Reaction of Nitric Oxide at the β-Carbon of Enamines. A New Method of Preparing Compounds Containing the Diazeniumdiolate Functional Group

Joseph A. Hrabie,*,† Ernst V. Arnold,‡ Michael L. Citro,§ Clifford George,⊥ and Larry K. Keefer‡

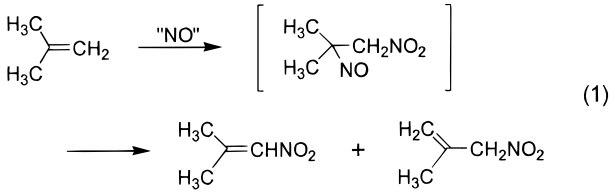
Analytical Chemistry Laboratory and Intramural Research Support Program, SAIC Frederick, National Cancer Institute—Frederick Cancer Research and Development Center, Frederick, Maryland 21702, Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute—Frederick Cancer Research and Development Center, Frederick, Maryland 21702, and Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375

hrabie@mail.ncifcrf.gov

Received April 19, 2000

The reaction of nitric oxide (NO) with enamines has been investigated. Unlike previously reported reactions of NO as a free radical with alkenes, the electrophilic addition of NO to the β-carbon of enamines results in the formation of compounds containing the diazeniumdiolate functional group (−[N(O)NO]⁻). This reaction between NO and enamines has been shown to be quite general and a variety of enamine-derived diazeniumdiolates have been isolated and characterized. While enamines derived from aldehydes and ketones whose structures allow for sequential multiple electrophilic additions tended to undergo overreaction leading to unstable products, it has been shown that this complication may be overcome by suitable choice of reaction solvent. The products obtained may exist as zwitterionic iminium salts or as neutral species depending upon the structure of the parent enamine. The diazeniumdiolate derived from 1-(*N*-morpholino)cyclohexene is unique among the new compounds in that it spontaneously releases NO upon dissolution in buffered aqueous solution at pH 7.4 and 37 °C. While the total quantity of NO released by this material (ca. 7% of the theoretical 2 moles) is apparently limited by a competing reaction in which it hydrolyzes to an α-diazeniumdiolated carbonyl compound and the parent amine, this feature may prove to be of great value in the development of multiaction pharmaceuticals based upon this new type of NO-releasing compound. Reports of enzymatic (oxidative) release of NO from previously known carbon-bound diazeniumdiolates also suggest that analogues of these compounds may be useful as pharmaceutical agents. This new method of introducing the relatively rarely studied diazeniumdiolate functional group into organic compounds should lead to further research into its chemical and biological properties.

The reaction of nitric oxide (NO) with simple olefins and dienes has been the subject of considerable study for some time. Following an early report[1] of the isolation of nitro compounds from the free-radical chain reaction of NO with cyclohexene, Brown[2] conducted an exhaustive study of the NO-isobutylene reaction and concluded that the major products of the reaction (eq 1) were formed via a free-radical mechanism initiated by addition of nitrogen dioxide (NO_2, present as an impurity in virtually all NO as a result of trace oxygen contamination) to the double bond. The often complex mixture of products that results



from such reactions was attributed in part to the formation of nitrosating species as byproducts. A subsequent study[3] of this reaction served to confirm Brown's suspicion that part of the complexity may also be due to the thermal instability of the initial products. Brown reported[2] that rigorously purified NO did not react with liquid olefins even on prolonged contact, a conclusion that is accepted to this day. Several other studies[4] have reinforced the conclusion that the reaction between "NO" and mono-olefins involves organic radicals. It is worth noting the tremendous difficulty involved in conducting reactions with truly pure NO, and this is probably the major reason that, to our knowledge, no definitive studies have been conducted to determine whether the reaction of NO with olefins can be initiated by other materials or photochemically although the gas-phase reactions of NO

† Analytical Chemistry Laboratory.
‡ Laboratory of Comparative Carcinogenesis.
§ Intramural Research Support Program.
⊥ Naval Research Laboratory.

(1) Bloomfield, G. F.; Jeffrey, G. A. *J. Chem. Soc.* **1944**, 120−124.
(2) Brown, J. F., Jr. *J. Am. Chem. Soc.* **1957**, *79*, 2480−2488.
(3) Phillips, L. V.; Coyne, D. M. *J. Org. Chem.* **1964**, *29*, 1937−1942.
(4) (a) Park, J. D.; Stefani, A. P.; Crawford, G. H.; Lacher, J. R. *J. Org. Chem.* **1961**, *26*, 3316−3319. (b) Birchall, J. M.; Bloom, A. J.; Haszeldine, R. N.; Willis, C. J. *J. Chem. Soc.* **1962**, 3021−3032. (c) Rockenbauer, A.; Györ, M.; Tüdös, F. *Tetrahedron Lett.* **1986**, *27*, 3425−3428. (d) Chiu, K. W.; Savage, P. D.; Wilkinson, G.; Williams, D. J. *Polyhedron* **1985**, *4*, 1941−1945. (e) Mukaiyama, T.; Hata, E.; Yamada, T. *Chem. Lett.* **1995**, 505−506. (f) Park, J. S. B.; Walton, J. C. *J. Chem. Soc., Perkin Trans. 2* **1997**, 2579−2583.

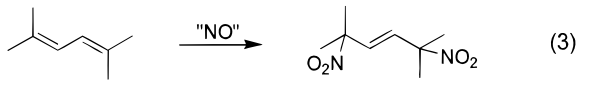
10.1021/jo000600g CCC: $19.00
Published on Web 08/08/2000

with olefins have been studied for decades as a result of their importance in atmospheric science.[5]

Similar radical reactions between NO and dienes have been described[6] with the additional feature reported being that, in these cases, initiation by NO itself might be possible. During the past decade, the central role of NO chemistry in biology has emerged,[7] prompting renewed interest in NO−diene interactions. One of the most interesting reactions discovered by these new investigations is clearly the reaction between NO and the *o*-quinodimethanes[8a] (eq 2) to form a persistent nitroxide which can be observed by ESR but not isolated although, in a similar reaction, *N*-hydroxyphthalimide was isolated from the reaction of NO with the corresponding bisketene.[8b] These two reactions, although electrophilic,

(2)

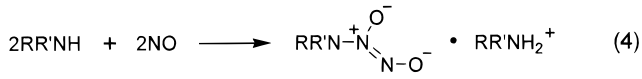
clearly represent special cases in which the strong bias toward formation of an aromatic ring determines the outcome of the reaction. The other polyenes whose reactions with NO have been studied thus far include *β*-carotene,[9a] retinyl acetate,[9b] ethyl linoleate,[9c] and 2,5-dimethylhexa-2,4-diene.[9d] The latter is the most complete study available, and the major product (eq 3) has been characterized by X-ray crystallography. The mechanistic



(3)

conclusions concerning the radical nature of this reaction represent an excellent summation of the current knowledge to date.[9d]

We have long been interested in the reaction of NO as an electrophile with amines[10] to produce the "nucleophile/NO adducts" containing the diazen-1-ium-1,2-diolate functional group bound to nitrogen (eq 4) since these materials have proven to be excellent NO donors for use in studying the role of NO in biology.[11] Despite the



(4)

growing importance of this reaction, NO has had an undeserved reputation as a very poor electrophile.[12] In fact, the above reaction of NO with amines and the analogous reactions with carbanions (enolates),[13] Grignards,[14] and imines[15] all appear to be examples of the reaction of NO with organic compounds which might be attributed to its electrophilic character.

In this paper, we report the discovery of a new electrophilic reaction of NO, demonstrate that this reaction of NO with the olefinic double bond of enamines produces compounds containing the diazen-1-ium-1,2-diolate functional group bound to carbon, and show that some of the compounds prepared in this way have the potential to serve as spontaneous NO donors.

Results and Discussion

The nitrogen-bound diazen-1-ium-1,2-diolates are useful NO donor compounds because the reaction which leads to their formation is reversible on dissolution in aqueous media. Traube[13a] reported observing some gas evolution from several of the analogous carbon-bound diazen-1-ium-1,2-diolates that he prepared from active methylene compounds. While several *N*-nitrosohydroxylamines (the name most commonly given to these materials in the past as a result of the most frequently employed method of preparation[16]) have been found to release NO as a result of enzymatic action (oxidation),[17] and one prepared by nitrosating *N*-(p-methoxybenzyl)-*N*-hydroxyguanidine has been found to do so in aqueous solution,[18] most of these compounds have not been reported to release NO spontaneously. We began our effort to discover carbon-bound diazen-1-ium-1,2-diolate NO donors with the assumption that the electronic structure of any such compound would need to provide for reversibility similar to that exhibited by the reaction of NO with amines. Additional guiding principles were the desire to find reaction conditions less harsh than the strong base used by Traube[13a] to generate carbanions of active methylene compounds and our belief that any electrophilic reaction between NO and olefins could be both enhanced and rendered more specific by the use of a polarizing electron-donating substituent. While the latter requirement could be met by a wide variety of Michael donor-type olefins, we were attracted to the enamines because an extensive literature exists for these compounds as a result of their use in the Stork alkylation reaction[19] and in some cases the Michael reaction of enamines with electrophilic olefins has been shown to be reversible.[19d]

(5) Heicklen, J.; Cohen, N. *Advances in Photochemistry*; Noyes, W. A., Jr., Hammond, G. S., Pitts, J. N., Jr., Eds.; Wiley: New York, 1968; Vol. 5, pp 157−328.

(6) Brindley, P. B.; Nicholson, S. H. *J. Chem. Soc., Perkin Trans. 2* **1975**, 1808−1813.

(7) Butler, A. R.; Williams, D. L. H. *Chem. Soc. Rev*. **1993**, *22*, 233−241.

(8) (a) Korth, H.-G.; Sustmann, R.; Lommes, P.; Paul, T.; Ernst, A.; de Groot, H.; Hughes, L.; Ingold, K. U. *J. Am. Chem. Soc*. **1994**, *116*, 2767−2777. (b) Adam, W.; Bottle, S. E.; Grice, I. D.; Pfeiler, D.; Wentrup, C. *Tetrahedron Lett*. **1996**, *37*, 2113−2116.

(9) (a) Gabr, I.; Patel, R. P.; Symons, M. C. R.; Wilson, M. T. *J. Chem. Soc., Chem. Commun*. **1995**, 915−916. (b) Afzal, M.; Walton, J. C. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 2329−2332. (c) d'Ishia, M. *Tetrahedron Lett*. **1996**, *37*, 5773−5774. (d) Kelly, D. R.; Jones, S.; Adigun, J. O.; Koh, K. S. V.; Hibbs, D. E.; Hursthouse, M. B.; Jackson, S. K. *Tetrahedron* **1997**, *53*, 17221−17234.

(10) (a) Drago, R. S.; Karstetter, B. R. *J. Am. Chem. Soc*. **1961**, *83*, 1819−1822. (b) Hrabie, J. A.; Klose, J. R.; Wink, D. A.; Keefer, L. K. *J. Org. Chem*. **1993**, *58*, 1472−1476.

(11) Keefer, L. K.; Nims, R. W.; Davies, K. M.; Wink, D. A. *Methods Enzymol*. **1996**, *268*, 281−294.

(12) Fontecave, M.; Pierre, J.-L. *Bull. Soc. Chim. Fr*. **1994**, *131*, 620−631.

(13) (a) Traube, W. *Liebigs Ann. Chem*. **1898**, *300*, 81−128. (b) Yandovskii, V. N.; Doshchechkina, T. I.; Tselinskii, I. V.; Kuznetsov, V. S. *Zh. Org. Khim*. **1983**, *19*, 489−493 (*Engl. Transl.: J. Org. Chem. U.S.S.R*. **1983**, *19*, 427−431).

(14) Zyuzin, I. N.; Lempert, D. B. *Izv. Akad. Nauk, Ser. Khim*. **1985**, 831−835 (*Engl. Transl.: Russ. Chem. Bull*. **1985**, 753−756).

(15) Hrabie, J. A.; Srinivasan, A.; George, C.; Keefer, L. K. *Tetrahedron Lett*. **1998**, *39*, 5933−5936.

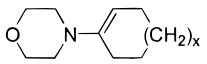
(16) Sandler, S. R.; Karo, W. *Organic Functional Group Preparations*, 2nd ed.; Academic Press: San Diego, CA, 1956; Vol. 2, pp 530−531.

(17) (a) Alston, T. A.; Porter, D. J. T.; Bright, H. J. *J. Biol. Chem*. **1985**, *260*, 4069−4074. (b) McGill, A. D.; Zhang, W.; Wittbrodt, J.; Wang, J.; Schlegel, H. B.; Wang, P. G. *Bioorg. Med. Chem*. **2000**, *8*, 405−412.

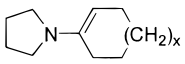
(18) Southan, G. J.; Srinivasan, A.; George, C.; Fales, H.; Keefer, L. K. *Chem. Commun*. **1998**, 1191−1192.

(19) (a) Whitesell, J. K.; Whitesell, M. A. *Synthesis* **1983**, 517−536. (b) Cook, A. G., Ed.; *Enamines: Synthesis, Structure, and Reactions*, 2nd ed.; Marcel Dekker: New York, 1988. (c) Szmuszkovicz, J. *Adv. Org. Chem*. **1963**, *4*, 1−113. (d) Hickmott, P. W. *Tetrahedron* **1982**, *38*, 1975−2050.

Nature of the Reaction of NO with Enamines. The compounds chosen for initial study were the readily available enamines **1**−**4** derived from condensation of cyclopentanone or cyclohexanone with morpholine or pyrrolidine. A previous study had shown that reaction

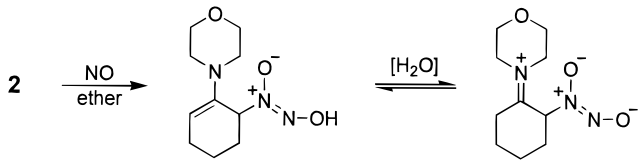


1: x = 0
2: x = 1



3: x = 0
4: x = 1

of these olefins with a mixture of NO and oxygen (nitrosating conditions) could be used to produce their 6-oximino derivatives,[20] but did not explore oxygen-free reactions. When any of these enamines were dissolved in acetonitrile and treated with NO gas (5 atm pressure) at room temperature (the identical conditions that were employed to synthesize the polyamine diazeniumdiolates[10b]), a vigorous exothermic reaction resulted accompanied by a large uptake of NO and the formation of a dark brown precipitate. Encouragingly, the reaction was not accompanied by the formation of the transient blue coloration associated with the free-radical processes described earlier. Attempts to isolate the solid precipitates often resulted in rapid decomposition with the release of large volumes of gaseous NO. In an attempt to bring the reaction under control the solvent was changed to diethyl ether, since NO is much less soluble in this solvent than in acetonitrile,[21] and the experiments were repeated at −78 °C without stirring (to further slow gas absorption). Although enamines **1**, **3**, and **4** still reacted uncontrollably under these conditions, the reaction of 4-(1-cyclohexen-1-yl)morpholine (**2**) afforded a good yield of crystalline NO addition product **5**. The



5 **6**

novelty of this reaction coupled with an increasing need to understand the fundamental structural features of the little-studied diazeniumdiolate functional group prompted an X-ray crystallographic study of this compound. The crystal structure (Figure 1) reveals the syn periplanar orientation of the N_2O_2 group consistent with previous reports for alkylated N-based,[22a] tosylated C-based,[22b] and nonderivatized C-based[22c] diazeniumdiolates. Examination of the relative bond lengths shown in Figure 1 also reveals that the compound is a true diazen-1-ium-1,2-diolate rather than an *N*-nitrosohydroxylamine, as evidenced, for example, in the 1.27 Å separation of the nitrogens consistent with considerable double-bond character. It is interesting to note that despite the presence of the morpholino nitrogen and nucleophilic carbon in **5**, the N_2O_2H proton remains on the oxygen. When a sample

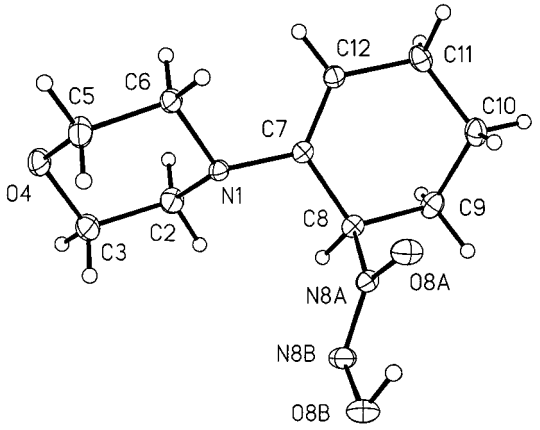


Figure 1. The molecular structure and numbering scheme for **5**. Selected bond lengths [Å (estimated standard deviation)]: N8A−O8A 1.274(2), N8A−N8B 1.268(2), N8B−O8B 1.369(2), C8−N8A 1.498(2).

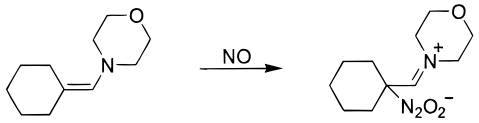
of **5** was dissolved in CD_3CN, the proton NMR spectrum obtained was fully consistent with the structure shown (it exhibited a single vinylic proton at 5.25 ppm) but when a sample of **5** was dissolved in D_2O the proton spectrum revealed that the preferred tautomer was zwitterion **6** (in which the vinylic proton was replaced by two allylic protons in the region of 2.1−2.6 ppm). Deuterium exchange in **6** was remarkably slow, and all carbon-bound protons could be observed initially. Exchange of the methine proton α to the diazeniumdiolate (which appeared at ca. 5.15 ppm in both tautomers) was complete within 24 h while little reduction of the signal attributable to the two allylic methylene protons had occurred.

The formation of **5** in the aprotic solvents employed in the NO reaction explains the vigorous nature and large uptake of gas observed earlier for enamines **1**−**4** in acetonitrile since it is still an enamine capable of further reaction with NO. If this process were carried to the extreme, one might expect up to eight molecules of NO to react with each enamine forming a tetradiazeniumdiolate. It is not surprising that these materials should prove too unstable to isolate. Since a change in solvent would slow but not stop such overreaction, we believe that our isolation of **5** was due to the fact that it is insoluble in ether and was kind enough to precipitate before additional reaction could occur. This large difference in solubility between the diazeniumdiolates and their precursors has been used to advantage previously to prevent the overreaction of polyamines with NO[10b] because they form as zwitterions which are insoluble in organic solvents. Since compound **5** as well as the compounds expected from enamines **1**, **3**, and **4** are not formed as zwitterions, it is not surprising that relative solubilities would prove to be of less help in this reaction. The successful isolation of the monodiazeniumdiolate of **2** but not of **1**, **3**, or **4** also correlates well with the known relative reactivities of these enamines in alkylation reactions,[23] in which **2** is least reactive. Enamine **2** has the lowest electron density at the β-carbon of these four compounds.[23] Overreaction is also the major problem encountered in the use of enamines in the Stork alkylation,[19] and it is usually overcome by avoiding substrates that are susceptible. Following this strategy, the remainder

(20) Fischer, R. H.; Weitz, H. M. *Synthesis* **1975**, 791−794.
(21) Shaw, A. W.; Vosper, A. J. *J. Chem. Soc., Faraday Trans. 1* **1977**, *73*, 1239−1244.
(22) (a) Saavedra, J. E.; Dunams, T. M.; Flippen-Anderson, J. L.; Keefer, L. K. *J. Org. Chem.* **1992**, *57*, 6134−6138. (b) White, E. H.; Todd, M. J.; Ribi, M.; Ryan, T. J.; Sieber, A. A. F.; Dickerson, R. E.; Bordner, J. *Tetrahedron Lett.* **1970**, 4467−4472. (c) Hickmann, E.; Hädicke, E.; Reuther, W. *Tetrahedron Lett.* **1979**, 2457−2460.
(23) Kuehne, M. E.; Garbacik, T. *J. Org. Chem.* **1970**, *35*, 1555−1558.

of the studies of this new reaction were conducted using enamines which could not react at more than one site.

Generality of the Reaction. First, the reaction of NO with more hindered enamines was confirmed by the direct formation of zwitterion **8** from 4-(cyclohexylidenemethyl)morpholine (**7**) in good yield. The only problem



7 **8**

encountered was the tendency of the product to darken with prolonged exposure to NO, and this could be overcome easily by conducting the reactions without stirring to produce well-formed colorless crystals.

The independence of this synthesis reaction from variations in the structure of the amine-derived half of the enamine was demonstrated by the preparation of diazeniumdiolate intramolecular iminium salts **9**−**14** from a series of isobutyraldehyde enamines. While some

9: NRR' = morpholino
10: NRR' = piperidino
11: NRR' = pyrrolidino
12: R = Me; R' = phenyl
13: R = Me; R' = p-tolyl
14: R = Me; R' = p-anisyl

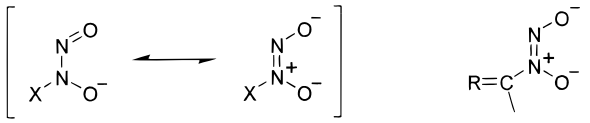
differences in both the relative rates (pyrrolidine enamine **11** reacted fastest; morpholine enamine **9** was slowest) and cleanliness (**9** cleanest; **11** dirtiest) of the reaction were observed, good yields of **9**−**14** were obtained. The relative rates of these reactions also correlate well with those found for the alkylation of enamines.[23] It should be noted that *N*-alkylation is often a serious complication when enamines are utilized to effect the alkylation of aldehydes and ketones.[19a] We speculate that some of the variation we have observed in the cleanliness of the diazeniumdiolation of enamines may be due to interference from reaction at the tertiary nitrogen and note the observation of Longhi et al.[24] that an unstable complex forms between trimethylamine and NO under pressure. While this does not appear to be a serious problem, results to date suggest that it can be minimized through the use of less reactive enamines such as those derived from morpholine and by the use of cold acetonitrile as the reaction medium.

The above results clearly demonstrate the general nature of the reaction of NO with enamines to produce carbon-bound diazeniumdiolates that may exist as either neutral molecules or zwitterions depending upon the structure of the carbonyl compound from which the enamine was derived.

Implications for NO Chemistry. As described earlier, it has been believed that pure NO does not react with mono-olefins and that nitrogen dioxide impurities are responsible for the previously observed radical reactions of alkenes with "NO". We are certain that traces of NO_2 are present in our reactions as well, and yet the electrophilic reaction prevails. This may have profound implications for interpreting the fate of NO produced in physiological processes. Despite the recent observations by Park and Walton[4f] that pure NO does not react with enones or enol ethers, studies in this area are still rather scarce. One fact that is certain is that O_2 is present in most of the environments, whether atmospheric or biological, where NO chemistry occurs, and the present study suggests the need to consider both ionic and radical chemistry in developing an understanding of these systems.

NO-Donor Properties of the Diazeniumdiolated Enamines. Unlike the *N*-diazeniumdiolates,[10b] these *C*-diazeniumdiolates are relatively stable to thermal decomposition and do not release copious volumes of NO upon addition to concentrated mineral acids. However, on dissolution of **5** in aqueous buffer at pH 7.4 and 37 °C, a slow release of NO could be detected by the chemiluminescence method used previously.[25] While the release of NO did not occur via a truly first-order process, rendering the assignment of a half-life meaningless, this compound produced a measurable NO flux for 170 h during which time the formation of approximately 7% of the theoretical quantity was observed. Similar results were obtained using citrate buffer at pH 3.0 with NO release occurring over 50 h. Zwitterionic enamine diazeniumdiolates **8**−**14** did not produce either NO or nitrous oxide (N_2O) spontaneously in aqueous solution at room temperature.

These results may be combined with the accumulating literature to begin to define the structural characteristics required to give a *C*-diazeniumdiolate spontaneous NO-releasing ability. It has recently been shown that adamantane-2-diazeniumdiolate can be forced (by heating in aqueous acid) to release N_2O and that this release occurs via protonation of the nitrosohydroxylamine resonance form (**15**).[26] In contrast, the NO-releasing *N*-



15 **16** **17**

diazeniumdiolates are known to be best represented by resonance form **16**. While there certainly are compounds whose solid-state X-ray crystal structures resemble **16** that do not release NO spontaneously (Cupferron, X = Ph, is one example) and the mechanism of NO release from **16** is not yet fully understood, the literature to date suggests that if **15** is favored over **16** dissociation to NO is inhibited. We note that both enamine diazeniumdiolate **5** (in one tautomeric form) and the previously known NO donor *N*-(*p*-methoxybenzyl)-*N*-nitrosohydroxyguanidine[18] exhibit the conjugated double-bond system shown in **17** while zwitterions **8**−**14** do not, and that this system is also the proposed NO-releasing intermediate arising from the dihydrodiazete 1,2-dioxides.[27] This suggests that future efforts to produce spontaneous NO releasers should be directed toward this type of system.

One explanation for the low total recovery of NO from the diazeniumdiolated enamines may be that the slow

(24) Longhi, R.; Ragsdale, R. O.; Drago, R. S. *Inorg. Chem*. **1962**, *1*, 768−770.

(25) Maragos, C. M.; Morley, D.; Wink, D. A.; Dunams, T. M.; Saavedra, J. E.; Hoffman, A.; Bove, A. A.; Isaac, L.; Hrabie, J. A.; Keefer, L. K. *J. Med. Chem*. **1991**, *34*, 3242−3247.

(26) Haider, J.; Hill, M. N. S.; Menneer, I. D.; Maskill, H.; Smith, J. G. *Chem. Commun*. **1997**, 1571−1572.

(27) Kirilyuk, I. A.; Utepbergenov, D. I.; Mazhukin, D. G.; Fechner, K.; Mertsch, K.; Khramtsov, V. V.; Blasig, I. E.; Haseloff, R. F. *J. Med. Chem*. **1998**, *41*, 1027−1033.

rate of release enables hydrolysis to the carbonyl compound and amine to compete favorably with dissociation to produce NO and enamine. The previously described observation that iminium tautomer **6**, rather than the tautomer containing the bonding shown in **17**, is preferred in aqueous solution strongly supports this interpretation. This hydrolysis would yield an α-diazeniumdiolated carbonyl compound of the type previously prepared by Traube[13] which appear to be less inclined to release NO spontaneously. Additionally, while extensive studies of the mechanism of NO release from *C*-diazeniumdiolates are currently underway, there is no reason to assume that they will produce 2 mol of NO per mol of compound just because the N-based compounds do so. In fact, the dihydrodiazete 1,2-dioxides produce only 1 mole of NO, being converted into oximes in the process.[27]

Potential Utility of Diazeniumdiolated Enamines. Although future synthetic and/or mechanistic studies may provide a means of overcoming the problem of low NO release, this may not unduly limit the potential pharmacological uses of these compounds. For example, a synergistic effect of NO administered in concert with several antimicrobial azoles has been observed,[28] and many physiological malfunctions can benefit from the simultaneous application of several corrective agents. Enamines have been proposed as a way to link two existing pharmaceuticals since hydrolysis can regenerate both active compounds.[29] Reaction of an enamine with NO to impart NO-donor properties while retaining the ability to regenerate its parent component molecules may prove a useful way to achieve a triple-action pharmaceutical. Alternatively, one of the halves of the enamine can be a targeting agent designed to guide the diazeniumdiolated enamine to a specific site in vivo. In these cases some loss of NO-releasing efficiency may be acceptable since the benefit is the concentration of the NO which is released at one specific site of action.

The fact that certain C-bound diazeniumdiolates have been shown to generate NO as a result of enzymatic oxidation[17,30] may also render these compounds useful pharmacologically as will the guarantee that the parent diazeniumdiolates cannot themselves be oxidized to highly carcinogenic *N*-nitroso compounds, a problem which may be associated with the decomposition of the N-bound diazeniumdiolates. Although their biological activities have not (yet?) been directly linked to NO release, the marine sponge metabolite poecillanosine[31] as well as synthetically produced 6-(*N*-nitrosohydroxylamino)purine[32] contain *C*-diazeniumdiolates, and both inhibit the proliferation of mouse leukemia cells in vitro. The natural products nitrosofungin[33] and alanosine,[17a] also containing this functional group, exhibit antifungal activity. There is thus ample reason, from a medicinal chemistry perspective, to devise new syntheses.

From the organic chemists' viewpoint, the diazeniumdiolate functional group is of interest as a powerful metal chelator[34] and the O^2-methyl derivative was briefly studied for its ability to stabilize adjacent carbanions as a powerful electron-withdrawing group.[35]

Conclusion. The reaction of NO with a wide variety of enamines produces C-bound diazeniumdiolates, some of which may serve as slow-release NO donors at physiological pH. In contrast to the Michael reaction of electrophilic olefins with enamines, which works best with the reactive enamines derived from pyrrolidine,[36] this reaction of NO with enamines works best when they are less reactive morpholine derivatives. While many methods have been developed for the conversion of amine-, thiol-, and hydroxyl-containing molecules into NO donors,[37] this appears to be a potential method for achieving this conversion for carbonyl-containing biomolecules and pharmaceuticals. This is one of a very few known electrophilic reactions of NO with neutral organic molecules.

The development of new methods such as this for the introduction of the somewhat orphaned diazeniumdiolate functional group into an ever-increasing variety of organic compounds should encourage further research into their chemical and biological properties.

Experimental Section

Caution! While no detonations have been observed, several NO derivatives of enamines which reacted at more than one site have spontaneously, and without warning, decomposed vigorously during reaction workup to generate large volumes of gas very rapidly. Newly prepared diazeniumdiolated enamines should never be placed in closed containers until after their stability has been verified.

General Information. The apparatus used for conducting reactions with oxygen-free NO has been described previously.[10b] Nitric oxide was obtained from Matheson Gas Products (Montgomeryville, PA) and used as received. Enamines **1**−**4** as well as all reaction solvents (anhydrous) were purchased from Aldrich Chemical Company (Milwaukee, WI). Unless otherwise noted, the NMR spectra of all compounds were recorded in D_2O (^1H at 200 MHz; ^{13}C at 50 MHz). Ultraviolet data were obtained in phosphate-buffered saline at pH 7.4 and room temperature unless reported otherwise. Chemiluminescence measurements of nitric oxide were performed with a Thermal Energy Analyzer model 502A or 610 (Thermedics, Inc., Woburn, MA), and possible nitrous oxide emissions were tested by GC. Melting points were obtained on a hot stage and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc. (Norcross, GA).

Enamine Preparation. All enamines derived from isobutyraldehyde were prepared by the azeotropic removal of water as described by Benzing.[38] Enamines derived from other aldehydes are most conveniently prepared via chemical dehydration.[39] The compounds were distilled in vacuo prior to use and exhibited ^1H NMR spectra representative of their structures.

General Procedure for the Preparation of Diazeniumdiolated Enamines. A solution of the enamine in the indicated solvent was placed in a standard Parr hydrogenation bottle. Nitrogen or argon was passed through the apparatus and bubbled through the solution for 5−10 min, the bottle was

(28) McElhaney-Feser, G. E.; Raulli, R. E.; Cihlar, R. L. *Antimicrob. Agents Chemother*. **1998**, *42*, 2342−2346.
(29) Bundgaard, H. *Methods Enzymol*. **1985**, *112*, 347−359.
(30) Kubrina, L. N.; Yakabovich, L. M.; Vanin, A. F. *Izv. Akad. Nauk SSSR, Ser. Biol*. **1988**, *6*, 844−850.
(31) Natori, T.; Kataoka, Y.; Kato, S.; Kawai, H.; Fusetani, N. *Tetrahedron Lett*. **1997**, *38*, 8349−8350.
(32) Giner-Sorolla, A.; Longley-Cook, J.; McCravey, M.; Brown, G. B.; Burchenal, J. H. *J. Med. Chem*. **1973**, *16*, 365−369.
(33) Dolak, L. A.; Castle, T. M.; Hannon, B. R.; Argoudelis, A. D.; Reusser, F. *J. Antibiot*. **1983**, *36*, 1425−1430.
(34) Middleton, A. R.; Wilkinson, G. *J. Chem. Soc., Dalton Trans*. **1980**, 1888−1892.
(35) Woodward, R. B.; Wintner, C. *Tetrahedron Lett*. **1969**, 2689−2692.
(36) Hickmott, P. W.; Miles, G. J.; Sheppard, G.; Urbani, R.; Yoxall, C. T. *J. Chem. Soc., Perkin Trans. 1* **1973**, 1514−1519.
(37) Feelisch, M.; Stamler, J. S. *Methods in Nitric Oxide Research*; Wiley: New York, 1996; pp 71−115.
(38) Benzing, E. *Angew. Chem*. **1959**, *71*, 521.
(39) Fisher, G. B.; Lee, L.; Klettke, F. W. *Synth. Commun*. **1994**, *24*, 1541−1546.

clamped, and the headspace was further deoxygenated by 20−25 repeat cycles of inert gas pressurization to 5 atm followed by venting. For reactions requiring cooling, the ice bath was placed around the pressure bottle at this point, and if called for, magnetic stirring was begun. After 5 min, NO gas was admitted to a pressure of 5 atm. At the end of the indicated reaction period, excess NO was vented and the inert gas was bubbled through the resulting slurry or solution (depending upon the solubility of the products) for 5 min. The products were then isolated as described in the individual sections. All are white crystalline solids unless noted.

Reaction of 4-(1-Cyclohexen-1-yl)morpholine with NO to Produce 5. A solution of 4-(1-cyclohexen-1-yl)morpholine (15.0 g, 89.7 mmol) in 150 mL of ethyl ether was cooled in dry ice and left unstirred during reaction with NO as described above for 20 h and then warmed to room temperature. The product was isolated by filtration, washed with ethyl ether, and dried in vacuo. The large clear crystals of **5** were directly suitable for later X-ray crystallographic analysis as described below. For **5**: yield 8.14 g (40%); mp 85−87 °C; ^{1}H NMR (CD_3CN) δ 1.5−2.3 (6H, m), 2.44−2.55 (4H, m), 2.85−2.96 (4H, m), 5.13−5.18 (1H, m), 5.23−5.27 (1H, t), 11.6 (1H, br s); ^{13}C NMR (CD_3CN) δ 19.2, 24.7, 28.6, 50.6 (2C), 67.1, 67.5 (2C), 112.5, 141.3; when a sample of **5** was dissolved in D_2O it showed ^{1}H NMR (D_2O) δ 1.4−2.2 (6H, m), 2.1−2.6 (2H, m), 3.2−3.3 (4H, m), 3.9−4.0 (4H, m) 5.1−5.2 (1H, m); UV λ_{max} (ϵ) 224 nm (sh, 9.5 mM^{-1} cm^{-1}), 244 nm (10.9 mM^{-1} cm^{-1}); exact mass calcd for $C_{10}H_{17}N_3O_3$ (M^+) 227.1269, found 227.1254.

Anal. Calcd for $C_{10}H_{17}N_3O_3$: C, 52.85; H, 7.54; N, 18.49. Found: C, 53.32; H, 7.63; N, 18.76.

Reaction of 4-(Cyclohexylidenemethyl)morpholine with NO to Produce 8. A solution of 4-(cyclohexylidenemethyl)morpholine (**7**, 10.0 g, 55.2 mmol) in 200 mL of CH_3CN was cooled to 0 °C in an ice bath and reacted without stirring with NO as described above for 6 h and then warmed to room temperature. The product was isolated by filtration, washed with CH_3CN and then ether, and dried in vacuo. For **8**: yield 7.13 g (54%); mp 115−117 °C; ^{1}H NMR δ 1.25−1.40 (2H, m), 1.48−1.70 (4H, m), 1.95−2.40 (4H, m), 3.20−3.26 (4H, m), 3.90−3.96 (4H, m), 5.05 (1H, s); ^{13}C NMR δ 24.1 (2C), 27.8, 31.3 (2C), 46.3 (2C), 67.1 (2C), 78.0, 95.7; UV λ_{max} (ϵ) 246 nm (7.0 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{11}H_{19}N_3O_3$: C, 54.76; H, 7.94; N, 17.41. Found: C, 54.93; H, 8.04; N, 17.60.

Reaction of 4-(2-Methyl-1-propenyl)morpholine with NO to Produce 9. A solution of 4-(2-methyl-1-propenyl)-morpholine (7.00 g, 49.6 mmol) in 100 mL of THF was cooled in dry ice, stirred, and reacted with NO as described above for 22 h and then warmed to room temperature. The product was isolated as an amorphous powder by filtering, washing with ether, and drying in vacuo. For **9**: yield 4.05 g (41%); mp 91−92 °C; ^{1}H NMR δ 1.48 (6H, s), 3.25−3.31 (4H, m), 3.92−3.98 (4H, m), 5.26 (1H, s); ^{13}C NMR δ 23.2 (2C), 46.1 (2C), 66.6 (2C), 75.7, 95.2; UV λ_{max} (ϵ) 244 nm (7.6 mM^{-1} cm^{-1}); exact mass calcd for $C_8H_{16}N_3O_3$ (MH^+) 202.1192, found 202.1137.

Anal. Calcd for $C_8H_{15}N_3O_3$: C, 47.75; H. 7.51; N, 20.88. Found: C, 47.74; H, 7.70; N, 20.13.

Reaction of 1-(2-Methyl-1-propenyl)piperidine with NO to Produce 10. A solution of 1-(2-methyl-1-propenyl)-piperidine (5.0 g, 35.9 mmol) in 150 mL of CH_3CN was stirred at room temperature and reacted with NO for 23 h as described above. The product was isolated as an amorphous powder by filtering, washing with ether, and drying in vacuo. For **10**: yield 3.25 g (45%); mp 84−85 °C; ^{1}H NMR δ 1.48 (6H, s), 1.66−1.83 (6H, m), 3.13−3.18 (4H, m), 5.25 (1H, s); ^{13}C NMR δ 23.2 (2C), 24.3, 25.1 (2C), 47.4 (2C), 75.5, 95.2; UV λ_{max} (ϵ) 246 nm (7.2 mM^{-1} cm^{-1}).

Anal. Calcd for $C_9H_{17}N_3O_2$: C, 54.25; H, 8.60; N, 21.09. Found: C, 54.69; H, 8.56; N, 21.28.

Reaction of 1-(2-Methyl-1-propenyl)pyrrolidine with NO to Produce 11. A solution of 1-(2-methyl-1-propenyl)-pyrrolidine (10.0 g, 79.9 mmol) in 200 mL of CH_3CN was cooled to 0 °C in an ice bath and reacted without stirring with NO as described above for 6 h and then warmed to room temperature. The product was isolated by filtration, washed with CH_3CN and then ether, and dried in vacuo. For **11**: yield 8.88 g (60%); mp 75−76 °C; ^{1}H NMR δ 1.48 (6H, s), 1.98−2.03 (4H, m), 3.23−3.32 (4H, m), 5.25 (1H, s); ^{13}C NMR δ 23.2 (2C), 26.5 (2C), 48.3 (2C), 75.6, 95.2; UV λ_{max} (ϵ) 246 nm (7.6 mM^{-1} cm^{-1}).

Anal. Calcd for $C_8H_{15}N_3O_2$: C, 51.88; H, 8.16; N, 22.69. Found: C, 51.97; H, 8.20; N, 22.76.

Reaction of *N*-Methyl-*N*-(2-methyl-1-propenyl)aniline with NO to Produce 12. A solution of *N*-methyl-*N*-(2-methyl-1-propenyl)aniline (5.0 g, 31.0 mmol) in 150 mL of CH_3CN was stirred at room temperature and reacted with NO for 20 h as described above. The resulting pale yellow solution was concentrated to dryness on a rotary evaporator, and the residual solid was recrystallized from absolute ethanol to yield 2.26 g (33%) of **12** as pale cream colored needles: mp 83−84 °C; ^{1}H NMR ($CDCl_3$) δ 1.59 (3H, s), 1.63 (3H, s), 2.75 (3H, s), 6.00 (1H, s), 6.96−7.37 (5H, m); ^{13}C NMR ($CDCl_3$) δ 17.4, 26.8, 34.4, 75.6, 101.1, 118.9 (2C), 122.7, 129.4 (2C), 149.3; UV λ_{max} (CH_3CN, ϵ) 244 nm (12.3 mM^{-1} cm^{-1}), 275 nm (sh, 7.2 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{11}H_{15}N_3O_2$: C, 59.71; H, 6.83; N, 18.99. Found: C, 59.77; H, 6.84; N, 19.01.

Reaction of *N*,4-Dimethyl-*N*-(2-methyl-1-propenyl)-aniline with NO to Produce 13. A solution of *N*,4-dimethyl-*N*-(2-methyl-1-propenyl)aniline (5.0 g, 28.5 mmol) in 150 mL of CH_3CN was stirred at room temperature and reacted with NO for 20 h as described above. The resulting pale yellow-orange solution was concentrated to dryness on a rotary evaporator, and the residual off-white solid was recrystallized from absolute ethanol to yield 2.21 g (33%) of **13** as white cotton-like needles: mp 127−128 °C; ^{1}H NMR ($CDCl_3$) δ 1.58 (3H, s), 1.61 (3H, s), 2.31 (3H, s), 2.71 (3H, s), 5.92 (1H, s), 6.90−7.15 (4H, m); ^{13}C NMR ($CDCl_3$) δ 17.3, 20.5, 26.8, 34.9, 75.4, 101.9, 119.7 (2C), 129.9 (2C), 132.7, 147.2; UV λ_{max} (CH_3CN, ϵ) 244 nm (12.9 mM^{-1} cm^{-1}), 275 nm (sh, 6.2 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{12}H_{17}N_3O_2$: C, 61.26; H, 7.28; N, 17.86. Found: C, 61.32; H, 7.35; N, 17.88.

Reaction of 4-Methoxy-*N*-methyl-*N*-(2-methyl-1-propenyl)aniline with NO to Produce 14. A solution of 4-methoxy-*N*-methyl-*N*-(2-methyl-1-propenyl)aniline (5.0 g, 26.1 mmol) in 150 mL of CH_3CN was stirred at room temperature and reacted with NO for 23 h as described above. The resulting pale brown solution was concentrated to dryness on a rotary evaporator, and the residual oil was crystallized from absolute ethanol to yield 4.89 g (75%) of **14** as colorless chunky crystals: mp 97−98 °C; ^{1}H NMR ($CDCl_3$) δ 1.58 (3H, s), 1.60 (3H, s), 2.67 (3H, s), 3.79 (3H, s), 5.80 (1H, s), 6.84−7.06 (4H, m); ^{13}C NMR ($CDCl_3$) δ 17.2, 26.8, 36.1, 55.5, 75.2, 103.0, 114.6 (2C), 122.8 (2C), 143.4, 156.3; UV λ_{max} (CH_3CN, ϵ) 242 nm (11.8 mM^{-1} cm^{-1}), 280 nm (sh, 6.7 mM^{-1} cm^{-1}).

Anal. Calcd for $C_{12}H_{17}N_3O_3$: C, 57.35; H, 6.82; N, 16.72. Found: C, 57.36; H, 6.87; N, 16.75.

Single-Crystal X-ray Diffraction Analysis of 5. The compound crystallizes in the monoclinic space group $P2_1/n$, with unit cell dimensions $a = 6.496(1)$, $b = 22.214(4)$, and $c = 8.218(1)$ Å, $\beta = 105.66(1)°$, $V = 1141.9(3)$ Å^3, $Z = 4$, $\varrho_{calcd} = 1.322$ mg mm^{-3}, and $T = 223$ °K. Data collection and structure refinement are described in the Supporting Information.

NO-Release Studies. NO release from **5** was measured by a chemiluminescence method described previously.[11] Briefly, solutions of **5** in the desired buffer were incubated continuously at 37 °C except for short intervals during which their contents were swept with inert gas into a chemiluminescence detector for quantification of NO. After a steady baseline was achieved, integration over several minutes provided the NO generation rate during that interval. Plots of these rates versus the times at the midpoints of the corresponding intervals gave a curve from which the yield of NO could be determined by integration with extrapolation to infinite time.[11]

Acknowledgment. We thank Mr. Stephen Fox for N_2O analyses, Mr. John Roman for HRMS data, and Mrs. Sandra Walker for help in the preparation of this manuscript. This project was funded in part by the

National Cancer Institute under Contract NO1-CO-56000 and in part by the Office of Naval Research.

Supporting Information Available: A description of the X-ray crystal structure determination for compound **5** including tables of crystal data, atomic coordinates, bond lengths and angles, and anisotropic thermal parameters. This material is available free of charge via the Internet at http://pubs.acs.org.

JO000600G