

Smectite-catalyzed conversion of 3-amino-4-hydroxyphenylarsonic acid to an azobenzene derivative

by

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Phenylarsonic acid derivatives are extensively used as feed supplements for broiler chickens. The most commonly used arsenic compound is roxarsone (3-nitro-4-hydroxyphenylarsonic acid). Roxarsone is fed to most broiler chickens raised in the United States to control coccidial intestinal parasites thereby improving weight gain per unit of feed (Muir, n.d.). Very little of the roxarsone is retained in the chicken meat (FDA limit is 0.5 ppm As in chicken muscle tissue); most of the roxarsone appears to be excreted unchanged (Morrison, 1969; Gregory, 1993). However, the degradation product, 3-amino-4-hydroxyphenylarsonic acid (3-amino-HPAA), has been detected in the urine of hens fed roxarsone (Moody and Williams, 1964).

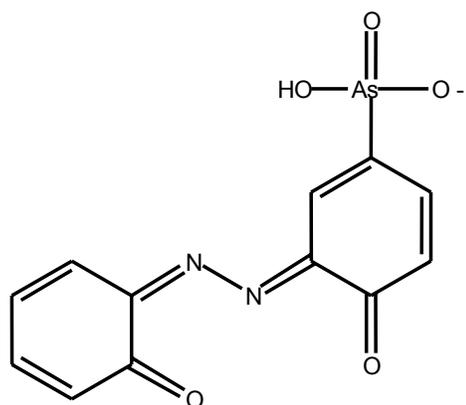
Nitro groups on substituted aromatic compounds typically undergo reduction to amino groups in soils. This reduction is illustrated by the reduction of trinitrotoluene (TNT) to its mono- and di-amino derivatives in soils and composts (Comfort and others, 1995); we expected that roxarsone would follow a similar degradation pathway in chicken litter to form 3-amino-HPAA. As part of our work on the environmental fate of roxarsone we measured the sorption of roxarsone and 3-amino-HPAA on soil minerals. During our sorptions studies we observed the formation of a red-colored product when smectite was added to 3-amino-HPAA solutions. The identity of this product and possible reaction mechanisms for its formation are the subject of this report.

Hauser and Leggett (1940) were the first to observe color-forming reactions between a smectite and various amines. They observed color formation both in dry systems and in the presence of water. A naturally-occurring Wyoming smectite was used in their study. It is not clear from this report if the colored products formed on the smectite surface or in solution, however, in subsequent published studies on the formation of colored products in presence of clay minerals the products generally have been detected sorbed on the clay-mineral surfaces (Ainsworth and others, 1991; Cloos and others, 1979; Fenn and others, 1973; Furukawa and Brindley, 1973; McBride, 1979; Moreale and others, 1985; Pillai and others, 1982; Tennakoon and others, 1974). In contrast, the pinkish-red product observed when SAz-1 smectite is added to solutions of 3-amino-HPAA developed in solution. The color was not detected immediately but took several hours to develop. The colored solutions were separated from the smectite and subjected to mass spectrometric analysis.

Solution samples were analyzed by flow injection analysis, using 25/75 water/methanol as the mobile phase, with positive- and negative-ion mode electrospray ionization/quadrupole-mass spectrometry (LC/ESI/MS), and liquid chromatography/

electrospray/ion-trap-tandem mass spectrometry (LC/ESI/MS/MS), in the positive- and negative-ion mode using acetonitrile and 10 mM ammonium formate buffer (50:50) as the mobile phase.

LC/ESI/MS spectra of the solution phase were measured at different times after addition of the smectite to a 3-amino-HPAA-smectite solution. These spectra show a peak at m/z (mass to charge ratio) of 335 that increases in relative intensity with time. This peak is consistent with the singly-ionized ketohydrazone shown in Figure 1. LC/ESI/MS/MS analysis provided additional evidence for this structure. Negative-ion fragmentation of the 335 m/z ions yielded arsenite ions ($m/z=123$) and ketohydrazone fragments. The ketohydrazone fragments were seen in both the positive- and negative-ion spectra. Kennedy and others (2000) have demonstrated by synchrotron radiation diffraction that the dominant tautomer of the solid azo pigment 4-(4-methyl-2-sulfophenyl)azo-3-hydroxy-2-naphthalic acid possesses a very similar structure. In addition, the infrared spectrum of the colored product was similar to published infrared spectra of known azobenzene compounds.



Mass: 335

Figure 1. Proposed chemical structure of 335 ion detected in mass spectra.

A possible reaction mechanism for formation of an azo compound from 3-amino-HPAA is shown in Figure 2. This reaction scheme involves oxidation of the amine group to a nitroso group. The first step in this reaction probably involves loss of two electrons and formation of a hydroxylamine; further loss of two electrons yields the nitroso (Corbett and Corbett, 1995). However, the redox reaction apparently is not enzymatically mediated as is often the case in the environment, but is brought about by reduction of Fe(III) in the smectite lattice (Stucki and others 1996) and by reduction of the As(V) to As(III). The nitroso can then condense with an unoxidized amine group to form the azo compound. Further oxidation of the azo to the ketohydrazone may take place in solution or in the source of the mass spectrometer. The detection of As(III) in freshly prepared reaction mixtures of 3-amino-HPAA and smectite provides further support for this reaction mechanism.

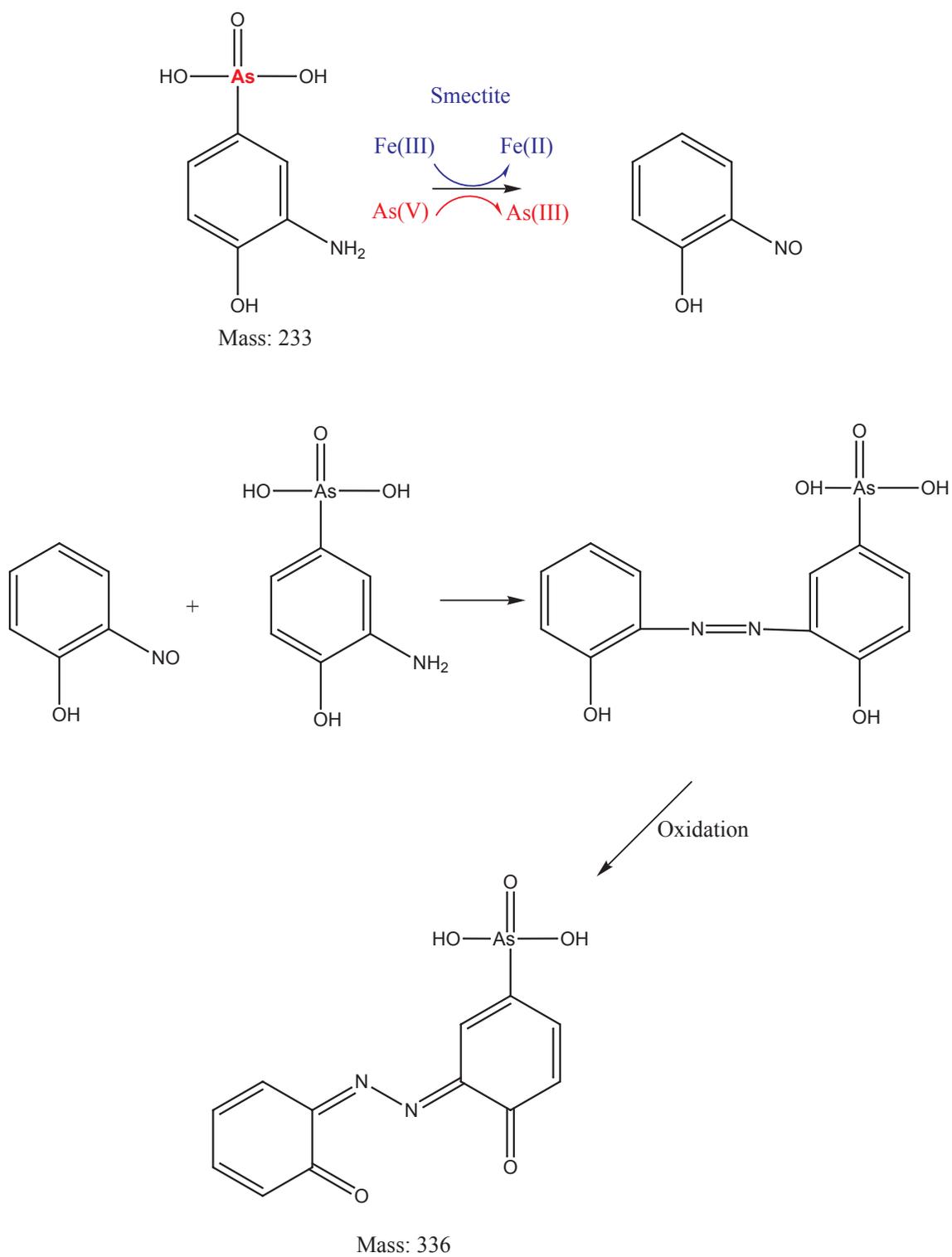


Figure 2. Proposed reaction mechanism for formation of the ketohydrazone product.

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