The Product Distribution of DNA Base Alkylation by *N*-Nitroso Compounds: an INDO SCF MO Theoretical Study

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Alkylation of DNA is regarded as a critical step involved in the mutagenic and carcinogenic action of *N*-nitroso compounds. The product distribution of DNA alkylation by *N*-nitroso compounds has been studied with several contributing factors in view. The theoretically computed values of these factors serve as clues to rationalise experimentally observed findings concerning this product distribution, and also allow for more generalised predictions that embrace a wider scope of application than the small list of compounds already studied experimentally. The relationship between product distribution and mutagenic and carcinogenic potency of *N*-nitroso compounds is briefly discussed.

Most *N*-nitroso compounds are well-known for their carcinogenic activity.[1–3] Their mutagenicity and carcinogenicity are attributable to alkylation of DNA at specific sites. *N*-Nitroso compounds alkylate DNA *in vitro* and *in vivo* at a large variety of nucleophilic sites located on the sugar–phosphate moiety as well as on the DNA bases.[4,5] A theoretical study is made here of the product distribution of the reaction of DNA alkylation by *N*-nitroso compounds which takes into account the various DNA base sites involved and also the changes in the alkyl group participating as the *N*-nitroso compound varies.

N-Nitroso compounds may be broadly classified into *N*-nitrosamines and *N*-nitrosamides. The former, exemplifed here by the dialkylnitrosamines, require metabolic α-hydroxylation for their activity as mutagens or carcinogens. The latter, represented here by the alkylnitrosoureas, can act by spontaneously decomposition *in vivo*. Metabolic or spontaneous activation yields the reactive electrophiles: alkyldiazo hydroxides, alkyldiazonium cations, and alkyl cations (R^+), which are regarded as the actual agents responsible for the alkylation of DNA. The activating and deactivating pathways postulated for the mutagenic and carcinogenic action of dialkylnitrosamines[6] and alkylnitrosoureas[7] are portrayed in the Figure. Alkylation of DNA by the reactive electrophile species can confer mutagenic properties on to the DNA bases, rendering them capable of inducing point mutations through aberrant base pairing during DNA replication. Point mutations at critical sites in certain genes called oncogenes have been found to provide a molecular basis for neoplastic transformation in numerous cases of human and animal cancer.[8]

Limited facts have been gathered from experiment about the product distribution of DNA alkylation by *N*-nitroso compounds. Of the various DNA base sites, attention is focused here on the N^7-guanine (N^7-G), O^6-guanine (O^6-G), and O^4-thymine (O^4-T) sites. The order of alkylation abundance with respect to DNA base site observed for methylation[9] and ethylation[10] is N^7-G > O^6-G > O^4-T. On administration of comparable doses of an *N*-nitroso compound, the methylating compound gives a net alkylation yield an order of magnitude higher than the ethylating yield.[11] However, with the transition from methylation to ethylation or butylation, the relative abundance of *O*-alkylated products (as compared with *N*-alkylated products) demonstrates a marked increase.[11–13]

Although the N^7-G site is the most abundantly alkylated DNA base site, it appears to be irrelevant for mutagenesis or tumourigenesis.[14,15] The promutagenic and procarcinogenic role seems to be fulfulled by the O^6-G and O^4-T sites, as several experimental findings would indicate.[16–19] Thus, it is the abundance of these *O*-alkylated products, rather than the net alkylation yield, which may be linked with the mutation-inducing or tumour-inducing power of the *N*-nitroso compound.

Three features of the product distribution of DNA base alkylation are considered for study here. These are (*a*) the net alkylation yield, (*b*) the product distribution with respect to the various DNA base sites, and (*c*) the relative abundance of *O*-alkylated products. The following factors may be invoked to rationalise the observed experimental findings relating to the product distribution, which can also provide a model to allow an attempt at prediction of the above features pertaining to this product distribution on extension to a larger set of hitherto untested compounds.

The Molecular Electrostatic Potential Minima Associated With the Various DNA *Base Sites.*—Pullman *et al.*[20] have computed their values, in descending order of magnitude: N^7-G > O^6-G > O^4-T, actual values being −2 858, −2 803, and −2 770 kJ mol^{-1}, respectively, in B-DNA.

The Steric Accessibilities Associated with the Various DNA *Base Sites.*—Using a water molecule probe, steric accessibilities have been calculated by Lavery *et al.*,[21] the order of magnitude being again N^7-G > O^6-G > O^4-T. Actual values are 4.1, 2.6, and 2.2 $Å^2$, respectively, in B-DNA.

The Heats of Alkylation of the DNA *Base Sites by the Reactive Electrophiles.*—These thermodynamic criteria determine the extent to which the DNA alkylation reaction would compete with other reactions like reactive electrophile hydrolysis to alcohols. Their order of magnitude with respect to the DNA base sites would be expected to contribute towards the experimentally observed trend. Their variation in magnitude as the alkylating group changes may also be expected to influence

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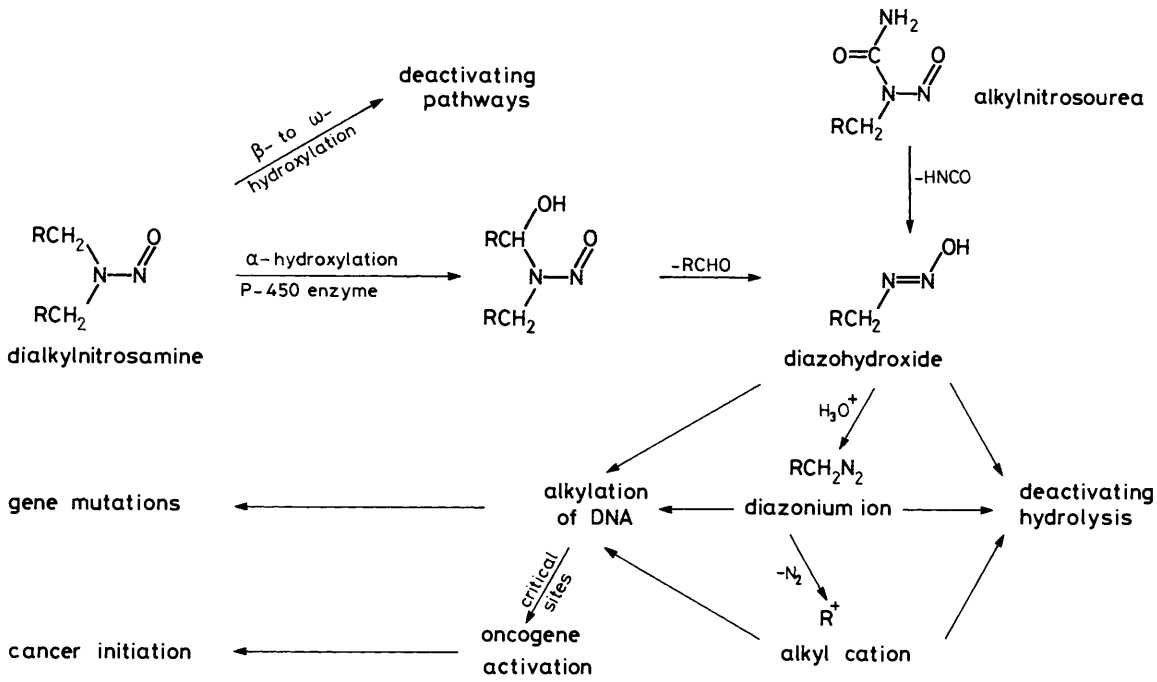

Figure.

the net alkylation yield observed upon administering the same dose of various parent *N*-nitroso compounds with different alkyl groups.

The Level of Preference for O-*Alkylation over* N-*Alkylation.*—This *O*-specific alkylation factor is of consequence in determining the relative abundance of *O*-alkylated products. This factor may be related to the degree of hardness or softness of the reactive electrophile participating in DNA alkylation. It is supposed here that the O^6-G and O^4-T sites are harder bases than the N^7-G site. By hard–soft acid–base concepts,[22] these oxygen sites would be more capable of attracting those reactive electrophiles possessing harder acid properties. The level of preference for *O*-alkylation would thus depend upon the degree of hardness of the reactive electrophile involved. Among the three types of candidate reactive electrophiles, the order of hardness may be assumed to be alkyl cation > alkyl diazonium > alkyl diazohydroxide. Hence, a relatively greater participation of the alkyl and alkyl diazonium cations in DNA alkylation would favour *O*-specific alkylation. The degree of this participation would be determined by the stability of the R^+ and RNN^+ species. Such reasoning links the preference for *O*-alkylation to the stability of the positively charged reactive electrophile.

The Competition between Activating and Deactivating Pathways at the Initial Step of Enzymatic Hydroxylation, which Applies to the Dialkylnitrosamines and not to the Alkylnitrosourea Carcinogens.—Cytochrome P_{450} tends to hydroxylate at the α-carbon, but there is substantial evidence for β- to ω-hydroxylation pathways.[3] Only the α-hydroxylation route is commonly regarded as activating (see Figure). Since the actual degree of hydroxylation at the various carbons is not known, it is assumed as a first approximation that the hydroxylation is random. The competition factor may then be expressed as in equation (1), where n_α is the number of hydrogen atoms at the

$$f = n_\alpha/n_t \qquad (1)$$

α-carbon of the alkyl group and n_t is the total number of hydrogen atoms in the dialkyltrinitrosamine which can be hydroxylated by enzyme. Increase in the alkyl group chain length would decrease the value of the competition factor *f*.

Nature of the N-*Nitroso Alkylating Agent.*—The alkyldiazo hydroxide species,[23] the alkyldiazonium ion species,[24] the alkyl cation species[25,26] and diazoalkane[27] were all considered as the possible alkylating agent in carcinogenesis due to *N*-nitroso compounds in the light of theoretical studies. The alkyldiazonium ion is regarded here as possessing the best compromise between electrophilicity and stability to act as the prime alkylating agent at least for nitrogen sites. But the existence of rearranged products (*viz.* O^6-isopropylguanine) upon action of dipropylnitrosamine would imply involvement of a carbocation species during alkylation at oxygen sites.[28] The oxygen sites being of significance for mutagenesis and cancer, it would seem appropriate to take into account both the RNN^+ and R^+ when investigating DNA alkylation of mutagenic and carcinogenic relevance. A possibly helpful approach (not implemented here directly) would be to consider the amount of carbocation character present in the transition state obtained on alkylation of the O^6-G/O^4-T sites by the alkyl diazonium cation. The MNDO activation energies for attack by alkyldiazonium cation upon various nitrogen and oxygen nucleophiles[24] suggests that the degree of preference for *O*-alkylation is much influenced by the relative value of this activation energy.

Theoretical

Methods of Calculation.—The INDO SCF MO method[29] was used for all calculations. Complete optimisation of all molecular geometries was carried out using an analytical gradient method[30,31] suitably modified to handle large molecules efficiently. The fairly large size and number of the molecules treated in this study necessitates the use of semiempirical MO theory which, although often not yielding accurate values of physical quantities, can be of use in predicting or analysing trends.

Table 1. Heats of alkylation by ultimate carcinogens at the N^7-G, O^6-G, and O^4-T sites.

Alkyl group	ΔH_{dh}/kJ mol^{-1}			ΔH_{dz}/kJ mol^{-1}			ΔH_{ac}/kJ mol^{-1}		
	N^7-G	O^6-G	O^4-T	N^7-G	O^6-G	O^4-T	N^7-G	O^6-G	O^4-T
Me	223.3	241.1	389.6	−357.6	−339.8	−191.3	−1 661.9	−1 644.1	−1 495.6
CH_2CN	240.1	266.7	420.9	−360.9	−334.3	−180.1	−1 396.7	−1 370.0	−1 215.9
Et	213.8	231.2	383.6	−329.3	−311.9	−159.5	−1 129.3	−1 111.9	−959.5
Pr	210.6	227.0	382.5	−319.3	−302.9	−147.4	−783.4	−767.1	−611.5
Bu	208.7	226.5	384.2	−316.3	−298.5	−140.8	−670.3	−652.3	−494.8
Pe	208.2	226.0	386.2	−314.1	−296.3	−136.1	−575.9	−557.3	−397.1
CH_2CHCH	247.7	267.3	419.2	−331.6	−312.0	−160.0	−1 412.5	−1 393.0	−1 241.0
CH_2CHOAc	235.7	260.4	417.8	−329.1	−304.4	−147.0	−1 283.2	−1 258.5	−1 101.1

Table 2. Theoretical indicators of the ease of alkylation compared with alkylation abundances at the N^7-G, O^6-G, and O^4-T sites.

Site	MEP/ kJ mol^{-1}	SA/Å^2	ΔH_{dz}(Me)/ kJ mol^{-1}	ΔH_{dz}(Et)/ kJ mol^{-1}	ΔH_{ac}(Me)/ kJ mol^{-1}	ΔH_{ac}(Et)/ kJ mol^{-1}	C_{Me}/pmol μmol^{-1} DNA	C_{Et}[a] (%)
N^7-G	−2 858	4.1	−357.6	−329.3	−1 661.9	−1 129.3	1 203	13.6
O^6-G	−2 803	2.6	−339.8	−311.9	−1 644.1	−1 111.9	147	9.2
O^4-T	−2 770	2.2	−191.3	−159.5	−1 495.6	−959.5	2	2.1

[a] In % of total alkylation.

Starting Geometries for Optimisation.—Starting geometries for the DNA bases were derived from crystal structure data.[32,33] The alkyldiazohydroxide geometries were constructed from standard data on molecular geometry,[34] locating the stable conformers by appropriate rotations. The MNDO optimised structure of the methyldiazonium[35] ion formed the basis for the alkyldiazonium ion geometries. A planar sp^2 and a protonated ethylene structure were used for the methyl and ethyl cations respectively. Protonated cycloalkane structures were employed for the propyl, butyl, and pentyl cations, being much more stable than the straight chain structures, as indicated by these INDO MO calculations.

The methylated DNA bases were constructed by imposing a pyramidal methyl group upon the DNA base moiety at each alkylation site after preliminary localised optimisation of internal co-ordinates. The most stable conformers for the longer chain homologues were located by appropriate rotations. In all cases, the orientation of the alkylating group was chosen *trans* to the Watson–Crick hydrogen bonding side, as this side is sterically favourable in double-stranded DNA for attack by the reactive electrophile.

Thermodynamic Indices.—The heats of alkylation of the DNA base sites by the alkyldiazohydroxide (ΔH_{dh}), by the alkyldiazonium ion (ΔH_{dz}), and by the alkyl cation (ΔH_{ac}) were obtained from the following equations, (2), (3), and (4).

$$\text{alkyldiazohydroxide} + B + H_3O^+ \longrightarrow RB^+ + N_2 + 2H_2O + \Delta H_{dh} \quad (2)$$

$$\text{alkyldiazonium} + B \longrightarrow RB^+ + N_2 + \Delta H_{dz} \quad (3)$$

$$R^+ + B \longrightarrow RB^+ + \Delta H_{ac} \quad (4)$$

where B and RB^+ stand for the free and alkylated DNA base at each site respectively.

Criteria for alkyldiazonium and alkyl cation stability (which represent the level of preference for *O*-specific alkylation) are provided by the heats of transformation ΔH_a and ΔH_b of the alkyldiazohydroxide to the alkyldiazonium, and alkyl cations respectively, and by the heat of dissociation ΔH_c of the alkyldiazonium to the alkyl cations as given by equations (5) to (7) below.

$$RNNOH + H_3O^+ \longrightarrow RNN^+ + 2H_2O + \Delta H_a \quad (5)$$

$$RNNOH + H_3O^+ \longrightarrow R^+ + 2H_2O + N_2 + \Delta H_b \quad (6)$$

$$RNN^+ \longrightarrow R^+ + N_2 + \Delta H_c \quad (7)$$

Results and Discussion

The heats of alkylation at the N^7-G, O^6-G, and O^4-T sites are presented in Table 1 for the RNNOH, RNN^+, and R^+ species. Values were calculated for the methyl (Me), cyanomethyl ($-CH_2CN$), ethyl (Et), propyl (Pr), butyl (Bu), pentyl (Pe), 2-hydroxyethyl ($-CH_2CH_2OH$), and 2-acetoxyethyl ($-CH_2CH_2OAc$) groups. Table 2 sums up the experimental and theoretical information on the product distribution with respect to the DNA base sites. Values of the stability criteria for the RNN^+ and R^+ species are presented in Table 3 for the alkyl groups of Table 1 as well as for the fluoromethyl ($-CH_2F$), difluoromethyl ($-CHF_2$), vinyl ($-CHCH_2$), and phenyl (Ph) groups. Table 4 gives various criteria that determine the product distribution of DNA alkylation for a number of dialkylnitrosamines. The symbol c_0 represents the actual concentration of O^6-G/O^4-T alkylated products obtained on reaction with the parent *N*-nitroso compound.

Product Distribution with Respect to DNA *Base Sites.*—Examination of the data in Table 1 reveals that the thermodynamic stability of the alkylated product with respect to the DNA base site is invariably N^7-G > O^6-G > O^4-T, regardless of the alkylating group. This order coincides with that displayed by the molecular electrostatic potential and steric accessibility indices in rationalising the experimentally observed product distribution for methylating and ethylating *N*-nitroso compounds as given in Table 2, where c_{Me} and c_{Et} refer to the product concentration obtained at each site when DNA is treated *in vivo* with methylnitrosourea[9] and ethylnitrosourea,[10] respectively. All these theoretical indicators point towards establishment of the order obtained by experiment. The steric accessibility index affords the best rationalisation for the diversity in alkylation abundance between the N^7-G and O^6-G sites. For the diversity between the O^6-G and O^4-T sites, the alkylation indices seem to provide the better explanation.

Net Alkylation Yield.—The heats of alkylation at all three DNA base sites by the RNN^+ and R^+ (but not the RNNOH species) provide in general the following order of ease of alkylation with respect to the alkylating group: Me > CH_2-CN > Et > Pr > Bu > Pe. The detoxification factor *f* (Table 4) has values in the order dimethyl- = dicyanomethyl- > diethyl- > dipropyl- > dibutyl- > dipentyl-nitrosamine.

Consideration of both these factors leads to the prediction that the net alkylation yield obtained on administering the same dose of parent dialkylnitrosamine under identical conditions would be in the same order as above. The limited experimental data[11] on alkylation yields by dimethyl- and diethylnitrosamine partly supports this conclusion since the order of yield is dimethyl- > diethyl-. Since the *f* competition factor does not apply to the alkylnitrosoureas, it is suggested that these compounds would exhibit a smaller range of values for net alkylation yields compared with the corresponding dialkylnitrosamine, although the order of yields may be expected to follow that given by the alkylation indices, as for the substituent order above. The position for dihydroxyethyl- and diacetoxyethyl-nitrosamine is not easy to determine. The former, having a β-hydroxy group, might be prone to elimination as a soluble glucuronide, leading to a loss of the potential alkylating species. The latter could be hydrolysed to the former and is thus also possibly susceptible to such elimination. These speculations lead one to suggest that the net alkylation yields for dihydroxyethyl- and diacetoxyethylnitrosamines (especially the former) might be expected to be appreciably lower than that which the above indices for competition (between activating and deactivating hydroxylation) and for DNA alkylation might indicate.

Relative Abundance of O-*Alkylated Products.*—The stability indices ΔH_a, ΔH_b, and ΔH_c of Table 3 [see equations (5) to (7)] give a measure of the level of preference for *O*-alkylation. The vinyl and phenyl groups may be classed separately from the rest because of differences in hybridisation at the α-carbon, and hence possibly in the degree of electrophilicity for the reactive electrophile. But the lower values of their stability indices (compared with the Me, hydroxyethyl, cyanomethyl, 2-acetoxyethyl, fluoromethyl, and difluoromethyl groups) would suggest a relatively high abundance of *O*-alkylated product.

Assuming a fairly constant range of electrophilicity for the other reactive electrophile, the stability indices give the order Pe > Bu > Pr > Et > CH_2CN > CH_2CH_2OH > Me for the level of preference for *O*-alkylation. The CH_2F and CHF_2 groups rank near the Me, CH_2CH_2OH, and CH_2CN groups in this order. The order for the relative abundance of the O^6-alkylguanines and O^4-alkylthymines may be expected to follow the above trends. This trend is borne out in part by experiment, since ethylation and butylation of *N*-nitroso compounds produce a substantially greater relative abundance of *O*-alkylated product than the methylating homologue.[11–13]

It may be better to define the mechanism of DNA alkylation, not so much in terms of individual participation of the discrete species (alkyldiazohydroxide, alkyldiazonium, and alkyl), but rather in terms of how much character of each species is present in the reaction. The stability indices of Table 3 could give clues to the extent of alkyldiazonium and alkyl cation character present in the alkylating agent involved, which is of consequence for *O*-specific alkylation (as the hard–soft acid–base argument would imply).

Product Distribution Related to Carcinogenic and Mutagenic Potency.—The procarcinogenic and promutagenic role proposed for the O^6-G/O^4-T alkylated bases would imply that the carcinogenic and mutagenic potency of an *N*-nitroso compound may be directly related to the actual concentration c_o of these products available at the time of DNA replication. Table 4 presents values for various factors which could determine c_o, comparing them for eight dialkyl nitrosamines. These factors are (*i*) the cpmpetition factor *f* influencing net alkylation yield, (*ii*) the stability indices ΔH_a and ΔH_b, (*iii*) the heats of alkylation $\Delta H_{dz}(O^6\text{-G})$ and $\Delta H_{dz}(O^4\text{-T})$ at the O^6-G and O^4-T sites respectively by the alkyldiazonium cation, (*iv*) the corresponding heats $\Delta H_{ac}(O^6\text{-G})$ and $\Delta H_{ac}(O^4\text{-T})$ for alkylation by the alkyl cation.

There is no simple correspondence in value between the above indices. The precise manner in which the various factors operate together to produce the actual value of c_o is not possible to gauge in this present study. The factors contributing to the net alkylation yield run contrary to those influencing *O*-specificity of alkylation. Clear-cut predictions concerning carcinogenic and mutagenic potency for these dialkylnitrosamines may be difficult to arrive at from this data. It may, however, be expected that since the alkylnitrosoureas involve no *f* competition factor, they might display a narrower range of genotoxic potency than the corresponding dialkylnitrosamines.

Table 3. Theoretical indicators of tendency towards *O*-specific alkylation.

Alkyl group	ΔH_a/kJ mol^{-1}	ΔH_b/kJ mol^{-1}	ΔH_c/kJ mol^{-1}
Me	580.9	1 885.2	1 304.3
CH_2CH_2OH	579.3	1 660.3	1 081.0
CH_2CN	601.0	1 636.8	1 035.8
CH_2CH_2OAc	564.8	1 518.9	954.1
CH_2F	677.7	1 754.9	1 077.3
CHF_2	737.1	1 692.7	955.6
Et	543.1	1 343.1	800.0
Pr	529.9	994.2	464.1
Bu	525.0	879.1	354.1
Pe	522.3	783.3	261.0
$CHCH_2$	550.4	1 545.0	994.6
Ph	470.3	1 180.6	710.3

Conclusions

Nucleophilic, steric, and thermodynamic considerations seem to co-operate to produce the experimentally observed trend of

Table 4. Theoretical factors influencing c_o (the available concentration of O^6-G/O^4-T alkylated bases) for various dialkylnitrosamines.

	Nitrosamine							
Factor	dimethyl	dinitromethyl	diethyl	dipropyl	dibutyl	dipentyl	dihydroxyethyl	diacetoxyethyl
f	1.000	1.000	0.400	0.286	0.222	0.182	0.500	0.286
ΔH_a/kJ mol^{-1}	580.9	601.0	543.1	529.9	525.5	522.3	579.3	564.8
ΔH_b/kJ mol^{-1}	1 885.2	1 636.8	1 343.1	994.2	879.1	783.3	1 660.3	1 518.9
$\Delta H_{dz}(O^6\text{-G})$/kJ mol^{-1}	−339.8	−334.3	−311.9	−302.9	−298.5	−296.3	−312.0	−304.4
$\Delta H_{dz}(O^4\text{-T})$/kJ mol^{-1}	−191.3	−180.1	−159.5	−147.4	−140.8	−136.1	−160.0	−147.0
$\Delta H_{ac}(O^6\text{-G})$/kJ mol^{-1}	−1 644.1	−1 370.0	−1 111.9	−767.1	−652.3	−557.3	−1 393.0	−1 258.5
$\Delta H_{ac}(O^4\text{-T})$/kJ mol^{-1}	−1 495.6	−1 215.9	−959.5	−611.5	−494.8	−397.1	−1 241.0	−1 101.1

alkylation abundance with respect to DNA base site. Joint consideration of the competition factor, f and of the alkylation indices provides a predicted order Me > $-CH_2CN$ > Et > Pr > Bu > Pe for net alkylation yield the corresponding dialkylnitrosamine, which experiment bears out in part. The order of magnitude Pe > Bu > Pr > Et > $-CH_2CN$ > Me predicted for the relative abundance of *O*-alkylated product is also borne out in part by experiment. In general, *N*-nitrosamides may be expected to exhibit a smaller range of values for carcinogenic and mutagenic potency than the corresponding dialkylnitrosamines.

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