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The Bioorganic Chemistry of the Nitroalkyl Group

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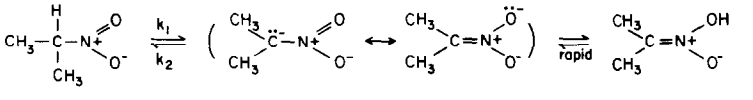
Nitroalkyl groups are conspicuously rare among pharmaceutical agents, and the bioactivity of substituted nitroalkanes has been described in few instances. This article examines the natural occurrence of such compounds and the reactions of nitroalkyl compounds with enzymes. The discussion is not limited to nitro hydrocarbons; the term "nitroalkyl" is intended to distinguish the compounds from nitroaromatic compounds, which are better known in pharmacology and involve different chemical considerations. Further study of the bioorganic chemistry of the nitroalkyl group may contribute to the understanding of biosynthetic strategies and enzymatic catalysis and may permit the rational design of useful bioactive molecules.

CHEMICAL FEATURES OF THE NITROALKYL GROUP

The chemistry of the nitro group is rich, and many detailed discussions are available (*1, 2*). Some enzymologically relevant features are outlined here.

Nitroalkyl compounds are carbon acids that ionize to nitronate anions in which the negative charge is delocalized. Nitronate ions can protonate on carbon to afford a nitro compound or protonate on oxygen to afford a nitronic acid as shown in [1].



[1]

2-nitropropone — propane-2-nitronote ion — propane-2-nitronic acid

Nitroalkyl compounds have been termed "pseudo acids" because their rates of ionization and reprotonation on carbon are not instantaneous. Representative rate and equilibrium constants (*3*) are given in Table 1.

Because the interconversion of nitro and nitronate species is slow, it can be possible to discern which species is acted upon by an enzyme. For instance, 3-nitroalanine **2** is an analog of asparagine and can ionize to a nitronate analog **1** of aspartate. Oxygen consumption occurs when the compound is presented to an

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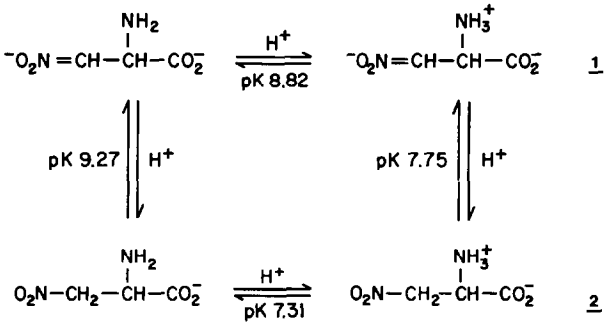
TABLE 1

IONIZATION OF NITROALKYL COMPOUNDS IN WATER AT 25°C (*3*)

	pK_a	k_1 (min^{-1})	k_2 ($M^{-1}\ min^{-1}$)
CH_3NO_2	10.2	2.6×10^{-6}	4.1×10^4
$CH_3CH_2NO_2$	8.6	2.2×10^{-6}	9.0×10^2
$CH_3CH(NO_2)CH_3$	7.7		
$CH_3CHClNO_2$	6.8	3.4×10^{-5}	
$CH_3COCH_2NO_2$	5.1	2.2	2.3×10^5

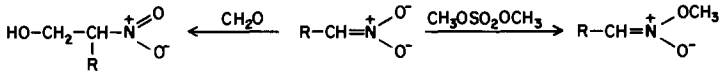
amino acid oxidase (that from rattlesnake venom) which accepts both asparagine and aspartate as substrates but preferentially oxidizes asparagine. When slightly acidic stock solutions of the nitro compound are diluted into enzyme assay mixtures, oxygen consumption occurs with a lag corresponding to the rate of ionization of the nitro compound. When alkaline stock solutions of the nitronate are diluted into enzyme assay mixtures, oxygen consumption starts at a rate which decays as the nitronate protonates. The enzyme thus oxidizes the nitronate analog of the poor substrate (aspartate) but not the nitro analog of the good substrate (asparagine). This observation can be reconciled with the inductive effects of the electron-withdrawing nitro group and the electron-donating nitronate group.

Nitronate ions exhibit strong ultraviolet absorbance at about 230 nm ($\varepsilon \simeq 10{,}000\ M^{-1}\ cm^{-1}$) while the (unconjugated) neutral nitro group absorbs weakly. This feature as well as the measurably slow rates of ionization and protonation permit determination of microscopic dissociation constants in nitro compounds bearing additional ionizable groups. The acid–base equilibria of 3-nitro-DL-alanine, for instance, can be dissected as described in [2] (*4*).



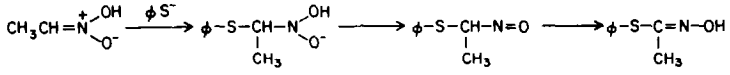
[2]

Nitronate ions are ambident nucleophiles as well as ambident bases so that either the carbon or oxygen atom of nitronate ions may covalently attach to electrophilic molecules. For instance, nitronates react as carbanions in the Henry version of the aldol condensation (*5*) while they react as oxyanions with dimethyl sulfate (*6*), affording nitronate esters in that case ([3]).



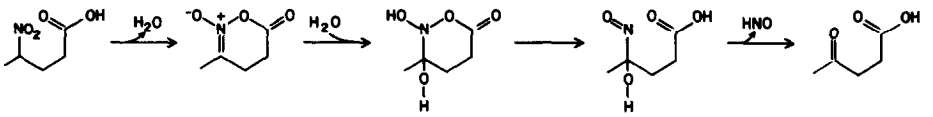
[3]

Whereas nitronate ions may react as nucleophiles (or reductants) toward enzymes, the protonated species may react as electrophiles (or oxidants). For instance, nitronic acids react with thiols to afford thiohydroximates (*7*, *8*) as shown in [4].



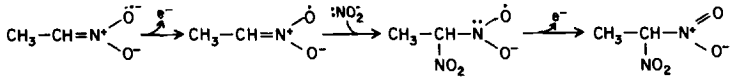
[4]

The low pK (~4.5) of nitronic acids and the very unfavorable nitro : nitronic equilibrium prevent effective covalent modification of proteins by nitronic acids. However, nitronate esters react similarly to nitronic acids with thiols and are potent enzyme-inactivating agents (*9*). Furthermore, nitronic acid formation can be subject to intramolecular catalysis. For instance, 4-nitrovaleric acid is attacked much more rapidly than its homologs by water in a Nef reaction (*10*). In that case, though, there may be transient formation of an electrophilic mixed acid anhydride ([5]).



[5]

The free radicals obtained upon one-electron oxidation of nitronates are also electrophilic so that nitronates can capture nucleophiles during oxidation by one-electron acceptors such as ferricyanide ([6]) (*11*).



[6]

Vinylogous addition to the electron-withdrawing nitro group may also occur, and nitro olefins are potent Michael-type alkylating agents.

Another relevant feature of the nitro group is its ability to function as a leaving group. Many nitroalkyl compounds are metabolized to inorganic nitrite, and the vasodilatory and hemoglobin-oxidizing properties of nitrite could obscure other pharmacological properties of a weakly bioactive nitroalkyl compound.

NATURALLY OCCURRING NITROALKYL COMPOUNDS

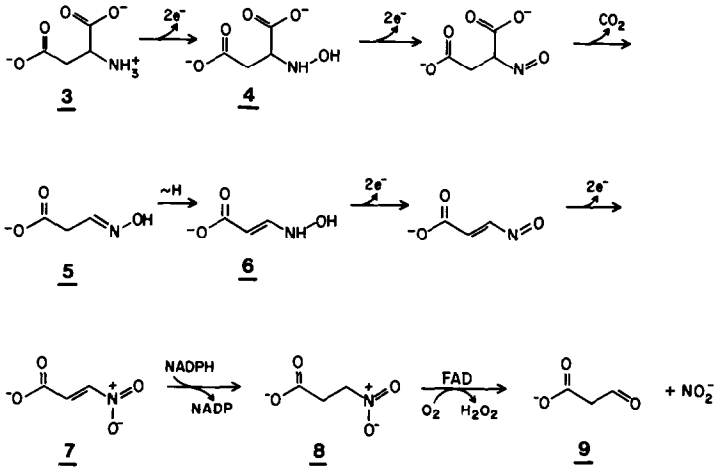
Few examples of nitroalkyl compounds have been found in nature, but they are produced by an impressive number of diverse species. Plants, fungi, and invertebrates produce the compounds discussed individually here.

3-Nitropropionic Acid

This was the first nitroalkyl compound to be found in nature. The compound **8** occurs as an ester in the glycosides hiptagin and karakin isolated from the bark of the Javanese tree *Hiptage mandoblata* and the berries of the karaka tree *Coryno-*

carpus laevigata, respectively. Although hiptagin was described in 1920, the nitropropionyl residue was not recognized as such until 1949 because of decomposition during harsh hydrolytic procedures (*12*). 3-Nitropropionic acid (hiptagenic acid) was then found to be identical to bovinocidin, the component of the legume *Indigofera endecaphylla* which proved responsible for toxicity to livestock (*13*). The nitro acid or its esters are widely distributed in nature. Toxic plant species of the genera *Astragalus, Coronilla, Heteropteris, Lotus,* and *Viola* (including the common garden violet) also produce the compound (*14, 15*), and it is synthesized by the fungi *Penicillium atrovenetum, Acremoniella atra,* and *Pestalotia palmarum* and by several species of *Aspergillus* (*16, 17*).

The biosynthesis of nitropropionate has been studied in best detail with *Penicillium atrovenetum* ([7]).



[7]

The most efficient incorporation of isotopically labeled carbon and nitrogen into nitropropionate was found with aspartate **3** as the labeled precursor (*18–20*). Ammonium ions are a more efficient nitrogen source than nitrite or nitrate ions and are presumably incorporated first into aspartate by the sequential actions of glutamate dehydrogenase and glutamate-oxalacetate transaminase. Radioactive bicarbonate can provide C-1 of 3-nitropropionate because of the action of phosphoenolpyruvate carboxylase, and C-4 of [4-^{14}C]aspartate can similarly provide C-1 of 3-nitropropionate. It is thus likely that nitropropionate arises by oxidation of the amino group of aspartate to a nitro group with concommitant loss of C-1 of aspartate as CO_2.

This six-electron oxidation of an aliphatic amino group to a nitro group poses an interesting problem in bioorganic chemistry. Curiously, the only enzyme so far identified in *Penicillium atrovenetum* which may be involved in the pathway by which aspartate is oxidized to nitropropionate uses NADPH to reduce a putative intermediate (*21*). Cell-free extracts possess a dehydrogenase which is highly specific for NADPH and 3-nitroacrylate **7**. The equilibrium lies far in favor of nitropropionate production so that the reaction is essentially irreversible. Further-

more, a separate enzyme exists for catabolism of nitropropionate to malonic semialdehyde **9** and inorganic nitrite (*22*). Perhaps an oxime intermediate **5** tautomerizes to an olefinic hydroxylamine **6** during the biosynthesis of nitropropionate. The hydroxylamine tautomer **5** might be a better reductant than the oxime **6**.

The *N*-hydroxylation reaction probably involves O_2 or H_2O_2 as a cosubstrate since another species of *Penicillium* has been shown to use glycine and $^{18}O_2$ to produce labeled *N*-hydroxyglycine (*23*). It is interesting to suppose that the chemical use of peracids to convert amines and oximes to nitro compounds is a biomimetic process (*24*).

The proposed intermediates are unstable or highly reactive and are probably sequestered from other cellular constituents. For instance, *N*-hydroxyaspartate **4** is a nucleophile which forms a stable nitrone with the coenzyme pyridoxal phosphate (*25*), and 3-nitroacrylate **7** is an electrophile which readily alkylates enzymes (*26, 27*). It is conceivable that reactive intermediates could be sequestered as esters or thioesters, but such a mechanism is not clearly available in the cases of other naturally occurring nitroalkyl compounds. At least one fungal peroxidase oxidizes amines to nitroso compounds without detectably releasing hydroxylamine intermediates, but that example (*Caldariomyces* chloroperoxidase) involves arylamines rather than alkylamines (*28*).

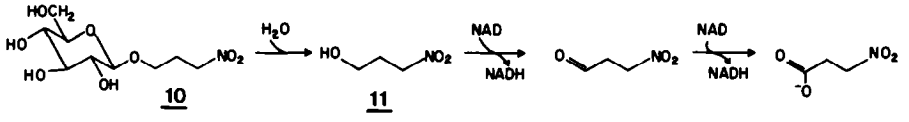
More than one pathway may exist for the biosynthesis of nitropropionate. The legume *Indigofera spicata* has been reported to make labeled nitropropionate from [2-^{14}C]malonic acid but not from labeled aspartate (*29*). Very low incorporation rates were measured in that case, however.

Nitropropionate has generally been found in nature because of investigation of its toxicity. Although rather high doses on the order of 100 mg/kg are generally required for fatality, the nitro compound can comprise several percent of the dry weight of some plants. Signs of nitropropionate poisoning are nonspecific (*14, 30, 31*). Poisoned animals exhibit general weakness and incoordination progressing to collapse and respiratory distress. Diffuse nerve degeneration is prominant on histological examination of chronically poisoned animals. Although the nitro acid is metabolized to nitrite and shares with nitrite the ability to cause methemoglobinemia, this feature does not fully account for the toxicity. Furthermore, the compound is an inhibitor of respiration in isolated cells (*32*).

Since nitropropionate is an isoelectronic analog of succinate, it was tested as an inhibitor of mitochondrial succinate dehydrogenase *in vitro*. Only weak competitive inhibition was appreciated with the nitro compound was presented to the enzyme in the presence of redox dyes which nonenzymatically scavenge the nitronate species (*33*). However, at equilibrium at pH 7, only about 1% of the compound (pK 9.1) exists as a nitronate ion. The nitronate form of the compound proved to be a potent irreversible inactivator of the enzyme. It is probably oxidized by the enzyme to nitroacrylate with consequent alkylation of an active-site nucleophilic group (*34–37*). The nitronate also inhibits the other Krebs-cycle enzymes succinic thiokinase (*27*) and fumarase (*4*). Inhibition of the Krebs cycle by the nitronate is probably the principal mechanism for the toxicity of nitropropionate (*31*).

3-Nitropropanol

Some poison vetches and locoweeds of the *Astragalus* genus produce 3-nitropropanol **11** rather than (or in addition to) 3-nitropropionate (*14, 30*). Like the nitro acid, the nitro alcohol occurs in plants as a glycoside. For instance, miserotoxin **10**, first identified in *A. miser*, is the β-D-glucopyranoside of the toxic nitro alcohol (*38*). Poisoning by plants containing nitropropanol glycosides resembles that by plants producing nitropropionate, and it is likely that the toxicity of miserotoxin is principally due to its metabolism in three steps to nitropropionate ([8]) and consequent blockade of the Krebs cycle (*14, 30, 39*).



[8]

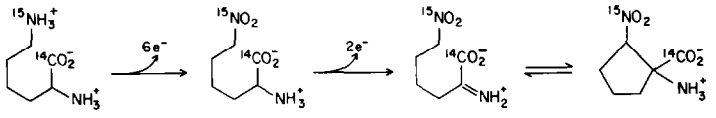
The toxicity of the nitro alcohol can thus be experimentally ameliorated by coadministration of either ethanol or inhibitors of alcohol dehydrogenase (*40*).

The presence of both nitropropanol and nitropropionate together in certain species of *Astragalus* raises the question as to whether the compounds share a common biosynthetic pathway or else arise by separate pathways. Few experiments have been reported in this regard although *Astragalus cibarius* has been shown to incorporate ^{15}N from [^{15}N]nitropropanol into nitropropionate (*14, 41*). Perhaps this leguminous plant synthesizes nitropropionate from homoserine while the fungus *Penicillium atrovenetum* employs aspartate. However, separate pathways in *Astragalus* are certainly plausible, especially since *Aspergillus wentii* synthesizes 3-nitropropionate together with 1-amino-2-nitrocyclopentanecarboxylate **12**. The latter two compounds cannot be easily reconciled with one biosynthetic pathway (*42*).

1-Amino-2-nitrocyclopentanecarboxylic Acid

This nitro amino acid, **12**, was discovered in an empirical program screening for fungal inhibitors of plant growth. Produced by *Aspergillus wentii*, it causes abnormal growth with production of short internodes, narrow leaves, diminished chlorophyll content, and loss of apical dominance in the development of branches. Its inhibition of mitosis in the roots of pea seedlings can be specifically reversed by comparable concentrations of leucine, but its inhibition of yeast cell growth is not reversed by leucine (*43*).

The compound is found in the racemic state because of either nonstereospecific biosynthesis or spontaneous racemization. Inspection of its structure and the proposed pathway for 3-nitropropionate production in *Penicillium atrovenetum* (Scheme 7) suggested that the nitro amino acid would be produced by oxidation of an amino group of diaminopimelic acid. However, labeled lysine (decarboxylated diaminopimelate) was found to be specifically incorporated into the nitro compound as indicated in [9] (*42, 44, 45*).

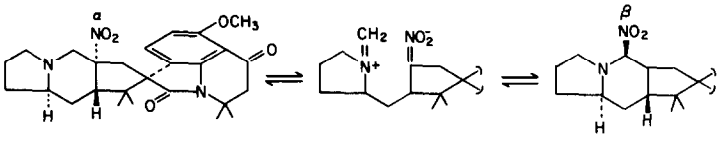


[9]

12

Cyclopiamine

Another cyclic nitro amine has been isolated from cultures of toxic strains of *Penicillium cyclopium* and *P. urticae* which can contaminate agricultural produce (*46*). Like aminonitrocyclopentanecarboxylate **12**, cyclopiamine **13** is found as a mixture of two isomers which are nonenzymatically interconvertible through reversible ring opening ([10]).



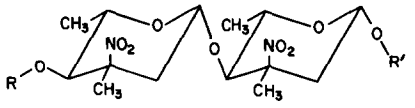
[10]

13

The equilibrium ratio of the α isomer to the β isomer is 1:7, but the α isomer predominates in fungal extracts so that the β isomer may be an artifact of the extraction process. The biosynthetic pathway is not established, but Bond *et al.* point out that inspection of the structure implicates tryptophan, proline, and two isoprene units as precursors (*46*).

Decilonitrose

Some anthracycline glycosides related to adriamycin have been found to bear nitro groups on the sugar moieties. For instance, decilorubicin (*47*) from *Streptomyces virginiae* carries two decilonitrose moieties in glycoside linkage ([11]).

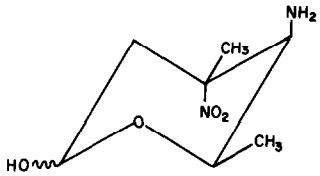


[11]

Arugomycin from *S. violochromogenes* is a structurally similar anthracycline which bears only one decilonitrose moiety (*48*). It is not established what influence the nitro groups have on the bioactivity of the anthracyclines. The nitro groups may arise by oxidation of aminoglycoside compounds.

Tetronitrose

N-Acetyl tetronitrose ([12]) is found in glycoside linkage in the tetrocarcins, which are antibacterial and antineoplastic compounds elaborated by the fungus *Micromonospora chalcea* (*49*).

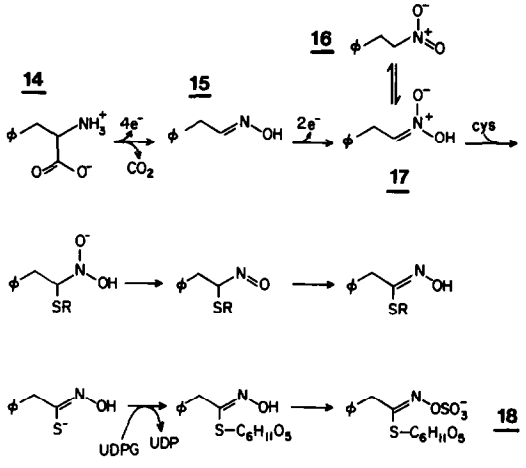


[12]

The role of the nitro group in the bioactivity of these compounds is not clear.

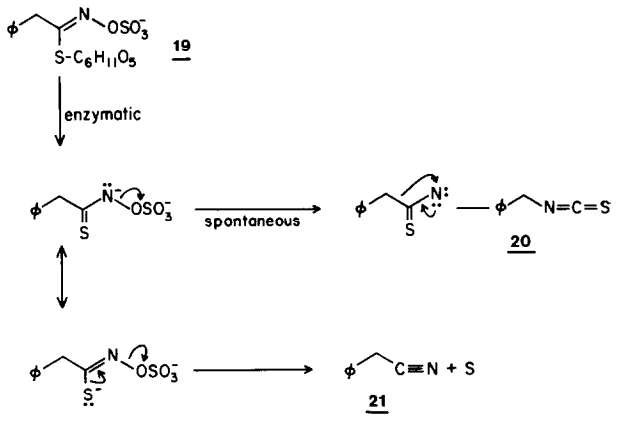
1-Nitro-2-phenylethane

This compound, **16**, is a fragrant cinnamon-like constituent of many plants (*50*). It is a major component of oil of dennettia, obtained from the fruits of the *Dennettia tripetala* tree (*51*). It is also found in species from the genera *Aniba, Citrus, Octotea,* and *Tropaeolum* (*7, 50–52*). Its biosynthesis ([13]) has been studied in the common garden nasturtium *Tropaeolum majus* (*7*), which can produce the nitro compound from labeled phenylalanine **14** from labeled phenylaldoxime **15**. The compound may serve to repel insect predators (*53*). It is interesting in this regard that structurally similar aralkyl nitro compounds such as 1,1-bis(4-ethoxypheny)-2-nitropropane have been artifically produced for insecticidal purposes (*54, 55*). In *T. majus,* however, 1-nitro-2-phenylethane (as its nitronic acid tautomer **17**) apparently serves as an intermediate in the biosynthesis of benzylglucosinolate **18** (*7, 8*).



[13]

Glucosinolates may serve a protective role against plant predators. They are converted to highly electrophilic and irritant isothiocyanates **20** by enzymes in plant tissues (*56*). Isothiocyanate production is prominant in the Cruciferae family of plants and affects the food flavors of members such as garlic, onion, horseradish, and mustard. After enzymatic hydrolysis of the thioglycoside bond, isothiocyanate production occurs in a nonenzymatic reaction that can be viewed as a Lossen rearrangement ([14]).



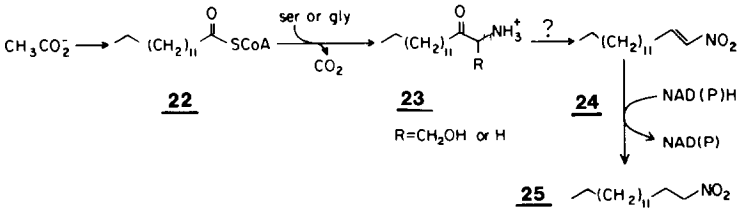
[14]

Interestingly, when the thioglycosidase reaction is studied at low pH (3.5–5), much of the glucosinolate is converted to a nitrile **21** rather than isothiocyanate (*56, 57*), the sulfur appearing in elemental form ([14]). This observation raises the question as to whether nitroalkyl (or nitronic acid) species are intermediates in the biosynthesis of nitriles as well as of glucosinolates. It is clear that many naturally occurring nitriles arise through oxidative decarboxylation of amino acids so that biosynthesis of the two functional groups is likely to exhibit much mechanistic analogy (*58*).

1-Nitro-1-pentadecene

Although the roles served by nitroalkyl compounds in the metabolism and ecology of plants and fungi are not certain, nitro compounds have a straightfoward role as products of specialized defense glands of invertebrate animals. Soldiers of the termite *Prorhinotermes simplex* spray the alkylating agent *trans*-1-nitro-1-pentadecene **24** at enemies (*59–61*). The nitroolefin is a lachrymator which readily engages in Michael reactions with nucleophilic cellular constituents. *P. simplex* workers, but not workers of closely related insect species, are protected by high levels of a dehydrogenase which uses NADH or NADPH to reduce the olefin to the nitroparaffin **25**.

The termites have been shown to incorporate labeled acetate into nitropentadecene, but the biosynthetic pathway is not established (*62*). Spanton and Prestwich have hypothesized that the nitro group arises by direct oxidation of the amino group of serine or glycine after the amino acid is first acylated by a fatty acyl coenzyme A ester **22**. This pathway ([15]) would resemble that of sphingosine biosynthesis.

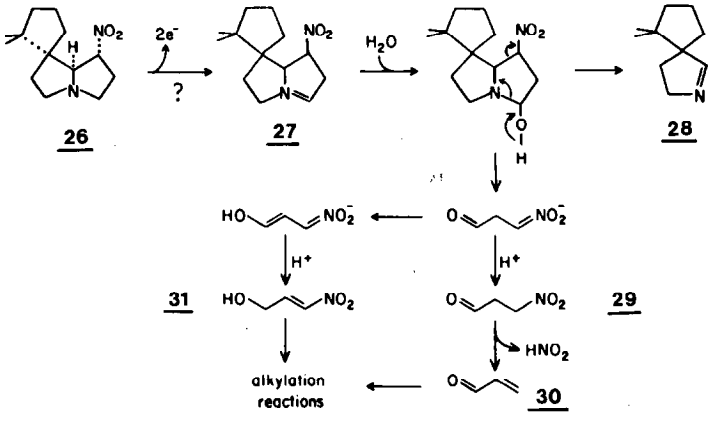


[15]

It is interesting to suppose that the 2-oxo amine **23** is first reduced to a 2-hydroxy amine and that the 2-hydroxy amine is then oxidized to a 2-hydroxy nitroalkane. The 2-hydroxy nitroalkane could be dehydrated to the nitro olefin **24** with the aid of ATP. A model for this ATP-driven dehydration reaction is provided by rabbit muscle pyruvate kinase *in vitro*. The enzyme can use ATP to phosphorylate 3-nitrolactate, which then spontaneously affords 3-nitro-acrylate (*63*). This reaction is discussed later (see [35]).

Nitropolyzonamine

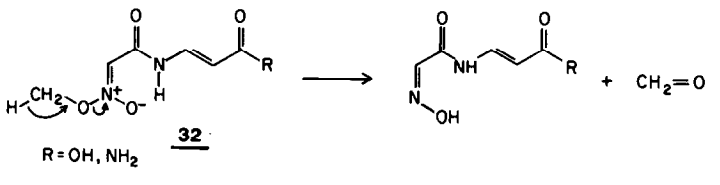
The millipede *Polyzonium rosalbum* is another invertebrate animal which secretes a nitro compound from a defense gland (*64*). The gland produces both nitropolyzonamine **26** and the fragment polyzonimine **28**. It is not established whether nitropolyzonamine arises from polyzonimine or vice versa. It is also not clear which compound serves to repel predators since neither is a good electrophile. It is tempting to speculate that the imine **28** arises through enzymatic oxidation of the nitro amine **26**. The oxidized nitropolyzonamine would decompose into polyzonimine and 3-nintropropionaldehyde **29** as shown in [16]. 3-Nitropropionaldehyde may be the actual bioactive agent from the millipede defense gland since the nitro aldehyde can spontaneously decompose into electrophilic olefins **30** and **31** (*39*).



[16]

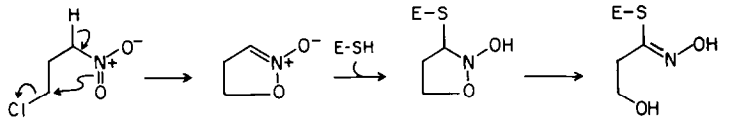
Enteromycin

Esters of nitronic acids also occur in nature. Enteromycin **32** is produced by *Streptomyces albireticuli* (*65*) while enteromycin carboxamide is produced by another streptomycete (*66*). The nitronate esters are unstable, and this feature may interfere with their detection in other organisms. One decomposition route of methyl nitronates affords formaldehyde and an oxime as indicated in [17] (*67*).



[17]

Nitronate esters are sufficiently electrophilic to capture thiol groups on proteins [18]. For instance, 1-chloro-3-nitropropane **33** can cyclize to a nitronate ester **34** shown to inactivate enzymes *in vitro* (*9*).



33 **34** [18]

A similar enzyme-inactivating reaction would be available to 3-nitropropanol (miserotoxin aglycone, **11**) upon its enzymatic esterification.

Nonenzymatic Production of Nitro Compounds

The number of nitroalkyl compounds discovered in nature has remained small since the identification of nitropropionate in 1949, but more examples are likely to be found. It is interesting that nitro olefins are among the lachrymatory factors in atmospheric smog (*68*). These arise through solar action on nitrogen oxides and organic material, much of which enters the atmosphere because of artificial activity. However, some of the nitric oxide and nitrogen dioxide in the atmosphere stems naturally from the effect of lightning on N_2 so that nitro compounds were probably among the organic nitrogen compounds available in the primordial soup. Nitro compounds may similarly arise nonenzymatically from nitrogen oxides produced during certain microbial fermentations (*69*, *70*). It is interesting in this regard that nitrohexane and 3-nonenylnitrolic acid, $CH_3(CH_2)_4CH{=}CHCH_2{-}C(NO_2){=}NOH$, have been identified in nitrous acid-treated corn (*71*, *72*).

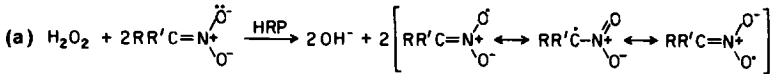
METABOLISM

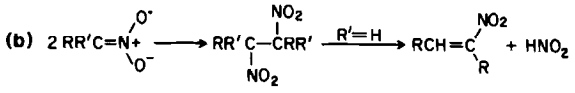
Nitroparaffins are generally metabolized by mammals to inorganic nitrite (*73*, *74*). The nitrite can cause methemoglobinemia and may be responsible for the low-grade mutagenicity detected in some nitroalkyl compounds (*75*). A minor mechanism for nitrite production involves nucleophilic displacement of the nitro group by the thiol group of the cysteinyl residue of glutathione in a reaction catalyzed by hepatic glutathione *S*-transferase (*76*, *77*). The alkyl chains are then renally excreted as mercapturic acids (*N*-acetyl-*S*-alkylcysteines). However, inorganic nitrite production from nitroalkyl compounds generally involves their concommitant oxidation to the corresponding oxoalkyl compounds.

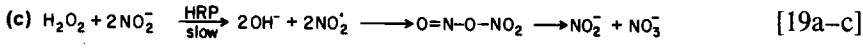
Oxidation by Hemoproteins

Several enzymes have been shown to catalyze the oxidation of nitroalkanes to nitrite and carbonyl compounds *in vitro*. Horseradish peroxidase provided one of the first examples (*78–80*). Of course, that enzyme has been the subject of much mechanistic investigation. In the classic mechanism, the resting hemoprotein is converted by H_2O_2 to a spectrally distinct oxidized species known as Compound

I. Compound I abstracts one electron from a molecule of reducing substate $A:^-$ to yield A· and Compound II. Compound II abstracts an electron from a second molecule of $A:^-$ to yield resting enzyme and a second molecule of A·. The nitronate anions are the active species when nitroalkanes are presented to the peroxidase as reducing substrates. The enzyme uses one molecule of H_2O_2 to convert two nitronate ions to two neutrally charged free radicals ([19a]). The free radicals obtained by enzymatic one-electron oxidation of nitronate ions are unstable and engage in subsequent nonenzymatic reactions. In the absence of oxygen, the radicals dimerize (*80*).

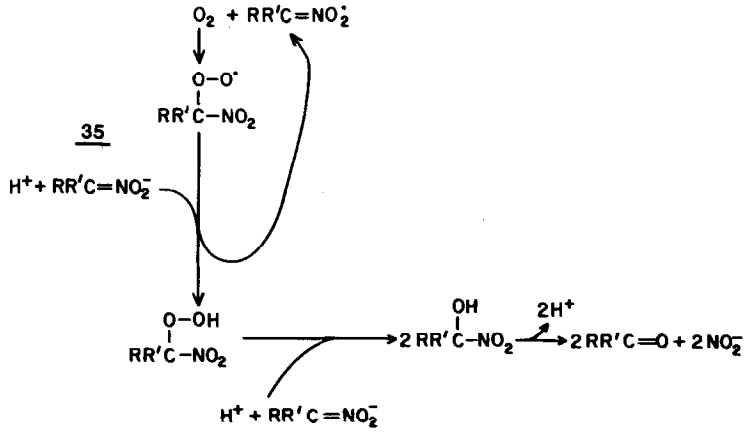






[19a–c]

The free-radical products of secondary nitroalkanes such as 2-nitropropane dimerize to inert products, but those of primary nitroalkanes such as 1-nitropropane dimerize to unstable species which eliminate nitrite to afford electrophilic nitro olefins ([19b]). Nitrite is also a substrate, albeit a poor one, for the peroxidase and is probably converted to nitrate through the intermediacy of the nitrosating species N_2O_4 ([19c]). In the presence of oxygen, the free radicals undergo autoxidation (*80, 81*). However, the free radicals are not only themselves oxidized but also initiate the autoxidation of the nitronate ions in a free-radical chain process. The chain reaction may proceed largely as shown in [20], but additional reactions contribute since chain length can be reduced by superoxide dismutase (*80, 81*).

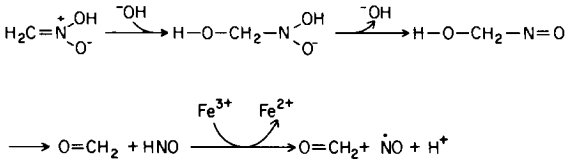


[20]

Particularly in the case of secondary nitroalkanes such as 2-nitropropane, very long chain reactions can occur before termination. Thus, a catalytic quantity of

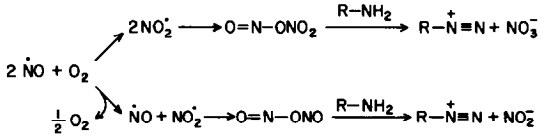
H_2O_2 can cause solutions of propane-2-nitronate to consume oxygen in the presence of the peroxidase. Much less efficiently, the ferrihemoprotein can also initiate the autoxidation reaction without added H_2O_2. If the largely nonenzymatic nature of this reaction is not appreciated, the horseradish enzyme appears to catalyze a dioxygenase reaction rather than a classic peroxidase reaction. Many oxidants can nonenzymatically abstract one electron from alkane-nitronate ions and so initiate autoxidation chain reactions (*80–82*). Strong oxidants such as $KMnO_4$, $C(NO_2)_4$, and $K_3Fe(CN)_6$ are particularly efficient, but the phenomenon can be observed with weaker oxidants such as ferric heme, $NaNO_2$ (at slightly acidic pH), and superoxide. Superoxide-generating enzymes can thus appear to catalyze nitroalkane dioxygenase reactions in the presence of catalytic quantities of reducing substrates.

Hepatic microsomal cytochrome *P*-450 is probably responsible for much of the metabolism of nitroparaffins in mammals. The monooxygenase has been shown to oxidize nitroalkyl compounds *in vitro* with NADPH and O_2 as cosubstrates (*83, 84*). α-Hydroxy nitroalkanes are presumably the initial products and would spontaneously afford nitrite and an oxoalkane. Nitromethane behaves anomalously with the enzyme in rat liver microsomes (*85*). In that case the ferric enzyme oxidizes nitromethane to formaldehyde and nitric oxide in the absence of NADPH. The enzyme is reduced to the ferrous state in this reaction and becomes inhibited as the spectrally distinct nitric oxide–ferroheme complex. Perhaps a Nef reaction occurs, and the iron is reduced by HNO [21]).



[21]

Nitric oxide would be a highly reactive metabolite of nitroalkanes if it were rapidly released free into solution from the enzyme (*69, 70*). For instance, in the presence of oxygen it can diazotize amines at neutral pH ([22]).



[22]

Nitroalkanes are potent inactivators of cytochrome *P*-450 in the presence of dithionite (*86, 87*). They are nonenzymatically reduced by $S_2O_4^{-2}$ to nitrosoalkanes which bind almost irreversibly to ferrous heme. This phenomenon may be relevant to considerations of the mechanism of the biosynthesis of nitroalkyl compounds.

There has been speculation that nitroalkyl compounds may occur as intermediates during mammalian microsomal drug metabolism (*88–91*). α-Substituted amines such as amphetamine (2-amino-1-phenylpropane) are not readily oxidized by flavin-dependent monoamine oxidase. 2-Nitro-1-phenylpropane may be an in-

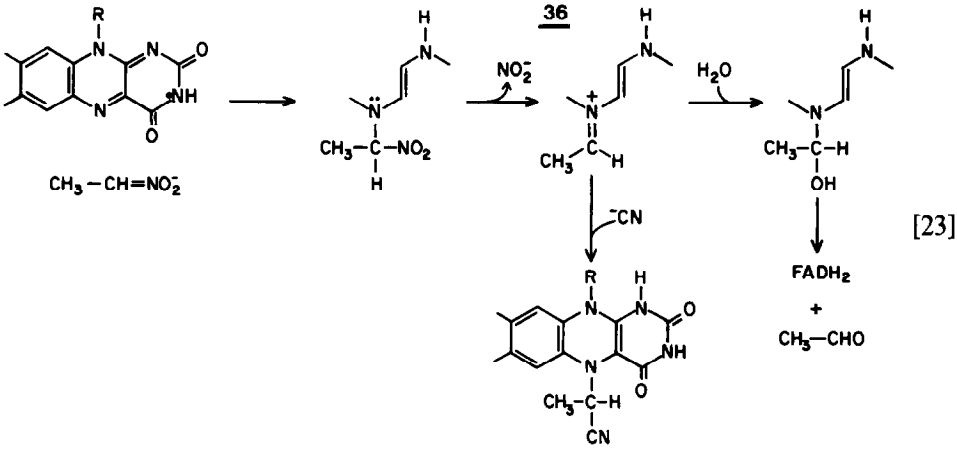
termediate in the hepatic microsomal oxidation of the drug to phenylacetone and nitrite.

The methane-oxidizing enzyme from *Methylobacterium organophilum* is another monooxygenase which adventitiously accepts nitroalkanes as substrates (*92*), but heme iron may not be required in that case.

Oxidation by Flavoproteins

Nitropropionate-synthesizing fungi contain enzymes which also oxidatively degrade the compound. 3-Nitropropionate oxidase has been purified from *Penicillium atrovenetum* (*22*) and found to contain a flavin cofactor (D. Porter, in preparation). Anaerobically, the oxidized resting enzyme is converted to its flavin semiquinone form by the nitronate of nitropropionate. The enzyme thus appears to oxidize the nitroalkyl compound by a one-electron-abstracting mechanism resembling that by which nitroalkanes are oxidized by horseradish peroxidase ([19], [20]). The enzyme is specific for 3-nitropropionate and 4-nitrobutyrate and does not accept nitroparaffins such as 2-nitropropane as substrates. However, because of its free-radical-mediated mechanism, the enzyme can use 3-nitropropionate to initiate the spontaneous autooxidation of 2-nitropropane. The nitropropionate radical (or its peroxide radical **35**), flavin semiquinone radical, or else flavin-generated superoxide radical abstracts one electron from propane-2-nitronate to initiate the chain reaction shown in [20].

Nitroparaffins are (presumably nonphysiological) substrates in the case of another flavoenzyme, D-amino acid oxidase from hog kidney (*93, 94*). All of our observations thus far with nitroethane as a substrate for that enzyme are consistent with an ionic two-electron-transfer mechanism although the mechanism may ultimately prove further dissectable into one-electron transfer steps. The enzyme reduces O_2 to H_2O_2 and oxidizes nitronates to carbonyl compounds and nitrite. With methanenitronate as the substrate the enzyme was noted to suffer inactivation and bleaching of its FAD cofactor. It was reasoned that a second molecule of methanenitronate may be trapping an iminium intermediate in the reaction pathway. Cyanide proved to trap the intermediate **36** with ethanenitronate as the

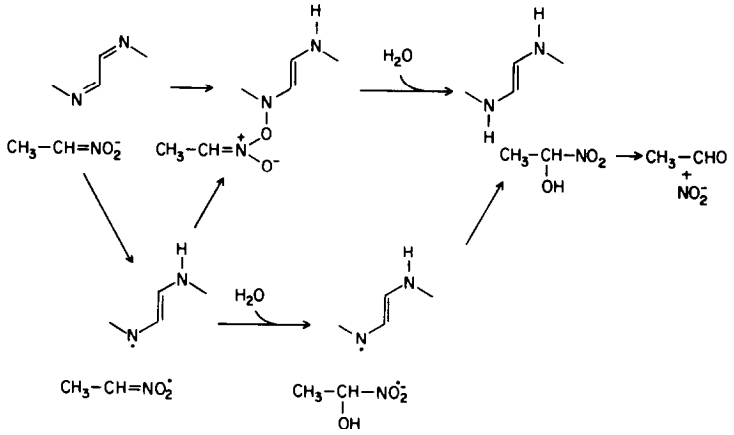


[23]

substrate. Cyanide does not inhibit the enzyme during the oxidation of D-amino acids but inactivates the enzyme and irreversibly bleaches its cofactor during the oxidation of nitronates. Stopped-flow kinetic analysis as well as the iminium-trapping experiment outlined in [23] established that the oxidation of ethanenitronate by D-amino acid oxidase proceeds through a kinetically competent coenzyme–substrate covalent intermediate (*93*).

The addition of nitroalkyl carbanions to N^5 of the flavin cofactor is actively catalyzed by the enzyme. We have not observed the reactions with free FAD nor with most of the other flavorenzymes which we have examined. Superoxide dismutase has been observed to slightly inhibit oxygen consumption when the hog kidney enzyme acts on propane-2-nitronate (*95*), but does not inhibit oxygen consumption when primary nitronates such as ethanenitronate are the substrates.

A free-radical-mediated mechanism is more apparent when nitronates are oxidized as adventitious substrates of another FAD-dependent enzyme, fungal glucose oxidase (*96*). That enzyme can be converted to its flavin semiquinone form by ethanenitronate, and its oxidation of ethanenitronate is attended by production of nitrate and 1,1-dinitroethane in addition to nitrite and acetaldehyde. Unlike hog kidney D-amino acid oxidase, *Aspergillus* glucose oxidase is not inactivated by cyanide or other iminium-trapping agents while processing nitroalkanes. If covalent coenzyme–substrate adducts are involved in the glucose oxidase reaction (and this question is unsettled), then the ambidently nucleophilic nitronates may attack the flavin as oxyanions ([24]) rather than carbanions ([23]).



[24]

As is also suggested in [24], a coenzyme–substrate adduct (if present) might also arise through radical coupling (*97*).

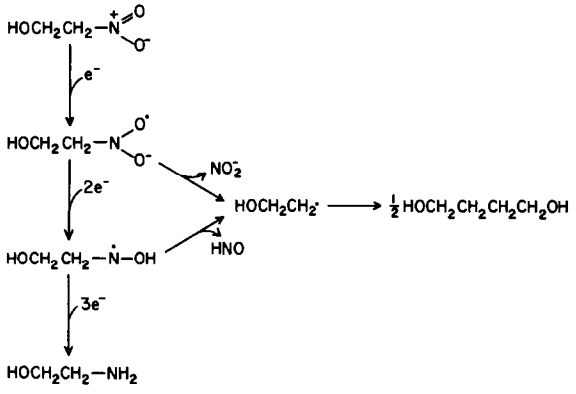
A large number of bacteria and fungi possess nitroalkane-oxidizing enzymes, and many can use nitroethane as their sole nitrogen source (*98–101*). In most cases examined nitroethane is metabolized to nitrite and acetaldehyde while 2-nitropropane is similarly converted to acetone. The enzyme purified from the fungus *Hansenula mrakii* has an FAD cofactor (*102*). This enzyme oxidizes 2-nitropropane with a dioxygenase stoichiometry and produces labeled acetone from $^{18}O_2$. The nitronate is the preferred form of the substrate, and the reaction is

partially inhibited by superoxide dismutase. A free-radical-mediated mechanism similar to that shown in [20] is thus implicated for that enzyme.

A mechanistically puzzling enzyme has been partially purified from *Streptomyces achromogenes* (*101*). The streptomycete enzyme also oxidizes 2-nitropropane with a dioxygenase stoichiometry. However, it is reported to use 2-nitropropane and propane-2-nitronate equally well as substrates, and its activity is not inhibited by superoxide dismutase. Dhawale and Hornemann favor a free-radical-mediated mechanism for the enzyme because the reaction exhibits a lag which is abolished by sodium peroxide and because the enzyme is inhibited by free-radical-scavenging agents. The presence of a non-protein cofactor is not established. Those authors point out that *S. achromogenes* synthesizes an *N*-nitroso antibiotic (streptozotocin) and suggest that nitroalkyl compounds may be intermediates through which fungi may generate nitrous acid or a similar nitrosating species.

Enzymatic Reduction

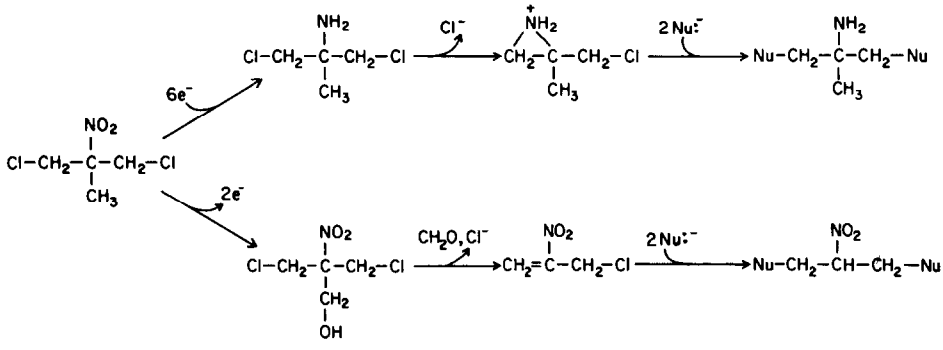
Many nitroaromatic antibiotic drugs are active against anaerobic microorganisms because the nitro group is enzymatically reduced to reactive species by the ferredoxins and flavodoxins required in some anaerobic fermentation pathways. The polarographic half-wave reduction potentials indicate that nitroalkyl compounds are generally less easily reduced than nitroaromatic compounds. However, clostridial enzymes have been shown to use H_2 to reduce nitroalkyl compounds *in vitro* (*103*). For instance, 2-nitroethanol is reduced by six electrons to 2-aminoethanol. The free-radical intermediates are unstable so that 1,4-butanediol is also found [25]).



[25]

The molybdoflavoprotein xanthine oxidase is a mammalian enzyme capable of reducing nitroaromatic drugs (*104*). The enzyme does not detectably reduce 1-nitro-2-phenylethane but can effect the one-electron reduction of 1-nitro-2-phenylethene (nitrostyrene), which thus undergoes reductive dimerization. Nitrostyrene derivatives exhibit antibiotic activity against anaerobic protozoa (*105*) but are more intrinsically reactive and have a less favorable therapeutic index than conventional nitroaromaic drugs.

It is interesting in this regard that 1,3-dichloro-2-methyl-2-nitropropane has been designed as a reductively enzyme-activated nitrogen mustard (*106, 107*). It is unlikely that mammalian tissues can reduce this nitroalkyl compound at a significant rate, but it is attractive to suppose that the compound is metabolically oxidized, on the other hand, to an alternate crosslinking agent ([26]).



[26]

Although nitroparaffins are not readily reduced by enzymes in mammalian tissues, nitroaryl compounds are metabolically reduced because of the radical-stabilizing influence of the aromatic ring (*108*). Nitroalkyl compounds with electron-withdrawing and radical-stabilizing substituents ought to similarly be subject to metabolic reduction by mammalian enzymes. Nitroalkyl nitriles, for instance, are reduced to cyano alkanes and inorganic nitrite in model nonenzymatic reactions with mild reductants such as dihydropyridines and thiols (*109, 110*). These reactions proceed through unstable nitro radical anions which decompose into inorganic nitrite and an alkyl radical as shown in [25]. When nitro radical ions are enzymatically generated in aerobic tissues, they can nonenzymatically react with O_2 to afford the superoxide anion and parent nitro compound (*108*). Suitably substituted nitroalkyl compounds might thus serve as catalysts for metabolic production of deleterious superoxide radicals.

INHIBITION OF ENZYMES

The ionized nitroalkyl group closely resembles the carboxylate group, and nitronates generally bind well to enzymes which ordinarily act on the analogous carboxylic acids (*26, 27*). However, chemical features of the nitroalkyl group permit interesting covalent and noncovalent reactions with enzymes.

Transition-State Analogs

One theory of enzymatic catalysis holds that enzymes lower the activation-energy barrier because the energy of (usually noncovalent) binding forces lowers the energy of transition-state species. It follows that good structural analogs of

TABLE 2

NITRONATE ANALOGS OF CARBANIONIC ENZYME INTERMEDIATES

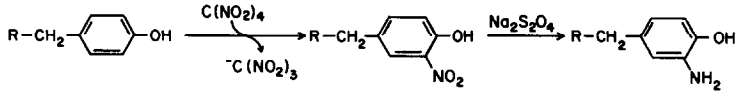
Nitronate	Enzyme	K_m/K_i	Ref.
$^-O_2N{=}CH{-}CHOH{-}CO_2^-$	Fumarase	900	(*4*)
$^-O_2N{=}CH{-}CH(NO_3^+){-}CO_2^-$	Aspartase	1,600	(*4*)
$^-O_2N{=}CH{-}COH(CO_2^-){-}CH_2{-}CO_2^-$	Aconitase	2,700	(*112*)
$^-O_2N{=}C(CH_2CO_2^-){-}CHOH{-}CO_2^-$	Aconitase	72,000	(*112*)
$^-O_2N{=}CH{-}CH_2{-}CO_2^-$	Isocitrate lyase	65,000	(*113*)
$^-O_2N{=}C(CH_2OH){-}CH_2{-}PO_3^{2-}$	Enolase	>1,000	(*114*)
$^-O_2N{=}C(NO){-}CH_2{-}PO_3^{2-}$	Enolase	>1,000	(*114*)
$^-O_2N{=}CH{-}CH(AMP){-}CO_2^-$	Adenylosuccinate lyase	28	(*115*)
$^-O_2N{=}CH{-}CH(arg){-}CO_2^-$	Argininosuccinate lyase	20	(*116*)

transition-state species should bind more tightly than analogs of either substrates or products to an enzymatic active site (*111*). Because enzymes accelerate reaction rates by orders of magnitude, a structural analog of the transition-state species theoretically should exhibit a K_i value that is orders of magnitude lower than the K_m value of the corresponding substrate. Although nitronate analogs of carboxylic substrates generally bind well to enzymes, the nitronates have been noted to bind particularly well to enzymes which generate, or are suspected to generate, α-carbanionic reaction intermediates. For instance, isocitrate lyase catalyses a reaction resembling a retro-Claisen condensation whereas the other enzymes in Table 2 catalyze α,β-elimination reactions. The nitronates may bind as analogs of the α-carbanionic intermediates which occur in those reactions. The best inhibitors shown in Table 2 bind so tightly that the onset and reversal of their inhibition is not instantaneous. For instance, the nitronate analog of the α-carbanion of succinate dissociates from isocitrate lyase in a reaction with a half-time exceeding 10 h (*113*).

In some cases the nitramino ($^-O_2N{=}N{-}R$) analogs have been compared with the nitronate ($^-O_2N{=}CH{-}R$) analogs shown in Table 2 (*27, 37, 117*). The carbon acids bind much more tightly than the nitrogen acids.

Intrinsically Reactive Compounds

A few electrophilic substituted nitroalkanes are of use as protein-modifying reagents. Tetranitromethane is the most commonly applied reagent of this class. The compound rapidly oxidizes sulfhydryl groups of proteins affording disulfides and other products (*118*). The reagent can also react with tryptophan (*119*) but a more useful reaction is its selective nitration of tyrosyl residues under mild conditions (on a time scale of minutes at room temperature and pH 8) (*120*). The nitrotyrosyl residue can be smoothly converted by dithionite to another analog with an altered pK ([27]).

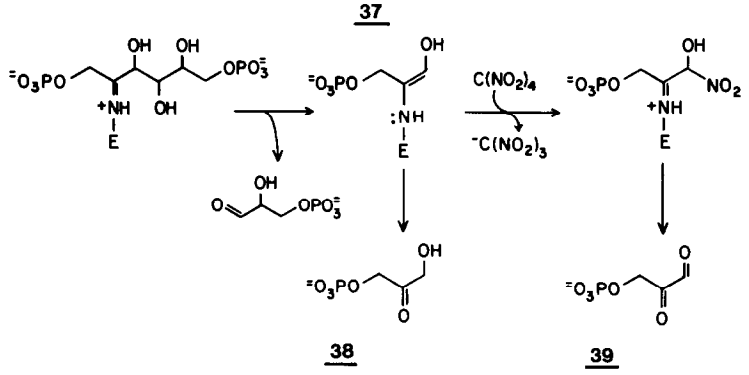


[27]

Crosslinking of protein can occur during reaction with $C(NO_2)_4$ because of one-electron oxidation of tyrosyl residues to free radicals which couple (*120*). The reagent also nitrates the carbanionic position of thiamine (*121*) and the vinyl groups of heme (*122*). Reactions of tetranitromethane can be followed by the absorbance of the orthonitroformate carbanion $C(NO_2)_3^-$ at 350 nm (ε = 14,400 M^{-1} cm^{-1}).

Tetranitromethane has been used to discriminate "exposed" tyrosyl residues from "buried" tyrosyl residues in enzymes. In a particularly interesting case, aspartate aminotransferase is rapidly inactivated by the reagent in the presence, but not in the absence, of substrates. A critical tyrosyl residue becomes reactive only while the enzyme is processing substrates. This reaction is an example of the phenomenon which Christen has termed "syncatalytic" enzyme modification (*123*).

Tetranitromethane can also serve as an electron-accepting substrate for enzymes. For instance, it oxidizes the reduced form of FAD-dependent D-amino acid oxidase (*124*). More interestingly, it can serve as an oxidant substrate for enzymes which ordinarily catalyze reactions that are not generally considered to be redox reactions (*123*). For instance, aldolase catalyzes the reduction of tetranitromethane by fructose 1,6-diphosphate because the aldolase reaction involves a carbanionic intermediate (the enamine **37**) which can be oxidized by the nitro compound ([28]).



[28]

The enzyme releases a reactive keto aldehyde **39** instead of dihydroxyacetone phosphate **38** in the presence of $C(NO_2)_4$. The keto aldehyde occasionally captures a critical active-site arginyl residue instead of diffusing out of the active site. Christen has referred to this type of catalysis-dependent inactivation as "para-catalytic" enzyme modification (*125*).

Attack by nucleophiles on the nitrogen atoms of tetranitromethane is sterically hindered, and nitration reactions by the compound probably proceed through free-radical intermediates ([29]). A charge-transfer complex may also be a preliminary intermediate in the reaction with phenols such as tyrosine (*120*).

$$\begin{aligned} R{:}^- + C(NO_2)_4 &\rightarrow R\cdot + (O_2N)_3C{-}NO_2^-\cdot \\ &\rightarrow R\cdot + NO_2\cdot + (O_2N)_3C{:}^- \rightarrow R{-}NO_2 \end{aligned} \qquad [29]$$

Electron-withdrawing halogen groups can also activate the nitro group for redox reactions. Examples include chloropicrin and bronopol. Chloropicrin, Cl_3CNO_2, is a lachrymator which was used as a chemical warfare agent during World War I and has been used as an agricultural pesticide. Bronopol is used to preserve solutions from microbial contamination (*126–128*). It rapidly oxidizes sulfhydryl groups of proteins ([30]). Dihydropyridines and ferrous heme also reduce halo nitro compounds in reactions mediated by free radicals (*109, 110, 129, 130*).

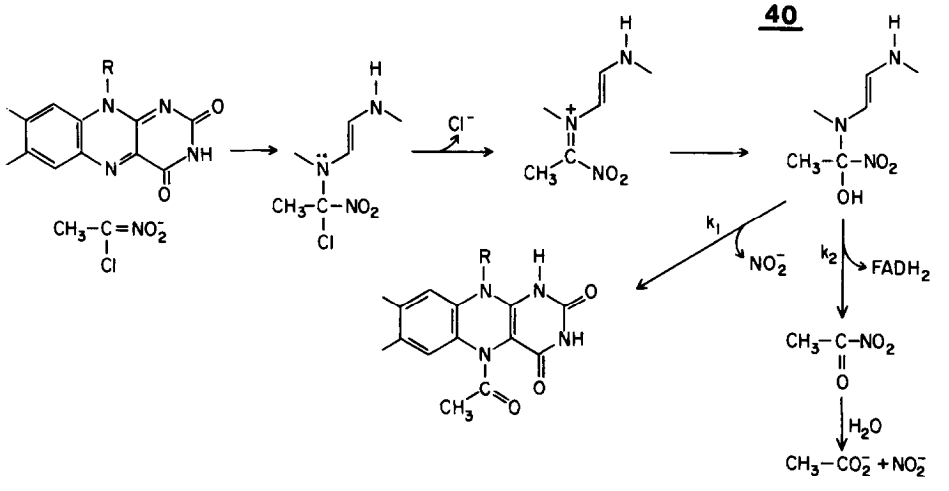
$$\begin{aligned} \text{R—SH} + (\text{HOCH}_2)_2\text{CBrNO}_2 &\rightarrow \text{R–S}\cdot + (\text{HOCH}_2)_2\text{CBrNO}_2^{-}\cdot \\ &\rightarrow \tfrac{1}{2}\text{R—SS—R} + \text{Br}^- + (\text{HOCH}_2)_2\text{C=NO}_2\cdot \end{aligned} \qquad [30]$$

Unsubstituted nitroalkyl compounds can serve as nucleophiles toward electrophilic groups on proteins. Disulfide groups of cystinyl residues are the most common electrophilic groups on enzymes, and some disulfides can be cleaved by carbanions such as cyanide (*131*). Nitro carbanions might be expected to react similarly. However, the reaction of alkanenitronates with disulfides is extremely slow in aqueous solution, even in the case of activated disulfides such as 5,5′-dithiobis(2-nitrobenzoate) (Ellman's reagent). The reaction of nitronates with Ellman's reagent, though, is dramatically accelerated in aprotic solvents such as dimethyl sulfoxide. Nitronates are stabilized by hydrogen bonds and thus appear more nucleophilic (and more basic) in aprotic solvents than in water (*132*). It is unlikely that binding to the active site of an enzyme would prevent hydrogen bonding and so permit nitronates to effectively cleave disulfide linkages; and electrophilic groups are otherwise rare in polypeptides. However, some enzymes contain electrophilic dehydroalanine residues which can be trapped with nitronate nucleophiles. Inactivation with nitromethane followed by identification of 3-(nitromethyl)alanine derivatives in hydrolysates helped to identify dehydroalanine residues in the active sites of bacterial histidine ammonia-lyase (*133*) and plant phenylalanine ammonia-lyase (*134*). (The nitromethylated amino acid is found as aspartate after acid hydrolysis or can be reduced to 2,4-diaminobutyrate before hydrolysis.) Other electrophilic groups of enzymes occur on cofactors, and nitronates readily form adducts with pyridinium compounds (*135*), for instance. Reaction with tightly bound electrophilic cofactors can permit suitably substituted nitronates to irreversibly inactivate certain enzymes.

Suicide Substrates and Reactive Products

Alkanenitronates are reductive substrates of renal D-amino acid oxidase because of their nucleophilic attack on N^5 of the flavin cofactor (*93*). Consideration of the reaction mechanism shown in [23] suggested that a nitronate bearing a second leaving group would inactivate the enzyme without the requirement of an additional reagent such as cyanide (*94*). 1-Chloro-1-nitroethane (p*K* 7) thus proved to inactivate the enzyme because of its reductive acylation of the flavin as shown in [31].

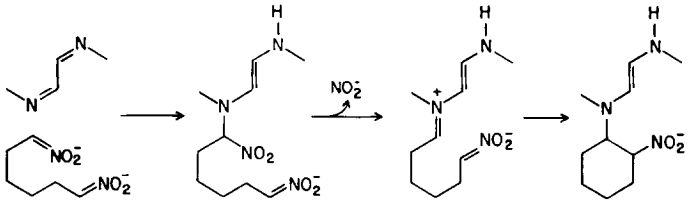
The intermediate **40** can eliminate enzymatically active $FADH_2$ or can eliminate nitrite to afford the catalytically inactive N^5-acetyl derivative of $FADH_2$. Since the rate constant k_1 is roughly equal to k_2, chloronitroethane is oxidized to acetate as



[31]

an ordinary substrate about half the times that it is processed by the enzyme. During the other half of the times that chloronitroethane is processed by the enzyme, it destroys the enzyme as a "suicide" substrate. Suicide substrates are enzyme-inactivating reagents which were so-termed by Abeles and coworkers (*136*) to emphasize the active catalysis by the target enzymes of their own inactivation. Suicide substrates are substrate analogs which are accepted into the active site and catalytically converted to a species which forms a stable linkage with the enzyme. The high specificity of enzymatic catalysis permits suicide substrates to be highly selective enzyme inactivators, and, for instance, we have not detected inactivation of any flavoenzyme other than hog kidney D-amino acid oxidase by 1-chloro-1-nitroethane.

Nitroalkanes might inactivate flavoenzymes by other variations of the reaction shown in [23]. For instance, since methanenitronate can replace cyanide as the iminium-trapping agent in that reaction, the dianion of 1,6-dinitrohexane might prove to inactivate a nitrohexane-oxidizing flavoenzyme as hypothesized in [32].



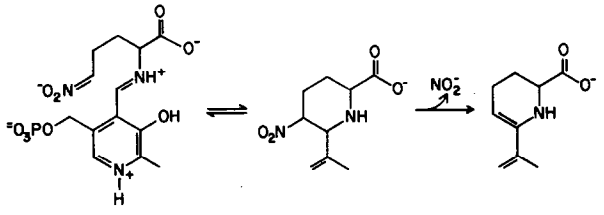
[32]

The dianion of the toxic antibiotic 3-nitropropionate is another suicide substrate for a flavoenzyme (*34, 35*). However, it labels the protein moiety rather than the flavin cofactor of mitochondrial succinate dehydrogenase (*36*). It is probably oxidized to 3-nitroacrylate, an electrophilic analog of fumarate. This species captures an active-site nucleophilic group without being released from the active site, and the enzyme can be completely inactivated by a nearly stoichiometric quantity of

nitropropionate *in vitro* (*36*). The inability of 3-nitroacrylate to diffuse out of the active site of the enzyme is a reflection of its high electrophilicity.

Nucleophilic nitronates can also attack the electrophilic cofactors of hemoproteins. For instance, horseradish peroxidase suffers inactivation while processing nitronate substrates ([19]). Methanenitronate was found to nitromethylate the activated heme cofactor of peroxidase Compound II at a methine bridge position (*80*).

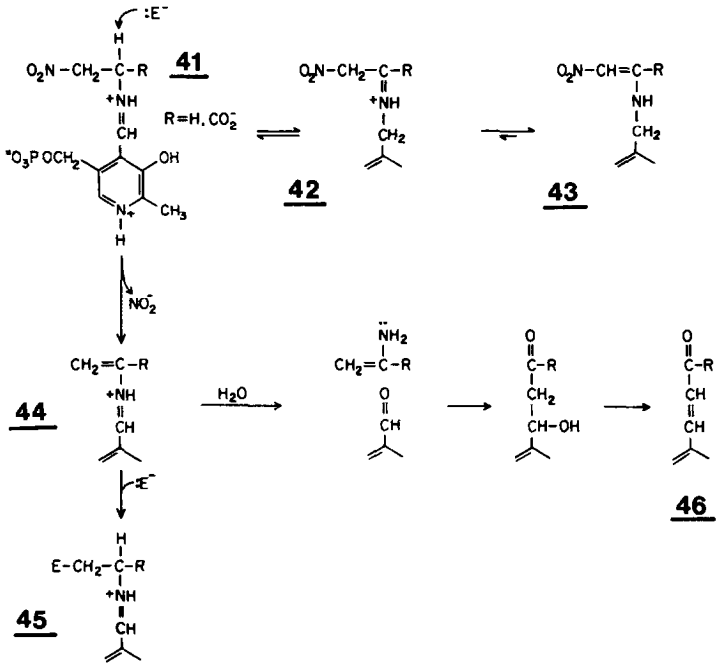
The nucleophilic nitronates should also add to the electrophilic carbonyl group of pyridoxal phosphate-dependent enzymes. Reaction of ethanenitronate with transaminases is rapidly reversible, but the nitronate of 5-nitro-L-norvaline can inactivate glutamate transaminases (*137*). It probably forms a metastable six-membered cyclic adduct from which nitrite can be slowly eliminated ([33]).



[33]

γ-Nitro amines should similarly form five-membered cyclic adducts with pyridoxal enzymes. We have thus tested 3-nitropropylamine as an inactivator of GABA-transaminase. The nitronate analog of GABA requires a few minutes to dissociate from the active site of the transaminase obtained from *Pseudomonas fluorescens*. However, the nitronate is oxidized as a substrate by the transaminase from porcine brain. The product is the nitronate of 3-nitropropionaldehyde. This analog of succinic semialdehyde is released free into solution where it spontaneously decomposes into electrophilic olefins which then nonspecifically inactivate the transaminase or other enzymes with which it is coincubated. The conversion of nitropropionaldehyde into reactive olefins (**30** and **31**) is shown in [16] in connection with the metabolism of the millipede defense agents nitropolyzonamine and polyzonimine. The locoweed toxin 3-nitropropanol ([8]) can similarly be converted to the reactive olefins upon oxidation by alcohol dehydrogenase (*39, 138*). Similarly, 3-nitropropionic acid occurs in plants in ester linkage to sugars (*12*) and its nitronate can be esterified to coenzyme A by mitochondrial succinic thiokinase (*27*). The nitropropionate esters can slowly eliminate nitrite to afford electrophilic acrylate esters.

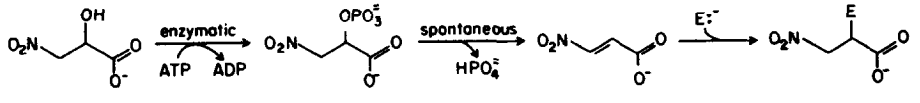
Many pyridoxal enzymes are inactivated by suicide substrates of the general formula X-CH_2—$CR(NH_3^+)$—R′ where R′ is H or CO_2^- and X is a leaving group such as fluoride or acetate (*136, 139–141*). The nitro group can leave as nitrite, and this feature probably accounts for the inactivation of glutamate-pyruvate transaminase by 3-nitroalanine (shown in [2]) and the inactivation of lysyl oxidase (which has a pyridoxal or pyridoxal-like cofactor) by 2-nitroethylamine (*137, 142*). However, the mechanism of inactivation of pyridoxal enzymes by β-nitro amines is not established, and at least the three shown in [34] are plausible (*136, 143–145*).



[34]

The aldimine adduct **41** can tautomerize to the ketimine adduct **42** as in ordinary transamination. However, the nitro group may cause the ketimine adduct **42** to tautomerize to a metastable conjugated nitroenamine **43**. Alternatively, nitrite elimination could occur to afford the reactive enamine **44**. This enamine could alkylate a nucleophilic group on the protein moiety of the enzyme to afford inactive enzyme **45**. The enamine could also be released from the coenzyme only to reattach as a carbon nucleophile and so afford derivative **46**.

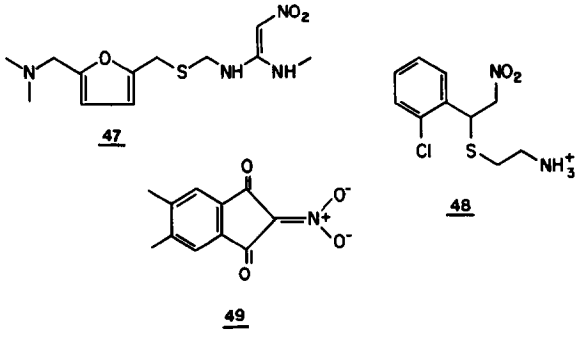
It was hypothesized that the nitronates shown in Table 2 might serve as suicide substrates for those of the enzymes which catalyze α,β-elimination reactions. The enzymes might convert the nitronates to electrophilic nitro olefins that would capture nucleophilic active-site groups which ordinarily function as general bases. However, the enzymes do not appear to be inhibited by that mechanism. For instance, the nitronate of nitropropionate cannot be converted to a nitro olefin by fumarase but binds about half as well as the nitronate analog of malate to that enzyme (4). Furthermore, fumarase is not readily inactivated by 3-nitroacrylate and does not detectably catalyze the hydration of nitroacrylate (4). However, the energetically unfavorable dehydration of 3-nitrolactate to 3-nitroacrylate is readily accomplished by another enzyme with the aid of ATP (63). Pyruvate kinase from rabbit muscle can mediate the phosphorylation of lactate *in vitro*. It also phosphorylates nitrolactate and is thereby inactivated in an ATP-dependent reaction ([35]).



[35]

NITROALKYL DRUGS

There are dozens of useful nitroaromatic drugs, but only a handful of nitroalkyl compounds have been assigned generic names in the United States. Of these ([36]), only ranitidine **47** is in clinical use (*146*). Ranitidine is active against peptic ulcer disease because it binds as an antagonist at H_2 receptors for histamine. The nitro group is not critical for such pharmacological activity, however, since other H_2-receptor antagonists such as cimetidine do not bear a nitro group.

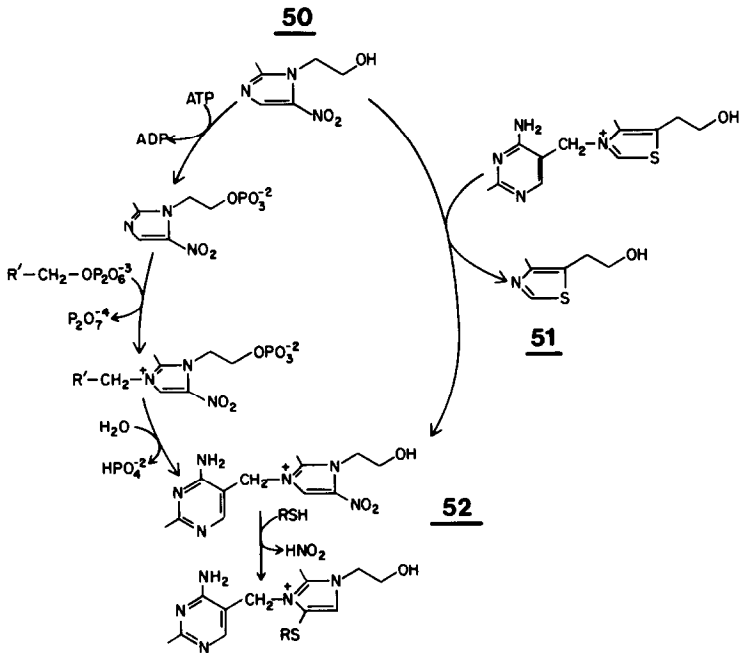


[36]

Nitroindandiones such as nivimedone **48** block the antibody–antigen-induced passive cutaneous anaphylaxis reaction in the rat and may be useful in the treatment of asthma (*147*). Its mechanism of action is not identified, but it is interesting to suppose that the nitronate **48** could undergo metabolic oxidation to nitrite and a reactive trioxo product (dimethyl ninhydrin). The structurally similar compound 5-nitrobarbituric acid is diabetogenic because it is metabolically oxidized to the trioxo compound alloxan (*148*). Other nitronates have been reported to have opiate-antagonist activity (*149*).

Nitralamine **49** exerts antifungal activity (*105*). It may be reduced to reactive free radicals or else eliminate cysteamine to afford a reactive nitrostyrene. Other nitrostyrene derivatives exhibit diuretic activity, probably because of structural analogy to the vinyl ketone ethacrynic acid (*150*).

Most of the pharmacologically useful nitro compounds have been aromatic, and, of these, metronidazole **50** is probably the most frequently employed nitroaromatic drug. Metronidazole is selectively toxic to anaerobic microorganisms because of metabolic reduction to reactive products (*103, 108*). However, the nitro group of metronidazole can be displaced by thiols in reactions that likely proceed through nonaromatic intermediates (*151*). The reaction of thiols with metronidazole occur slowly. However, enzymatic alkylation of the nitroimidazole would provide a nitroimidazolium cation which ought to react more readily than the parent compound with nucleophilic cellular constituents. There is no evidence that metabolic alkylation of the drug does occur, but the close structural analogy of the drug to the thiazole precursor **51** of thiamine makes it attractive to speculate that gut flora could metabolize the drug to an electrophilic analog of thiamine as hypothesized in [37]. At least two routes to the putative metabolite are plausible ([37]).



[37]

The drug might be accepted as a substrate for the thiamine-biosynthetic enzymes (*152*) and thus be converted in two steps to an analog of thiamine monophosphate. Another route might involve the thiamine-destroying enzyme, gut bacterial thiaminase. Thiaminase catalyzes the replacement of the thiazole moiety of thiamine by various heterocyclic nitrogenous bases (*153*) and might thus convert the drug to the spurious vitamin **52** in one step. The hypothesis that metronidazole may be metabolized to an affinity label for thiamine-binding proteins is particularly interesting because the drug exhibits neurotoxic side effects (*154–156*) that have been likened to the effects of hypovitaminosis B_1 (*157*).

CONCLUDING REMARKS

Many bioorganic questions with regard to nitroalkyl compounds remain to be answered. The chemical details of the enzymatic six-electron oxidation of the amino group to the nitro group remain vague, and it is not established that all nitro groups arise in nature by that route. Furthermore, although toxic products can confer ecological advantages, it is not clear why nitroalkyl compounds are secondary metabolites of diverse organisms.

The nitroalkyl group can serve as a one- and two-electron reductant, oxidant, ambident nucleophile, electrophile, ligand, and leaving group in reactions with enzymes and other biomolecules. The richness of its reactivity may have hindered the application of the nitroalkyl group in medicinal chemistry. However, the interesting reactions that have been observed with enzymes *in vitro* may prove applicable to the design of inhibitors useful *in vivo*.

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