Nitric Oxide-Releasing Fabrics and Other Acrylonitrile-Based Diazeniumdiolates

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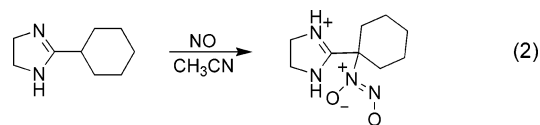Received December 4, 2006; E-mail: hrabie@ncifcrf.gov

Nitric oxide (NO) is a highly reactive diatomic molecule with so many important physiological functions and such a wide spectrum of activity that prodrugs acting via production of NO must be carefully targeted either to specific metabolic processes or to specific physical locations within a living organism.[1] Thus, the development of NO-releasing polymers is of considerable current interest.[1,2] We have previously shown that the reaction of NO with carbanions generated via deprotonation of nitriles can be used to produce a variety of small molecules containing the NO-releasing diazeniumdiolate (represented as $-N(O)=N-O^-$ or $N_2O_2^-$) functional group.[3,4] We have now been able to prepare NO-releasing diazeniumdiolated derivatives of poly(acrylonitrile) (PAN) and a variety of related materials that can form the basis of NO-releasing textiles, rubbers, and plastics.

While, to our knowledge, the acidity of isobutyronitrile has never been measured in solution, its gas-phase acidity has been shown to be substantially below that of acetonitrile.[5] This is reflected in its very low rate of deprotonation,[6] as well as the fact that we have never been able to perform a successful reaction between its anion and nitric oxide (NO). Accordingly, PAN might be expected to be a poor candidate for reaction with NO under basic conditions were it not for the tendency of the 1,3-oriented cyano groups to undergo intramolecular reaction.[7] These reactions, which are both thermal and base-initiated, have been investigated extensively because they are responsible for the discoloration and general degradation of acrylic fabric.[8] They lead to the production of short sections of condensed rings (as shown by **1**) which have been viewed as conjugated imines, but which may also be considered to be amidines that can exist in an enediamine tautomeric form (shown by **2**).
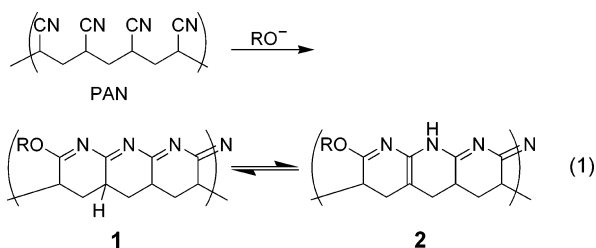
The protons α to the cyano groups of PAN are thus far more easily removed than would at first be anticipated. Additionally, recent results have shown that amidines can react directly with NO via their enediamine tautomers (without the need for additional base) to produce carbon-bound diazeniumdiolates (eq 2).[9]
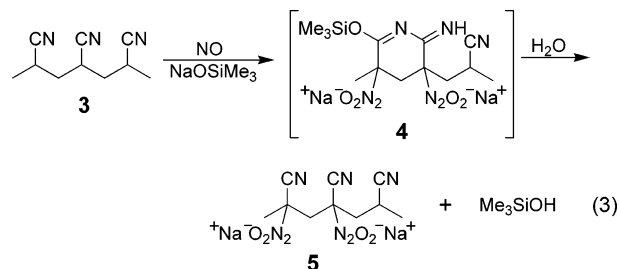
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Treatment of PAN powder with NO gas in the presence of nucleophilic bases such as sodium methoxide or sodium trimethylsilanoate resulted in the production of a tan powder (PAN/NO) which released NO for more than 80 days when suspended in pH 7.4 phosphate buffer at 37 °C. Even after this time, a chemilumi-

nescence signal could be detected upon treatment of a stream of nitrogen that had been passed through this suspension with ozone in a nitric oxide analyzer. Reaction of a clear film of PAN suspended in a sodium methoxide solution in methanol with NO gas resulted in the production of diazeniumdiolate functional groups bound to carbon as evidenced by the observation of a previously absent absorption at 266 nm in the UV spectrum of this material. Long duration (>5 days) NO release was also detected from NO-treated PAN in the form of 100% acrylic yarn and as a hollow fiber removed from an Asahi PAN-03 hemofilter.

We have used this reaction to prepare NO-releasing derivatives from the most commercially significant PAN copolymers including the textile poly(propylene-*co*-acrylonitrile) (known as PROPAN), the thermoset resin poly(styrene-*co*-acrylonitrile) (known as SAN), and the rubber poly(butadiene-*co*-acrylonitrile) (known as BUNA-N or "nitrile rubber"). The NO-release characteristics of these materials are shown in Table 1.

We have explored the mechanism of this reaction using 2,4,6-tricyanoheptane (**3**)[10] as a model compound. Reaction of **3** with NO in the presence of sodium trimethylsilanoate produced a material that, consistent with previous observations of base-treated PAN,[8] proved to be extremely insoluble in any solvent except water, in which it was converted to 2,4-bisdiazeniumdiolated-2,4,6-tricyanoheptane (**5**). While **3** is prepared as a mixture of three

isomers which have been separated and used in the study of the tacticity of PAN,[11] the need to use strongly basic conditions in the NO reaction could possibly lead to epimerization, so we did not

Table 1. NO Release Data for Diazeniumdiolated Acrylonitrile-Based Materials[a]

material	% acrylonitrile	total NO release (mol/mg)[b]	duration of NO release (days)[b]
PAN/NO	100	1.5×10^{-7}	>80
SAN/NO	25	7.2×10^{-9}	>7
PROPAN/NO	73	3.9×10^{-8}	70
BUNA-N/NO	33	2.3×10^{-8}	>7
5		6.6×10^{-6}	>200

[a] See the expanded discussion of this table in the Supporting Information. [b] Measured in pH 7.4 phosphate buffer held at 37 °C. For comparison, the endothelium generates ca. 4 pmol $NO/mm^2/min$.
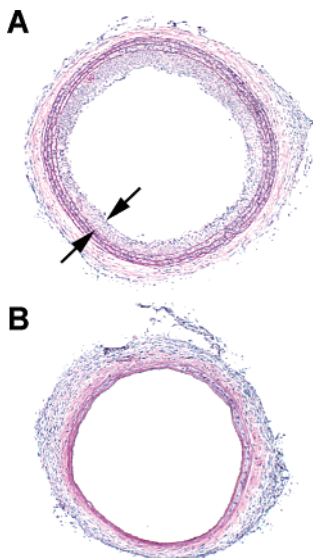

Figure 1. Hematoxylin- and eosin-stained cross sections of rat carotid arteries 14 days after injury with a balloon catheter without (A) and with (B) simultaneous periadventitial application of PAN/NO powder. Arrows point to neointimal hyperplasia seen in the untreated animals.

separate these isomers. The NMR spectra of **5** suggest that it is a single isomer, but its isolation in 64% yield does not permit us to draw any conclusions about the possible role of stereochemistry in this reaction. Compound **5** (PAN3/NO) is an extremely long-lived NO donor which continued to generate NO even after 200 days in pH 7.4 phosphate buffer at 37 °C, at which point it had released a total of approximately 2 mol NO/mol of compound. Quantitative measurement of NO release from this ultra-long-lived diazenium-diolate (and, indeed, from any of the polymers as well!) does not follow a first-order process and is subject to unavoidable errors because of interference by slow infiltration of oxygen, carbon dioxide, and even bacteria into the test solutions. The UV spectrum of **5** ($\lambda_{max} = 256$ nm; $\epsilon = 16.9$ mM^{-1} cm^{-1}) exhibits an absorption intensity double that of a typical monodiazeniumdiolate, and its composition is supported by both high-resolution mass spectral and elemental analysis data.

PAN and all of its copolymers of which diazeniumdiolated versions have been prepared are hydrophobic polymers, and the NO reactions are conducted in rigorously dried solvents. Accordingly, it is likely that the isolated diazeniumdiolated polymers contain intact condensed ring structures, as evidenced by the tan color of PAN/NO. SAN is a copolymer that has only a small content of acrylonitrile and thus cannot form the larger-sized conjugated rings upon reaction with NO, so it is gratifying to note that SAN/NO is colorless. Similarly, although the processes involved in the penetration of water into the polymer matrix (as is required to cause hydrolysis to NO) are complex,[12] it should be noted that both the total quantity and the duration of NO release shown in Table 1 show a direct correlation to the number of nitrile groups in the polymer backbone.

Many applications of this chemistry in vascular surgery and other medical procedures can be envisioned. To test for such utility, we subjected carotid arteries of rats to balloon angioplasty and studied the effect of simultaneously applying 20 mg of PAN/NO powder around the outside of the affected vessel. As illustrated in Figure 1, arterial cross-sections evaluated 14 days later showed abundant scarring in the untreated animals that was substantially reduced in those receiving PAN/NO treatment.[13] Other possible applications of these polymers include arterial stenting, bypass grafting, and surgical endarterectomy.

These diazeniumdiolated acrylonitrile-based polymers are stable at room temperature for many months and may thus represent a broad class of commercially viable materials to be developed as NO donors. PAN is easily spun into fibers for fabric production (and the dialysis membrane/hemofilter mentioned above) and can form graft copolymers with most of the other polymers of industry. SAN resin can be injection molded and extruded, presenting the possibility of manufacturing a wide variety of medical devices that require either antibacterial or antithrombotic activity.[14] Further exploration of the applications of these novel materials is underway.

Supporting Information Available: Preparations of all five materials listed in Table 1 as well as characterization data (NMR, UV, MS, elemental analysis), details of the NO-release studies, and a description of the rat carotid artery injury studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0686864