half-life of about 23 h in deionized water and about 6 h in deionized water containing 2% acetone as a triplet sensitizer. Photolysis, especially in the presence of natural sensitizers in lakes or ponds, may thus be an important factor influencing the dissipation of fluridone from aquatic environments.

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Identification of Nitrohexane in Corn Treated with Nitrous Acid

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Nitrohexane has been identified as a major product following deliberate nitrosation of corn. Identification was based on chromatographic and mass spectral data. This finding is discussed in relation to the high incidence of stomach cancer in a region of southern Colombia. We also show that nitroalkanes give a positive response in the thermal energy analyzer, a device which is used as a specific detector for *N*-nitroso compounds.

The occurrence of nitrosamines in nitrite-treated meat products has been widely investigated (Scanlan, 1975; Gough et al., 1977). Nitrosamines have been shown to form from secondary amines and nitrite in human gastric juice (Sander, 1967; Sen et al., 1969; Lane and Bailey, 1973) and in the stomachs of animals (Sander et al., 1968; Sen et al., 1969; Alam et al., 1971; Mysliwy et al., 1974). The possibility of gastric nitrosation has assumed an increased importance in view of recent findings of constant, and sometimes high, levels of nitrite in human saliva (Tannenbaum et al., 1974, 1976; Spiegelhalder et al., 1976). However, little work has been done on the determination of compounds that form by deliberate nitrosation of food material as an indication of the kinds of compounds that may form in the gastric environment.

We chose to initiate our research in this area by investigating the nitrosation of corn. There were two reasons for this choice. First, nitrosamine formation from foods of plant origin has not received much attention. Secondly, we are attempting to determine environmental factors related to the high incidence of stomach cancer in a region of southern Colombia (Correa et al., 1975; Cuello et al., 1976). We have already shown a higher average intake of nitrate in the population at risk compared to control populations (Cuello et al., 1976; Tannenbaum et al., 1979). Consumption of corn also has a positive association with risk for stomach cancer in this region (Haenszel et al., 1976).

In this study, corn samples obtained locally and from the high risk area in Colombia were reacted with nitrite under acidic conditions and we have determined the structure of a principal product.

EXPERIMENTAL SECTION

Materials. Yellow corn was obtained from a region of Nariño in Colombia which has a high incidence of gastric cancer. Whole yellow corn from a local (Cambridge, MA) natural foods store and an enriched, degerminated, commercial corn meal were used for comparison. All solvents were either pesticide grade (Mallinckrodt, St. Louis, MO) or high-pressure LC grade (Fisher, Pittsburgh, PA). 1-Nitrohexane was purchased from ICN (Plainsview, NY). Hexyl nitrite was synthesized from 1-hexanol and sodium nitrite (Vogel, 1948).

Analytical Methods. All gas chromatographic procedures used a 2 m × 2.1 mm (i.d.) stainless steel column packed with 3% OV-17 on Chromosorb G-HP (100–120 mesh), at 120 °C (100 °C for amine analysis). Gas chromatography–thermal energy analysis (GC–TEA) was performed as described by Fine and Rounbehler (1975). The TEA pyrolysis oven temperature was 350 °C and the trap was cooled with acetone/dry ice. GC–mass spectrometry was performed on a Hitachi-Perkin Elmer RMU-6 instrument; GC–chemical ionization mass spec-

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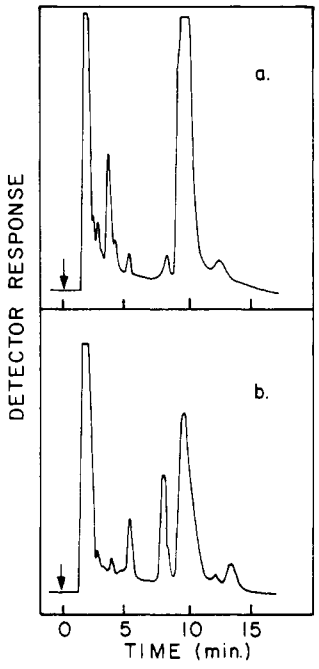

Figure 1. Gas chromatography using the TEA detector of the dichloromethane extract of the distillate following nitrosation of (a) Colombian yellow corn and (b) local corn.

trometry was performed on a Hewlett Packard 5980 quadrupole instrument using methane as the carrier/reactant gas. GC–high-resolution mass spectrometry was performed on an AEI-MS9 instrument at the Epply Institute for Cancer Research, Omaha, NB. High pressure liquid chromatography was performed using a 30 cm × 4 mm (i.d.) column packed with μ-Porasil (Waters Associates, Milford, MA); the eluant was dichloromethane/hexane in various proportions at a flow rate of 1 mL/min. The column effluent was monitored at 254 nm.

Analytical Sample. Whole corn was ground in a stainless steel hammer mill. Twenty-five grams of this material or the corn meal were slurried with 50 mL of aqueous 0.1 M sodium nitrite. The slurry was acidified to pH 3 with concentrated HCl and allowed to react for 24 h with occasional stirring. After adding 50 mL of mineral oil, the sample was distilled under vacuum, and volatile material was trapped in liquid nitrogen (Fine et al., 1975). Trap contents were extracted twice with 50-mL portions of dichloromethane. The dichloromethane fractions were then combined, dried with anhydrous magnesium sulfate, and concentrated to about 5 mL in a Kuderna-Danish evaporator. Heptane (1 mL) was added and the sample was further concentrated to 1 mL under a nitrogen stream. Local and Colombian corn yielded the gas chromatograms shown in Figure 1, and we decided to identify the major TEA-positive peak that was present in both samples.

Preparative Sample. In order to prepare enough material for analysis by mass spectrometry, 25 samples of yellow Colombian corn were prepared as described above except that the amounts of corn and water were doubled, and the nitrite concentration was increased to 1 M. This sample was subjected to clean-up procedures, during which the presence of the TEA-responsive compound of interest was monitored by GC–TEA.

The concentrated extracts from the 25 preparative samples were combined, then washed with equal volumes of distilled water, acid (saturated KCl adjusted to pH 2 with HCl), and base (20% K_2CO_3). The base wash removed much of the yellow color from the sample, so this was repeated twice more. The sample was then dried, reduced to 5 mL with nitrogen, and applied to a 1 × 20 cm column of silicic acid. The column was washed with hexane and eluted with dichloromethane. Analysis of the eluate by gas chromatography with a flame ionization detector (FID) showed that it contained multiple overlapping peaks in the region of the compound of interest and indicated the need for additional clean-up before analysis by GC–MS.

Further purification was achieved using high-pressure LC with a μ-Porasil column and dichloromethane/hexane (1:9) as eluant. Five-milliliter fractions were collected and, using GC–TEA, the 20–25-mL fraction was shown to contain only the compound of interest. Using this high-pressure LC system, the entire preparative sample was chromatographed in successive portions. The derived fractions were pooled and used for GC–MS analysis.

We performed a control reaction in which corn was treated exactly as described above except that no nitrite was added. Gas chromatography of the extract from this sample yielded no TEA-positive peaks. In another control, we nitrosated a sample of white Colombian corn and purified the extract as before. Although this sample gave a few small TEA-positive peaks upon analysis, it did not yield any of the material we chose to identify in the yellow corn samples.

Hexylamine Analysis. One-hundred grams of ground Colombian corn was combined with 200 mL of water and adjusted to pH 10 with 5 N NaOH. The sample was steam-distilled into a receiver containing 5 mL of 1 N HCl. Two-hundred milliliters of condensate was collected and then reduced to 10 mL on a rotary evaporator. This fraction was washed with two 10-mL portions of dichloromethane to remove acids and neutrals, and the aqueous phase was divided into two 5-mL portions. One of these was basified to pH 10 with NaOH and extracted with dichloromethane, and the organic phase was prepared for amine analysis by derivatization with trifluoroacetic anhydride. A sample of hexylamine was similarly derivatized. These samples were analyzed by GC. The other portion of the aqueous phase was mixed with 5 mL of 30 mM $NaNO_2$ and adjusted to pH 3. After reaction overnight, the sample was extracted with two 10-mL portions of dichloromethane. The dichloromethane fractions were combined, reduced to 0.1 mL with a stream of nitrogen, and analyzed by GC–TEA.

RESULTS

Typical chromatograms of the distillate from nitrosated corn are shown in Figure 1. The quantitative difference between Colombian and local corn was quite reproducible and may be caused by differences in varieties, growing conditions, or storage conditions. There was no difference between the local whole-grain corn and the commercial corn meal. We also noticed no differences in the ratio of peaks between the analytical and preparative samples of nitrosated corn.

Since the major peak in both Colombian and local corn following nitrosation appeared to be the same compound, we chose it as our first target for identification. Recovery of this compound was greater than 95% throughout the isolation and purification procedures.

Cleanup using a conventional silicic acid column followed by high-pressure LC on μ-Porasil enabled us to collect a fraction that contained the compound of interest in essentially pure form, as determined by GC–FID and GC–TEA. Figure 2 shows the liquid chromatogram of the eluate from the silicic acid column monitored by a UV detector at 254 nm. Figure 3 shows the GC–FID chro-

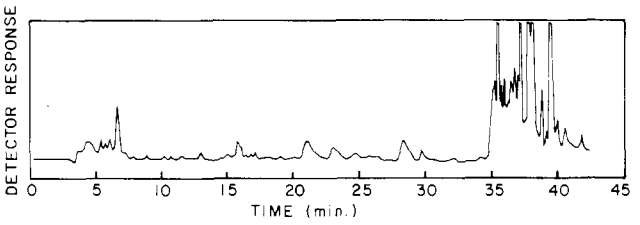

Figure 2. Liquid chromatogram of eluate from silicic acid column using UV detector at 254 nm. Flow rate was 1 mL/min. Eluant was changed from dichloromethane/hexane (1:9) to dichloromethane at 30 min.

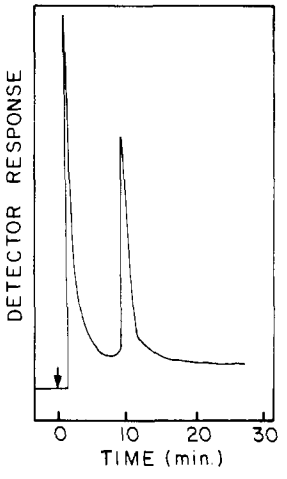

Figure 3. Gas chromatography using FID detector of the 20–25-mL fraction shown in Figure 2.

matogram of the derived fraction. It is clear that this fraction contained no contaminating peaks. Analysis by GC–TEA yielded a substantially identical chromatogram.

GC–MS of the purified sample gave the spectrum shown in Figure 4. The highest mass peak observed (m/e 85) and most of the other peaks appeared to be due to hydrocarbon fragmentation (McLafferty, 1973). The peak at m/e 30 could have been due to NO^+, a common fragment of nitro and nitroso compounds. On the basis of this information, however, a structure assignment could not be made and we, therefore, examined the chemical ionization mass spectrum and high-resolution mass spectrum of the compound.

The chemical ionization spectrum had a base peak of m/e 85, the same as the highest mass peak seen by electron impact mass spectrometry. Higher mass peaks were seen at m/e 132 and 172, indicating a parent mass of m/e 131. Chemical ionization mass spectra with methane as the reactant gas typically give peaks at M + 1 ($M + H^+$) and M + 41 ($M + C_3H_5^+$) (Arsenault, 1972). High-resolution mass spectrometry identified the electron impact fragment at m/e 85 as $C_6H_{13}^+$, at m/e 30 as NO^+, and most of the other peaks as hydrocarbon fragments. Two low intensity peaks not shown in Figure 4 were identified as $C_3H_6NO_2^+$ and $C_4H_8NO_2^+$.

Taken together, these data suggest a structure in which a C_6H_{13} alkyl group (mol wt 85) is attached to an NO_2 group (mol wt 46). The two possibilities for this combination are hexyl nitrite and nitrohexane. There are no published spectra for these compounds and the mass spectra of lower homologues were not helpful in distinguishing between them. We therefore obtained authentic samples of the two compounds and compared their chromatographic characteristics and mass spectra with the unknown. The GC retention time for nitrohexane was 9.7 min, which was identical with that of the unknown

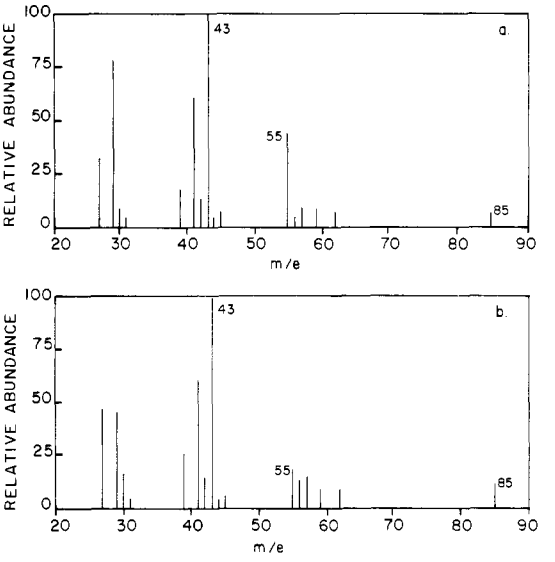

Figure 4. Mass spectrum of (a) purified extract from nitrosated corn and (b) 1-nitrohexane.

compound. Under the same conditions, hexyl nitrite eluted at the solvent front. Figure 4 illustrates that the electron impact mass spectrum of nitrohexane, obtained by direct sample introduction, was almost identical with the compound isolated from nitrosated corn. The mass spectrum of hexyl nitrite showed a completely different fragmentation pattern. The high-pressure LC retention times for nitrohexane and the unknown were also identified, as were their molar responses in the TEA. The latter were determined by comparing GC–TEA and GC–FID peak areas of the unknown and nitrohexane standard with *N*-nitrosodipropylamine. Nitrohexane and the unknown gave a 2% molar response, while hexyl nitrite and the nitrosamine gave a 100% molar response. Neither nitrohexane nor the unknown was destroyed by long-wavelength UV irradiation; alkyl nitrites and nitrosamines are destroyed by this procedure. UV irradiation has been suggested by Doerr and Fiddler (1977) to distinguish *N*-nitroso compounds from other TEA-positive compounds. We calculated that 25 g of corn nitrosated as described for the analytical sample yielded 125–250 μg (equivalent to 5–10 mg/kg) of nitrohexane.

Analysis of amines in the steam distillate of a Colombian corn sample indicated that no hexylamine was present (detection limit 0.2 mg/kg). Palamand et al. (1969) also found no hexylamine in corn. When the basic fraction of the steam distillate from the corn sample was nitrosated, nitrohexane was formed at a level of about 1% of that which would have been formed by direct nitrosation of an equivalent amount of whole corn.

DISCUSSION

We have shown that a major product formed following the deliberate nitrosation of corn is nitrohexane. The mass spectrum of the corn product was virtually identical with 1-nitrohexane although we have not ruled out formation of other isomers. Since nitrohexane formation was shown to be dependent on the presence of nitrite, we postulated that it might form in corn by nitrosation of hexylamine. The hexyl diazonium ion thus formed could react with another molecule of nitrite to yield the nitro compound. Nitrite salts have been shown to form nitroalkanes by nucleophilic attack on alkyl halides in nonaqueous systems (Vogel, 1948). The postulated reaction, however, does not occur in corn since we found no evidence for the presence of hexylamine. A small amount of nitrohexane was formed

by nitrosation of the steam distillate from whole corn, but the origin of the compound in nitrosated corn remains obscure.

The toxicology of nitroalkanes has not been extensively studied, but nitroalkanes are strong irritants, particularly for the upper respiratory tract and the gastrointestinal tract (Machle et al., 1940). Conjugated nitro olefins are stronger irritants than nitroalkanes and at least one nitro olefin, 3-nitro-3-hexene, is carcinogenic (Deichmann et al., 1965). We found nitrohexane in both local and Colombian corn that had been nitrosated. People in the area of high risk for stomach cancer in Columbia, however, are exposed to higher levels of nitrate and nitrite and, hence, potentially to higher levels of nitrosation products than people from low risk areas (Cuello et al., 1976; Tannenbaum et al., 1979).

Nitrite levels up to 3.6 mM have been found in gastric juice samples from individuals in the high risk area (Tannenbaum et al., 1979). We are currently investigating the nitrosation of corn and other food products at similar nitrite concentrations.

Finally, we have shown that nitroalkanes represent a new class of compounds that may be present in certain foods or form in the gastric environment in the presence of nitrite and which give a positive TEA response. Nitroalkanes may consequently interfere in the analysis of *N*-nitrosamines. However, since the molar response of nitrohexane is low, our sample containing 5 mg/kg of nitrohexane would give approximately the same size peak as a sample containing 0.1 mg/kg of a nitrosamine with the same molecular weight. Use of an auxiliary method such as UV photolysis would distinguish the two classes of compounds. Work is under way to identify other products formed following deliberate nitrosation of foods.

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Determination of Volatile Nitrosamines in Crops and Soils Treated with Dinitroaniline Herbicides

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Volatile nitrosamines have been reported to be contaminants in several dinitroaniline herbicides. Since these nitrosamines are known to be carcinogenic in laboratory animals, it became necessary to analyze crops and soils treated with these herbicides for the presence of nitrosamine residues. In the procedures described, plant tissue was extracted with methanol, and soil was extracted with methanol/water (3:1). Sample extracts were purified by liquid–liquid extraction and alumina column chromatography. Measurement was accomplished by means of a gas chromatograph–thermal energy analyzer. The sensitivity of the methods was 0.2, 0.05, and 0.01 ppb for *N*-nitrosodi-*n*-propylamine in crops, soil, and water, respectively. No detectable nitrosamine residues were observed in any crops treated with the herbicides trifluralin, benefin, or oryzalin.

The development of the thermal energy analyzer as a sensitive and selective detector for *N*-nitroso compounds (Fine et al., 1973) led to the discovery that certain pesticide products contained trace quantities of volatile nitrosamines (Fine et al., 1976; Ross et al., 1977). Among these was the herbicide Treflan, a registered trademark of Elanco Products Co., Division of Eli Lilly and Co., for the her-

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