model reaction. Cucullu et al. (1976) report not only 10% remaining aflatoxin B_1, but 10% conversion to aflatoxin D_1 in the reaction of pure B_1 with ammonium hydroxide. Results reported in Table IV, obtained on meal spiked with aflatoxin B_1 and ammoniated in the 450-mL Parr reactor, closely paralleled those obtained on cultured meals ammoniated in the 450-mL Parr bomb (Tables II and III). These results indicate that meal, rather than mold constituents, influence both decrease of aflatoxin B_1 and formation of aflatoxin D_1.

The average conversion of aflatoxin B_1 to D_1 was 0.36% for cottonseed meals. Although the measured amount of D_1 formed from B_1 varies considerably within a given set of conditions, when data are considered on the basis of the amount of toxin in the original meal (approximately 1000 μg of B_1/g), the final content of both B_1 and D_1 are similar. Even more pertinent is the similarity of these results to those obtained for the pilot plant ammoniation of cottonseed meal containing only 330 μg of B_1/kg (Table III).

Feeding studies on ammoniated cottonseed meals (Vohra et al., 1975; Waldroup et al., 1976) indicate that ammoniated cottonseed meals can safely be fed to laying hens and broilers. The meal fed these birds was ammoniated under the harshest conditions of heat and pressure (115 °C, 45–50 psig). Our results show a similar conversion of B_1 to D_1 under all conditions of ammoniation, as well as comparable amounts of unreacted B_1.

Further studies are presently being conducted at this laboratory in which we are evaluating the protein and carbohydrate quality of aflatoxin contaminated meals ammoniated under the three conditions. The results of that study, along with those reported here, will give processors a more complete profile of meal quality.

ACKNOWLEDGMENT

The authors are grateful to A. O. Franz, Jr., for quantitative aflatoxin analyses, S. P. Koltun and E. T. Rayner for the ammoniated cottonseed meal prepared in the pilot plant, and W. A. Pons, Jr., for valuable advice.

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Nitramines as Thermal Energy Analyzer Positive Nonnitroso Compounds Found in Certain Herbicides

Joseph H. Hotchkiss,* James F. Barbour, Leonard M. Libbey, and Richard A. Scanlan

Certain dialkyl nitramines have been shown to elicit a false positive response on the thermal energy analyzer (TEA). The TEA is a detector specifically employed in the analysis of samples for trace amounts of nitrosamines. Dipropylnitramine (DPNO) was responsible for a TEA false positive peak in the analysis of a widely used herbicide formulation containing α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine. In addition, the presence of dipropylnitrosamine at a concentration of 130 mg/kg in the herbicide was confirmed. The molar response ratio of DPNO compared to dipropylnitrosamine was 0.50. In addition, DPNO was Griess reagent positive, UV sensitive, and had a similar retention time to dibutylnitrosamine on Carbowax 20M. Nitramines are almost certainly responsible for unknown TEA positive peaks in other herbicide formulations. Nitramines might be mistakenly identified as *N*-nitroso compounds, especially where mass spectral confirmation is not made.

The thermal energy analyzer (TEA) is widely employed as a specific detector for the analysis of nitrosamines (NAs) in a variety of substances. The TEA was first used as a GLC detector (Fine and Rounbehler, 1975) and more recently as a LC detector (Fine et al., 1976). The principles of operation have been described in detail elsewhere (Fine et al., 1975).

The high selectivity of the TEA detector over more conventional detectors is well established, but recent work indicates that certain samples contain non-NAs which can elicit a positive TEA response. Unidentified TEA positive peaks which elute prior to dimethylnitrosamine (DMN) have been reported (Stephany and Schuller, 1978) as well as unidentified peaks eluting after DMN in tobacco smoke

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condensate, cheese, and fish products (Fiddler et al., 1978). The instrument manufacturer (Thermo Electron Corp.) reports that certain organic nitrites and nitrates, plus some inorganic nitrites produce a positive TEA response, particularly when the instrument is operated in the direct injection mode. For this reason many authors consider confirmation by an independent technique manditory (Fan et al., 1978). Most often this confirmatory technique is mass spectrometry (MS). The greater sensitivity of GC–TEA over GC–MS (Gough, 1978) could lead to reporting NAs in food and environmental samples without MS confirmation.

Ross et al. (1977) have reported the occurrence of NAs in certain herbicide formulations. Their report is based on GC–TEA as well as GC–MS data. Subsequent work by the EPA has confirmed the presence of dialkyl nitrosamines in certain dinitroaniline-type herbicides (Cohen et al., 1978). This paper also reported the occurrence of other unidentified TEA positive peaks in some herbicides. Our own analyses of the herbicide formulation containing α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine produced an unidentified TEA positive peak which prompted us to further investigate the nature of this peak. We report here the identity of a non-NA compound which is TEA positive and found in Treflan.

METHODS AND MATERIALS

Dipropylnitrosamine (DPN), diethylnitrosamine (DEN), and trifluoroacetic anhydride were purchased from Eastman Organic Chemicals (Rochester, N.Y.), and 50% hydrogen peroxide was obtained from Fisher Scientific Co. (Fair Lawn, N.J.). All solvents were high purity grade.

The method of Emmons (1954) was used with the following modifications for the synthesis of dipropylnitramine (DPNO): An equal molar amount of 50% H_2O_2 was substituted for 90% H_2O_2, and 1-μL aliquots of the reaction mixture were taken during heating and analyzed by GC–FID until the DPN peak was less than 1% of the DPNO peak; the total heating time was 2.5 h. Distillation at 92–94 °C (4 mm) [literature value, 105–106 °C (10 mm); Robson, 1955] yielded 7.6 g of colorless DPNO (52% theoretical). The synthetic DPNO was analyzed by IR spectrometry neat between NaCl discs, using a Beckman Model IR-18A spectrophotometer.

One-half-milliliter aliquots of the herbicide were cleaned up prior to analysis on 15 g of activity II alumina in a 14-mm i.d. glass column. The column was washed with 100 mL of hexane, followed by 50 mL of 10% dichloromethane (DCM) in hexane. The compounds of interest were eluted from the column by 100 mL of DCM; the eluate was collected in a Kuderna-Danish apparatus fitted with a three-ball Snyder column and concentrated to ca. 3 mL. The DCM was further concentrated to 0.5 mL under a stream of nitrogen and then analyzed by GC–TEA. One-tenth-milliliter aliquots were further concentrated to ca. 10 μL for GC–MS analysis. Recovery studies for DPN and DPNO in hexane gave essentially quantitative recovery. The concentrated eluent was analyzed by GC–TEA on a Model 502 TEA analyzer (Thermo Electron Corporation) coupled to a Varian Model 1400 GC. Instrument parameters were as follows: (GC) injection port, 200 °C; column, 0.125 in. o.d. × 20 ft stainless steel packed with Carbowax 20M on 60/120 mesh Chromosorb G, isothermal at 200 °C; (TEA) furnace, 400 °C; trap, isopentane and liquid N_2 slurry.

The molar response ratio, *r*, of DPNO was determined by injecting a solution of DPN and DPNO in hexane into the GC–TEA, correcting for mole content, and dividing the area of the DPNO peak by the DPN peak.

Quantification of the DPN and DPNO content in the herbicide was accomplished by using DEN as an internal standard (previously it had been determined that detectable levels of DEN were not present in the herbicide).

Mass spectral data were obtained with a Finnigan Model 1015C quadrupole mass spectrometer coupled to a Varian 1400 GC. Instrument parameters were as follows: (GC) injection port, 200 °C; column, 0.03 in. i.d. × 500 ft stainless steel, wall-coated with SF-96, isothermal at 130 °C; (MS) filament current, 350 μA; electron voltage, 70 eV; analyzer pressure, 10^{-6} Torr. Data were collected by a System Industries System 250 computerized data system.

Thin-layer chromatography (TLC) was accomplished on 0.25-mm thick Silica Gel G plates (Macherey-Nagel and Co.) using hexane–ethyl ether–DCM, 4:3:2 (v/v/v) as the developing solvent. Griess reagent, as modified by Fan and Tannenbaum (1971), and UV irradiation were used for visualization.

To determine the stability of hexane solutions of DPN and DPNO to UV light, irradiation was carried out in 1-cm^2 fused silica stoppered cuvets (Beckman) placed 40 cm from a sunlamp (General Electric, RSK/3).

RESULTS AND DISCUSSION

In analyzing two separate lots of the herbicide formulated with α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine we found both lots contained sufficient DPNO to give a positive TEA response. Figure 1 is a GC-TEA chromatogram of the concentrated eluent derived from the herbicide. Ross et al. (1977) in their original report on the DPN content of a formulated sample of α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine did not report the occurrence of extraneous TEA positive peaks. In a recent report (Cohen et al., 1977) unknown peaks were not reported in an α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine-containing herbicide but were reported in other similar dinitroaniline herbicides. In addition, analysis of a different herbicide formulation containing α,α,α-trifluoro-2,6-dinitro-*N*-butyl-*N*-ethyl-*p*-toluidine in our laboratory, in the same manner as outlined above, indicates that the nitramine derivative of ethylbutylamine is almost certainly responsible for one of the unknown TEA positive peaks observed. The occurrence of nitramines in this type of herbicide may be general and due to the manufacturing process.

The structure of the authentic DPNO was confirmed by both IR and MS analyses. The IR spectrum of DPNO showed characteristic nitramine absorptions at 1280 and 1520 cm^{-1} (Colthup et al., 1964). The mass spectrum of the authentic DPNO was nearly identical with the mass spectrum of DPNO found in both lots of the α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine containing herbicide (Figure 2). The authentic and the herbicide DPNO spectra both matched closely to a standard spectrum (Stenhagen et al. 1974).

Comparison of the peak areas of DPNO to DPN over five trials gave an average molar response ratio, *r*, of 0.50 on our GC–TEA (compared to DPN where $r = 1.0$). Fiddler et al. (1978) have reported that a peak with the same retention time as diethylnitramine (DENO) was found in smoked salmon and that DENO has an *r* of 0.35 compared to DEN. The difference in *r* values between DENO and DPNO may be due to differences in instrument parameters.

Comparison of the peak areas of the unknowns to the internal standard DEN gave concentrations of 130 and 10 mg/kg for DPN and DPNO, respectively, in the α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine-containing herbicide. It must be noted that these values may not

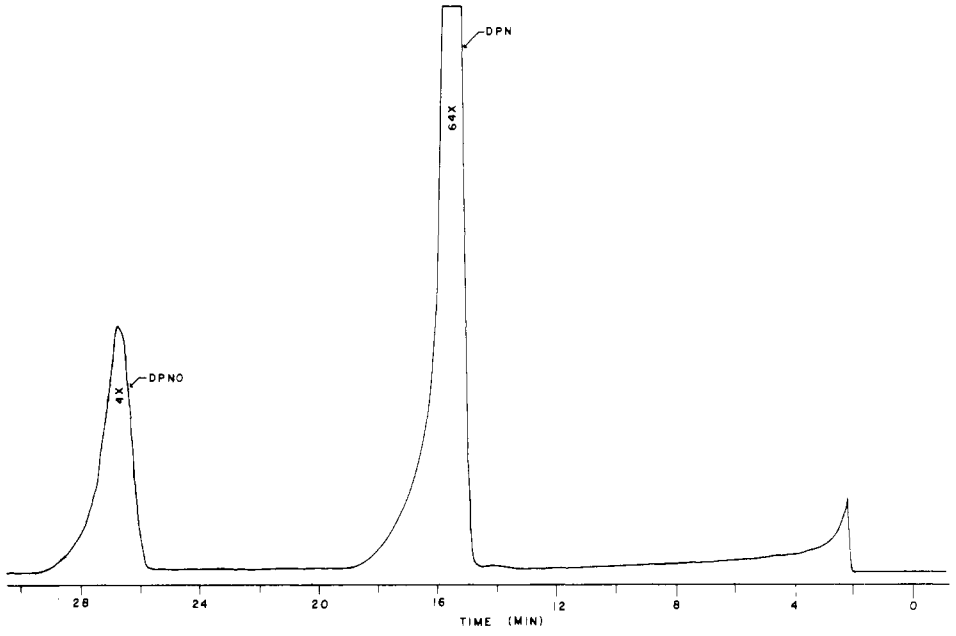

Figure 1. GC–TEA chromatogram of the concentrated eluent derived from a herbicide formulation containing α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine. Dipropylnitrosamine (DPN), dipropylnitramine (DPNO). GC conditions: injector, 200 °C; column 0.125 in. o.d. × 20 ft stainless steel packed with Carbowax 20M on 60/120 Chromosorb G, isothermal at 200 °C.

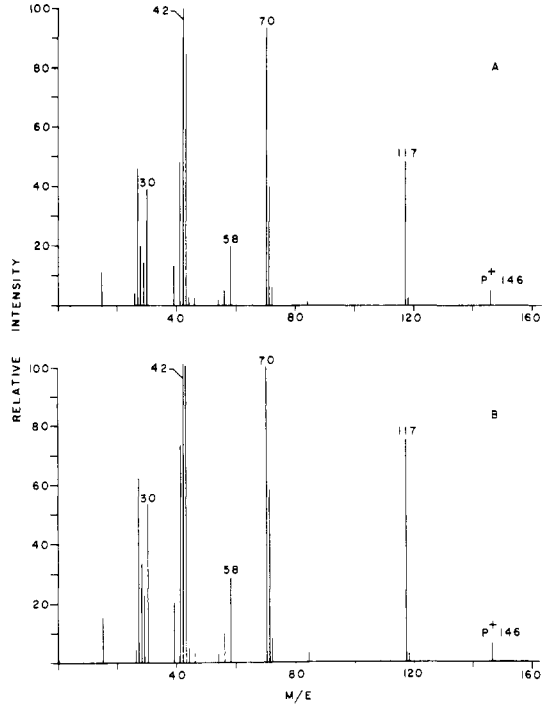

Figure 2. Mass spectra of dipropylnitramine from (A) a herbicide formulation containing α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine; (B) authentic compound.

reflect concentrations produced by current manufacturing practices. This is further indicated by the fact that a DPN concentration of 154 mg/kg was first reported by Ross et al. (1977) but more recent EPA work on registrant supplied herbicide gave DPN levels of 121 mg/kg for one sample and 13 mg/kg for another, reflecting changes in manufacturing processes (Cohen et al., 1977).

The TLC R_f values of DPN and DPNO standards were 0.43 and 0.45, respectively. The nitramine was Griess reagent positive although increased amounts of compound or increased irradiation times were necessary.

The possibility that DPNO was formed as an artifact of the analysis was considered. First, DPN in hexane at the same order of magnitude concentration as in the herbicide formulation was subjected to the same analytical procedure and analyzed by GC–TEA for DPNO content. Second, DPN was added to the herbicide formulation in sufficient amount to double the DPN content of the herbicide. The sample was then subjected to the analytical procedure and analyzed by GC–TEA to determine if the DPNO content had increased. Both procedures indicated that DPNO was not an artifact.

While it should be noted that at least one dialkylnitramine (dimethylnitramine) has been reported carcinogenic in rodents (Goodall and Kennedy, 1976), our main concern is that these compounds may be mistakenly identified as NAs, especially in cases where GC–MS confirmation is not made. This possibility becomes real when one considers that these compounds are TEA positive, Griess positive, and have ions at the low end of the mass spectrum that are very similar to the corresponding nitrosamine ions. In addition, the retention times of certain NAs are very close to those of some nitramines. On our Carbowax 20M column, DPNO and dibutylnitrosamine had retention times of 26.25 and 25.50 min, respectively. Althorpe et al. (1970) have also reported close retention times for certain NA and nitramine combinations on Carbowax 20M, which is a commonly used stationary phase in NA analyses.

Fiddler et al. (1978) have suggested that samples containing TEA positive peaks be photolyzed after TEA analysis and reanalyzed with the assumption that nitrosamines will decompose under UV irradiation and disappear from the chromatogram, while false positive compounds will not. To test this procedure with regard to nitramines we exposed hexane solutions of DPN and DPNO in separate cuvets to artificial sunlight. The solution was sampled periodically and analyzed by GC–TEA. In as little as 15 min a decrease in the DPN peak and the appearance of a DPNO peak was seen in the hexane containing only DPN. In 5 h the DPN peak had disappeared. The DPNO-containing solution was more stable but did show signs of decreasing concentration after 8 h. At the end of 24 h the DPNO peak was ca. 20% of the original value. This indicates DPNO is a product of the

UV decomposition of DPN which is in agreement with the findings of Althorpe et al. (1970). It appears, however, that nitramines may also be degraded by UV but at a much slower rate than NAs. The most reliable method for confirmation of nitrosamines continues to be high resolution mass spectral analysis of not only the NO^+ ion but also the molecular (M^+) ion, or complete low-resolution spectra.

Continuing work at this laboratory indicates that the TEA may respond positively to nitramine compounds in general.

SAFETY: *N*-Nitroso compounds must be handled with caution, as many are potent carcinogens.

ACKNOWLEDGMENT

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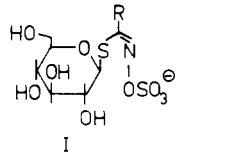
Studies of Some Nonenzymatic Reactions of Progoitrin

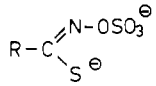
Salo Gronowitz,* Leif Svensson, and Ragnar Ohlson

The hydrolysis of isolated progoitrin (2-hydroxy-3-butenylglucosinolate) at different pHs and in different buffers has been investigated. The rate of disappearance of the glucosinolate was determined, as well as the formed hydrolysis product. No goitrin could be detected. Rapid hydrolysis was observed in the presence of borax and a new product, 5-vinyl-2-oxazolidinone, previously not isolated from progoitrin, was obtained, besides the known hydrolysis product 1-cyano-2-hydroxy-3-butene.

In connection with the development of a process for the preparation of a protein concentrate for human consumption from *Brassica napus* rapeseed (Ohlson, 1972), it became of importance to transform the glucosinolates present in rapeseed, especially progoitrin (Ia) and glu-



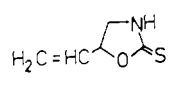
I



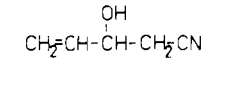
II

a R = CH_2=CH-CH(OH)-CH_2-
b R = CH_2=CH-CH_2-CH_2-

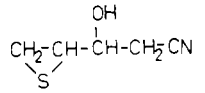
conapin (Ib), to safe degradation products as fast as possible. The glucosinolates appear in the leach water from the protein process. The enzymatic hydrolysis of glucosinolates by myrosinases (thioglucoside glucohydrolases) (Björkman, 1976) gives the aglycon II, which is unstable and by chemical reactions is transformed to isothiocyanates and nitriles. Low pH favors nitrile formation, indicating that nitriles are formed via a S- or N-protonated form of II (van Etten et al., 1966). Nitrile formation could also be promoted by some metal cations, especially Fe(II) ions (Austin et al., 1968; Tookey and Wolff, 1970; Kirk et al., 1971). From Ia, (*R*)-1-cyano-2-hydroxy-3-butene (IV) (Daxenbichler et al., 1966) and



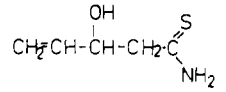
III



IV



V



VI

(*S*)-5-vinyl-2-oxazolidinethione (III) (Astwood et al., 1949) are formed, the latter by further reaction of the isothio-

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