI should not result in significant changes in the overall description of the LUMOs.

Table VI. LUMO Energies and Atom Contributions for (*Z*)- and (*E*)-Methanediazohydroxides[a]

atom	*Z*-I	*Z*-II	*E*-I	*E*-II
N_1[b]	0.394	−0.387	0.395	0.405
	0.546	0.540	0.598	0.603
N_2[b]	−0.459	0.464	−0.468	−0.455
	−0.604	0.611	−0.637	−0.631
O[b]	0.174	−0.162	0.166	0.161
	0.224	−0.213	0.221	0.207
C	0.233	−0.276	0.000	0.000
CH_A[c]	−0.362	0.419	0.230	0.243
CH_B[c]	0.362	−0.419	−0.230	−0.243
CH_c[c]				
energy, au	0.16075	0.16320	0.15771	0.16299

[a] Obtained by SCF 6-31G*. [b] Contribution of p_y orbital with axis orthogonal to the molecular plane. [c] 1s orbital contribution.

Table VII. HOMO Energies, Atom Contributions, and Net Atomic Charges for Guanine[a]

atom[b]	HOMO contribution	net atomic charge, au
N_1	−0.229	−0.361
C_2	0.248	0.375
N_3	0.453	−0.336
C_4	−0.323	0.197
C_5	−0.457	−0.017
C_6	0.025	0.310
O^6	0.362	−0.267
N_7	0.189	−0.249
C_8	0.391	0.125
2-NH_2	−0.279	−0.419

[a] Obtained by STO-3G calculations. [b] Energy = −0.215 50 au.

R which has the CH_3 group orthogonal to the NNO plane (see Table IV). The rotational energy barrier is estimated to be 42.6 kcal mol^{-1}.

Table V gives the net atomic charges for the four configurations of methanediazohydroxide. It may be noted that there is an increase in negative charge on N_1 in structure *E*-I compared with *E*-II. In addition the geometry optimization and energy calculations summarized in Table IV predict a slight stabilization of conformer *E*-I compared with *E*-II by 0.005 28 au. This information combined with the observed increase in negative charge on N_1 in *E*-I may be accommodated by weak hydrogen bonding between the OH and the N_1 lone pair in structure *E*-I. This corresponds to a 0.02-electron overlap by the Mulliken population analysis derived from the GAUSSIAN 80 program.[30]

Another conclusion to be drawn is that the two *Z* conformers possess bigger net charges on the carbon than either of the two *E* conformers.

The LUMO energies and individual atom contributions for the four principal conformations of the *Z* and *E* methanediazohydroxides were calculated with 6-31G* basis sets and are given in Table VI. The corresponding main contributions to the HOMO of guanine, required for the HSAB treatment of the approach of electrophiles, to a principal DNA nucleophilic site, are given in Table VII. Since guanine is a bigger molecule it cannot be treated with the large basis set employed for **5aB** and **6aB** so the STO-3G[34] basis set was used. The geometry used for guanine is the one obtained by Del Bene,[35] with STO-3G[34] calculations.

Discussion

One of the major unifying hypotheses in the field of chemical carcinogenesis, for which there is considerable experimental support, is that all carcinogens exert their effects after transformation into electrophiles which react with nucleic acids.[36]

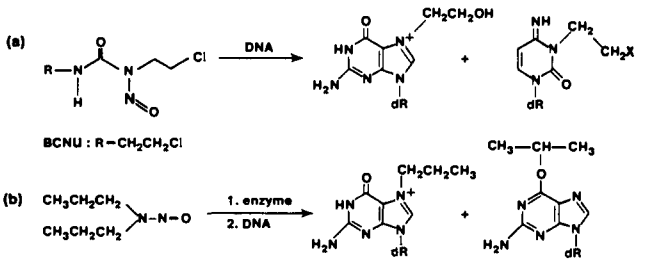

Figure 1. (a) Representation of the isolated products from the in vitro reaction of bis(2-chloroethyl)nitrosourea [BCNU] with polydeoxyribonucleotides $(dG)_n$ and $(dC)_n$.[9,10] (b) Representation of the isolated products from the in vivo reaction of the carcinogen dipropylnitrosamine following metabolic α-hydroxylation and dealkylation to monopropylnitrosamine, then alkylation of guanine sites on DNA.

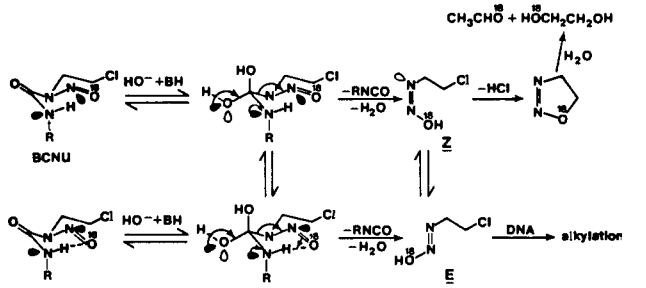

Figure 2. Decomposition of BCNU under physiological conditions resulting in reversible amide carbonyl hydration, stereoelectronically controlled formation of (*Z*)- and (*E*)-chloroethanediazohydroxides, and evidence, via specific ^{18}O labeling, of the cyclization of the former to an intermediate 1,2,3-oxadiazoline thence to the isolated ^{18}O-labeled products.

However, it has been pointed out recently by Swann that "the concentration upon alkylation of nucleic acids, [and] the consequences of metabolism, has diverted attention from the nature and chemistry of the reactive intermediates".[36b] The possible electrophiles to which *N*-nitroso compounds give rise in vivo, and which therefore may ultimately be responsible for the characteristic biological properties of carcinogenic alkyl nitrosamines,[1,12,37] mutagenic nitrosamides,[37] and certain antitumor nitrosoureas,[4,9,10,13,38-40] include the (*Z*)- and (*E*)-alkanediazohydroxides, the alkanediazonium ion,[41] and the corresponding carbenium ion. The reaction illustrated in Figure 1a shows that when the clinical anticancer agent **BCNU** [1,3-bis(2-chloroethyl)-1-nitrosurea] reacts with DNA the isolated alkylated bases contain unrearranged alkyl groups.[9,10] Experiments with selectively deuterated **BCNU** demonstrate that less than 10% of the products of decomposition of BCNU under physiological conditions (and in the absence of DNA) corresponded to rearrangement, i.e., hydride shift in an intermediate carbenium ion.[42] In the second example in Figure 1b in the reaction of dipropylnitrosamine in vivo, and following enzymatic α-hydroxylation and conversion to propylnitrosamine, a mixture of alkylated guanosine derivatives was obtained corresponding to alkylation without rearrangement at G-N_7 and with rearrangement to an isopropyl group at G-O^6.[11,12] The authors concluded that the amounts of the different adducts indicated that attack at both N7 and O^6 of guanine proceeded by bimolecular substitution but that the transition state for reaction at O^6 may

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be much looser than that for reaction at N_7 permitting a hydride shift.[11] A very recent study examined the reaction of 1-*n*-propyl-1-nitrosurea with calf thymus DNA in vitro.[13] The authors observed more guanine N7-alkylation than O^6-alkylation in a ratio of 1.37:1. The proportion of unrearranged product to rearranged was (93–90):(7–10) for N_7-alkylation and (77-72):(23–28) for O^6-reaction. These examples strongly indicate a predominantly S_N2 process and militate against a free carbenium ion for the main component of the reaction, although a more critical test would involve use of an electrophile with a chiral methyl group. The alkyldiazonium ion is at first sight an attractive candidate[41] in terms of reactivity although S_N1 processes are perhaps more likely with these species.[43,44] However, recent evidence on the stereoelectronically controlled[14] decomposition of (2-chloroethyl)-nitrosoureas under physiological conditions employing specific $N^{18}O$ labeling[45] favors the alkanediazohydroxide instead, at least for this important class of *N*-nitroso compounds. Thus, as illustrated in Figure 2, the ^{18}O labeling proved the intermediacy of the 1,2,3-oxadiazoline arising from cyclization of the (*Z*)-2-chloroethanediazohydroxide to the extent of ~20% for **BCNU** and ~80% for α,α-Me_2**BCNU** of the overall reaction pathways.[14] The diazonium ion is clearly not involved. Although these experiments do not rule out the alkanediazonium ion as a minor electrophile responsible for alkylating the DNA, they do indicate that the (*Z*)- and (*E*)-alkanediazohydroxides are present as major reactive species and should be considered as candidates for the role of key electrophile. The ^{18}O-labeling experiment with **BCNU** indicated a ratio of about 80:20 for *E*/*Z* diazohydroxide configurational isomers and also indicated, as is reflected in the present results, that the isomers do not interconvert readily.[45] These above considerations prompted the present studies of the properties and interrelationships of alkyldinitrogen species. We focused here on the methanediazohydroxides and related species because of the well-documented carcinogenic properties of methanenitrosamine.[1,12,20]

The conclusion that the (*Z*)-methanediazoate in both polar protic and aprotic solvents is best represented by **2aC** (Scheme II), in which the potassium counterion is located on average between N_1 and the O atoms is in accord with X-ray diffraction evidence on (*Z*)-methanediazoate[22] and for the corresponding arenediazoates.[23] This indicates equilibration between species **2aA** and **2aC** which is fast on the NMR time scale. The lifetime $\tau = \tau_A\tau_C/(\tau_A + \tau_C) << {}^1/_2\pi(\nu_C - \nu_A)$ (where ν_A and ν_C are, for example, the N_2 chemical shifts).[46]

In contrast the NMR data (^1H, ^{13}C, and ^{15}N) for the (*E*)-methanediazotate indicates two discrete species in MeSO-d_6 with a sufficiently slow interconversion rate in which the potassium ion moves in position across the N⇌N⇌O system so that two signals are observed. Under these conditions $\tau >> {}^1/_2\pi(\nu_C - \nu_A)$.[46] Substituting the ^{15}N chemical shift differences for **4aA** and **4aB** this becomes

$$\tau >> (1/2\pi)(564.7 - \delta 539.8) \equiv 1/2\pi(24.9 \times 20.283 \text{ Hz})$$

$$\tau >> 3.15 \times 10^{-4} \text{ s}^{-1}$$

The two species could be σ coordinated at O or N or π coordinated and may represent two types of ion pairs or clusters.[47] There are precedents for association of ions of this type and for their slow interconversion.[48,49]

The corresponding pairs of (*Z*)- and (*E*)-alkanediazoates (**2** and **4**) are relatively stable at ambient temperatures in Me_2SO-d_6 and do not interconvert. This is in accord with the estimate of the substantial optimized energy barrier for configurational inversion of the corresponding diazohydroxide or even compared with the lower value calculated on the basis of rotational interconversion of the corresponding methanediazoate anions.

The greater reactivity of the (*Z*)-alkanediazoates and -diazohydroxides in proton exchanging solvents in being rapidly converted into diazoalkanes may be accounted for either by syn selectivity[50] or stereoelectronic elimination of hydroxyl due to participation of the anti-N_4 lone pair.[51] It may be noted in this connection that the small additional stabilization in the *E*-I structure afforded by the 0.02-electron overlap between the N_1 lone pair and the OH hydrogen and amounting to 1.6 kcal/mol^{-1} is not available to the more reactive *Z*-I and *Z*-II forms. The higher energies and reactivity of the *Z* isomers may contribute to the "looseness" in the transition state for nucleophilic substitution by guanine base sites mentioned by other reports[11] permitting some rearranged products.

The net atomic charges on the carbon atom indicate the somewhat more electrophilic nature of these centers in the *E* forms. The ab initio calculations predict significantly greater stability for the *E* isomers by their relative energies which is also reflected in the corresponding LUMO energies.

The results on the alkyldinitrogen species are embodied in the Scheme II, the utility of which may be judged by its ability to interpret some biological events. Thus in the case of the (2-chloroethyl)nitrosoureas the stereoelectronically controlled collapse of tetrahedral intermediates affords discrete (*Z*)- and (*E*)-2-chloroethanediazohydroxides.[14] The results summarized in Scheme II indicate that little interconversion occurs between the *Z* and *E* diazohydroxides. This ensures that they will react more or less independently. In addition the higher reactivity and selective removal of the *Z* isomer by the cyclization pathway to the 1,2,3-oxadiazoline[45] (Figure 2a) combined with the greater thermodynamic stability of the *E* form may explain why the *E* isomer accounts for ~80% of the reaction pathways for **BCNU**.[45]

At present a controversy exists concerning the significance of the configurational differences between alkyldinitrogen species as it relates to their reactivity.[20,27] The observation of distinctly different residence times for the metal counterion on the nitrogen and oxygen centers of (*E*)- and (*Z*)-alkanediazoates may be significant in this regard. The elusive nature of the alkanediazohydroxides pointed out by others[20,27] is borne out by the present results. However, the integration of these species into Scheme II, together with the analogies with the more accessible corresponding aryldinitrogen compounds, permits some inferences to be drawn regarding their properties. While the pathway **2aB** → **5aB** → **8a** → **7a** is plausible, an alternative direct elimination of hydroxide from **2aB** to give **7a** is also possible. In addition the relatively slow interconversion of **4A** ⇌ **4B** ⇌ **4C** may provide a pool for production of the (*E*)-alkanediazohydroxide, which is not accessible to the more reactive *Z* isomer owing to the more rapid exchange between **2A** ⇌ **2B** ⇌ **2C**.

Recent evidence suggests that the key metabolite of the carcinogen, *N*,*N*-dimethylnitrosamine is (hydroxymethyl)methylnitrosamine,[36b] which loses formaldehyde to give methylnitrosamine.[52,55] Supporting evidence is that the formation of (*E*)-methylnitrosamine (**6aA**) and (*E*)-methanediazohydroxide (**6aB**) have been inferred in the aqueous decomposition of acetoxynitrosamine.[53] The (hydroxymethyl)methylnitrosamine is considered to be the only metabolite likely to have sufficient stability

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(47) The restrictions imposed by the requirements to employ $Cr(AcAc)_3$ as a relaxing agent to observe the ^{15}N signals prevent accurate measurements of relative concentration of species **4aA** and **4aB** by ^{15}N NMR at the moment. Future work designed to quantify these equilibria will therefore employ ^{13}C NMR.
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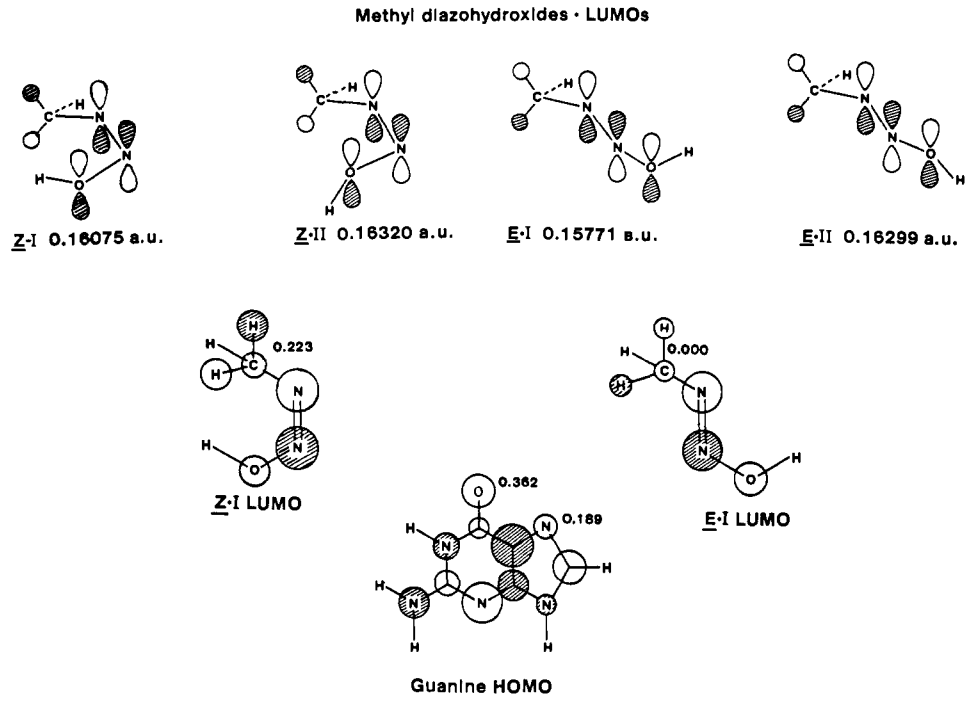

Figure 3. Representation of the principal conformers of the (*Z*)- and (*E*)-methanediazohydroxides derived from the geometry optimization calculations, their relative energies, and the orbital signs of their respective LUMOs. The lower figures give depictions of the signs and relative magnitudes of the atom coefficients on the LUMOs of *Z*-I and *E*-I methanediazohydroxides and those of the HOMO of guanine permitting prediction of situselectivities. The diameters of the circles are approximately proportional to the magnitudes of the orbital coefficients.

to diffuse from the cytoplasm, where it is formed, to the nucleus where it can generate methanediazohydroxide to react with DNA.[36b,54] Once released, methylnitrosamine will equilibrate with the diazohydroxide because the energy barrier in the interconversion of **6aA** and **6aB** is estimated to be ca. 12 kcal/mol^{-1}.[55] By contrast the barrier to rotation about the N–N bond in dimethylnitrosamine is 23 kcal mol^{-1}.[56]

The present ^{1}H and ^{15}N NMR evidence suggests an equilibrium between the *Z* and *E* conformers of the unstable key carcinogen methylnitrosamine at least in aprotic solvents. Tautomerization of these forms would be expected to give rise to a mixture of the (*Z*)- and (*E*)-methanediazohydroxides (**5aB** and **6aB**). A single or preferred conformer of carcinogenic alkylnitrosamine may conceivably exist in vivo. However, this seems unlikely and indeed the results on alkylation of guanine by dipropylnitrosamine in vivo leading to clean unrearranged propylation at N_7 and incorporation of an isopropyl group at O^6 argue in favor of two related electrophilic species subject largely to bimolecular processes.[11,12] The present results indicate these species may be the (*E*)- and (*Z*)-alkanediazohydroxides.

The situselectivity of these species was examined using the concept of hard and soft acids and bases (HSAB)[57-59] and employing the concepts of frontier orbital theory.[60] The critical sites of alkylation of the DNA bases in terms of accessibility, frequency of reaction, and biological response are the O^6 and N_7 positions in guanine of which the former appears to be the more significant.[61,62] For example, neonatal rat brain is more sensitive to ethylnitrosourea carcinogenicity because of the formation and longer persistence of O^6-ethylguanine.[63] In addition the induction of angiosarcomas upon exposure to dimethylnitrosamine or 1,2-dimethylhydrazine has been attributed to the accumulation and persistence of O^6-methylguanine in nonparenchymal cells.[64,65] Guanine O^6-alkylation specifically changes base pairing since it can form a wobble pair if the alkyl group is anti to the Watson–Crick side.[66] For alkylation of DNA one needs to consider the LUMOs of the electrophiles[33,60] and the HOMOs of the nucleophiles[57] (Figure 3). It may be seen from Table VII that the main contributions of N_7 and O_6 are 0.189 and 0.362, respectively. From the rule that the larger the coefficient in the appropriate frontier orbital at the reaction center the softer the reagent[58,60] the O^6 position will be softer than N_7. Table VI, shows the LUMO of structure *E*-I has the lowest energy at 0.157 71 au, and that structure *Z*-I has the higher energy of 0.160 75 au. The main contributions of the carbons to the LUMOs of *E*-I and *Z*-I are, respectively, 0.000 and −0.276. Invoking the rule that soft centers prefer to react at soft sites, one may predict preferential reaction of *Z*-I methanediazohydroxide at O^6 where the higher reactivity may favor a looser transition state.[11] Conversely one predicts preferential reaction of the more stable and less reactive *E*-I diazohydroxide at N_7. These results may therefore provide a rationale for the in vivo situselectivity in the reaction of the carcinogen dipropylnitrosamine with DNA.[11,12]

It will have been noted that this initial approach to interpret the carcinogen electrophile situselectivity has not yet addressed the problem of Watson–Crick or Hoogsteen base pairing. In this regard Singer[67] has observed that the extent of alkylation at oxygens including the critical G-O^6 position is not a function of strandedness of the DNA, since the O^6 of G (and O^4 of T and the O^2 of C) all possess a reactive unbonded electron pair.

The utility of this approach of combined ^{15}N NMR characterization of relevant alkyldinitrogen species with appropriate ab initio calculations to explore the situselectivity of other *N*-nitroso compounds observed in vivo will be reported in due course.

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Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The ^1H NMR spectra of the intermediates were recorded on Perkin-Elmer 90 and Varian HA-100 analytical spectrometers and those of the final nitrosoureas were recorded on Bruker WH-200 and WH-400 spectrometers. The spectra were measured on approximate 5–10% (w/v) solutions depending upon the spectrometers, in appropriate deuterated solvents with tetramethylsilane as internal standard. Line positions are recorded in ppm from reference.

The ^{15}N spectra were recorded on a Bruker WH-200 spectrometer operating at 20.283 MHz. The spectra were obtained by using dimethylformamide as external reference, and chemical shift values were reported relative to ammonia at 0.0 ppm and 10–20% with the respective deuterated solvent as lock signal.[53] Most of the proton-decoupled natural-abundance ^{15}N spectra were taken by using 1 M solutions containing 0.05–0.1 M $Cr(AcAc)_3$ in a 20-mm diameter tube after 85–86K scans. The spectra of specifically labeled compounds, were recorded on 0.01 M solutions with 0.01–0.1 M added $Cr(AcAc)_3$ and in approximately 1–4K scans.

Materials. Sodium nitrite-^{15}N (95–99%), butyl nitrite-^{15}N (99%), methylamine-^{15}N, ethylamine-^{15}N, and propylamine-^{15}N (99%) were obtained from Merck Sharp and Dohme. Methyl, ethyl, and propylhydrazines were obtained from Aldrich Chemical.

Preparation of Doubly ^{15}N-labeled (*Z*)-Alkanediazoates (2). The required specifically $^{15}N,^{15}NO$ doubly ^{15}N-labeled nitrosourea (99% enriched) precursors were prepared as described previously.[14] Solutions of the potassium (*Z*)-alkanediazoates (**2**) were prepared freshly as required by treating 5 mM solutions of the labeled nitrosoureas in anhydrous butanol with 1.1 equiv of KO-*t*-Bu at −20 °C.[15] Inorganic salts were removed by centrifugation, and the solvent was removed in vacuo at low temperature with shielding from the light. The appropriate anhydrous cold solvent, CD_3OD, MeSO-d_6, or ethanol was added to provide an approximately 5% solution for the NMR experiments.

Preparation of Singly ^{15}N-Labeled (*E*)-Alkanediazoates (4). The required (*E*)-alkanediazoates (**4**) were prepared from the corresponding alkylhydrazines by treatment with 2 equiv of *n*-BuO^{15}NO (99% enrichment) and 1 equiv of KO-*t*-Bu in butanol according to a literature procedure for the unlabeled materials.[16]

Compound **3a** could be isolated[17] as a solid: mp 45 °C; ^1H NMR (pyridine-d_5) 3.80 (d, 3 H, CH_3, $^3J(^{15}N–H)$ = 2.20 Hz), 7.60 (br m, 2 H, NH_2); ^{13}C NMR (pyridine-d_5) 39.72 (d, CH_3, $^2J(^{15}N–^{13}C)$ = 6.2 Hz); ^{15}N NMR (Me_2SO-d_6) 474.1 (N=O); ^{15}N NMR (THF) 92.4 (NH_2), 252.0 (NCH_3), 487.0 (N=O).

Preparation of Doubly ^{15}N-Labeled (*E*)-Alkanediazoates (4). (a) (^{15}N)Methylethylcarbamate. A mixture of 1 g (15 mmol) of methylamine hydrochloride in 2 mL of water was added to 10 mL of ether and the mixture cooled in an ice bath. Ethyl chloroformate (1.62 g, 15 mmol) was added followed by a solution of sodium hydroxide (1.2 g, 30 mmol) in 1.5 mL of water with continuous stirring. Stirring was continued for a further 15 min then the ether layer was removed and the water layer extracted twice with ether (2 × 15 mL). The combined ether extract was dried (Na_2SO_4) and concentrated. The residual oil was purified by distillation under reduced pressure: 1.35 g (85% yield), bp 71–73 °C (18 mm);[68] ^1H NMR ($CDCl_3$) 1.25 (t, 3 H, J = 8 Hz), 2.75 (d, 3 H, $J(^{15}N–^1H)$ = 7.6 Hz), 4.1 (q, 2 H, J = 8 Hz), 4.8 (brs, 1 H).

(b) Doubly ^{15}N-Labeled N-Methyl-*N*-nitrosourethane. A solution of (^{15}N)methylurethane (1.35 g, 13 mmol) and $Na^{15}NO_2$ (1.8 g, 26 mmol) in 5 mL of water and with 10 mL ether was cooled in an ice bath. Concentrated hydrochloric acid (2.6 mL, 26 mmol) was added slowly with stirring. After a period of 15 min the ether layer was removed and the aqueous layer was extracted with ether (3 × 10 mL). The combined ether extract was dried ($MgSO_4$) and the solvent removed under reduced pressure. The residual yellow oil was purified by chromatography on florisil with ether as eluant: 1.7 g (95% yield); ^1H NMR ($CDCl_3$) 1.45 (t, 3 H, ^1J = 7 Hz), 3.15 (q, 3 H, $^2J(N_1–H)$ = 1.4, $^3J(N_2–H)$ = 0.6 Hz), 4.55 (q, 2 H, ^1J = 7 Hz).

(c) Potassium ($^{15}N_2$)-(*E*)-Methanediazoate. A solution of the doubly labeled *N*-methyl *N*-nitrosourethane (0.135 g, 1 mmol) in 1 mL of dry ether was cooled to −20 °C and KO-*t*-Bu (0.12 g, 11 mmol) added with continuous stirring. After 30 min the resulting solid was quickly filtered under vacuum and washed well with dry ether and stored cold under a He atmosphere. The NMR spectral data are given in the Tables I–III.

Doubly ^{15}N-Labeled Methylnitrosourea. A solution of (^{15}N)methylurea (0.75 g, 10 mmol) and $Na^{15}NO_2$ (1.4 g, 20 mmol) in 5 mL of water with 10 mL of ether was cooled to 0 °C, and concentrated hydrochloric acid (1.9 mL, 20 mmol) was added gradually with stirring. After 15 min the ether layer was removed and the aqueous layer was extracted with ether (2 × 15 mL). The combined ether extracts were dried ($MgSO_4$), and the solvent was removed under vacuum. The residue was purified by chromatography on florisil with ether as eluant: 0.9 g (90% yield), mp 118 °C (lit. mp 112–113 °C);[69] ^1H NMR ($CDCl_3$) 3.2 (d, 3 H, $J^{15}(N_1–H)$ = 0.8 Hz), 5.6 (br, 2 H).

($^{15}N_2$)Diazomethane (7a). This was prepared by reaction of (*Z*)-methanediazoate (**2a**) with 2 equiv of KO-*t*-Bu in CD_3OF at −80 °C and was characterized by ^{15}N NMR (CD_3OD): 289.3 (N_1), 395.9 (N_2), $^1J(^{15}N_1–^{15}N_2)$ = 7.5 Hz.[70]

The (^{15}N)Nitrosyl chloride required for nitrosation of methylamine in ether and at −50 °C was prepared by addition of hydrogen chloride gas to a 10% aqueous solution of $Na^{15}NO_2$.

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