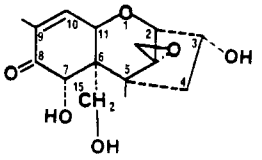
Negative Ion Chemical Ionization Mass Spectrometry of Deoxynivalenol (DON): Application to Identification of DON in Grains and Snack Foods after Quantitation/Isolation by Thin-Layer Chromatography

William C. Brumley,* Mary W. Trucksess, Scott H. Adler,[1] Clara K. Cohen,[1] Kevin D. White, and James A. Sphon

The negative ion (NI) mass spectrum of deoxynivalenol (DON) under resonance electron capture conditions is discussed. Certain fragmentations were studied by collision-induced decomposition/mass-analyzed ion kinetic energy spectroscopy. The NI technique was applied to the identification of DON in extracts of grains and snack foods by using on-column injection capillary gas chromatography. The sample extract was obtained by extraction with acetonitrile–water and was subjected to thin-layer chromatography (TLC) as a quantitative screening or isolation step. DON levels in wheat, corn, corn meal, flour, and snack foods as determined by TLC are reported.

Deoxynivalenol (DON, vomitoxin, 3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) is a mycotoxin produced by several genera of mold including several species of *Fusarium* (Sato and Ueno, 1977). According to the



classification of Sato and Ueno, DON is a trichothecene of class B by virtue of its ring structure and the presence of a conjugated carbonyl group. DON was first reported in Japan in contaminated barley (Morooka et al., 1972; Yoshizawa and Morooka, 1973) and later was found in the United States in corn that had caused vomiting in swine (Vesonder et al., 1973). The occurrence of DON in grains has been reported more recently in Canada (Trenholm et al., 1981; Scott et al., 1981) and the United States (Eppley et al., 1984). DON was also identified in "yellow rain" from Southeast Asia (Rosen and Rosen, 1982; Mirocha et al., 1983).

The toxic effects of DON in animals have been studied (Pathre and Mirocha, 1979; Trenholm et al., 1981; Scott et al., 1983; Ueno, 1984). Because of interest in the toxicity and the extent of natural contamination associated with DON, several methods have been used for its determination. Early reports on analysis of cultures and feedstuffs used gas chromatography/mass spectrometry (GC/MS) with electron ionization (EI) for identification of DON as the trimethylsilyl (Me_3Si) ether derivative (Tatsuno et al., 1973; Mirocha et al., 1976; Pathre and Mirocha, 1978). GC determinations of DON as the Me_3Si ether derivative (Vesonder et al., 1978; Kuroda et al., 1979) and as the heptafluorobutyryl derivative (Scott et al., 1981; Bennett et al., 1983) have been reported. DON has been determined by thin-layer chromatography (TLC) in corn and wheat (Trucksess et al., 1984) and in more limited applications (Takitani et al., 1979; Sano et al., 1982). High-performance liquid chromatography (HPLC) has been used to purify DON standards, which led to the identification of a new trichothecene that lacks the hydroxyl group at C_7 (Bennett et al., 1981). HPLC was also used to quantitate DON in wheat (Chang et al., 1984).

In the present paper we describe the application of resonance electron capture (EC) in negative ion chemical ionization mass spectrometry (NICIMS) (Hunt and Crow, 1978) for the identification of DON isolated from various matrices by TLC. The technique uses on-column capillary GC for sample introduction (Knauss et al., 1981). The overall simplicity of this approach and its compatibility with a rapid TLC screening and quantitation procedure are discussed. In addition, this technique is compared to other reported confirmations of identity. The rather extensive fragmentation found in the NI spectrum is discussed with reference to the OH^- spectrum previously reported (Brumley et al., 1982) and to additional collision-induced decomposition/mass-analyzed ion kinetic energy spectroscopy (CID/MIKES) results. The TLC quantitation procedure is used to determine DON levels in grain and finished food products made predominantly from wheat or corn.

EXPERIMENTAL SECTION

TLC Isolation. Extraction of grain and food samples with acetonitrile–water and TLC fluorodensitometric quantitation have been described (Trucksess et al., 1984). For isolation, a 20-cm silica gel plate was divided into 3-cm channels with a pencil. Sample extracts containing 100–200 ng of DON were spotted in the channels; a 200-ng standard was spotted in a separate channel. The plate was developed for 1 h and the solvent (chloroform–acetone–isopropyl alcohol, 8:1:1) was evaporated. While the sample channels were covered with a glass plate, the standard channel was sprayed with $AlCl_3$ solution (20 g $AlCl_3.6H_2O$ in 50 mL of H_2O and 50 mL of C_2H_5OH). The sprayed portion of the plate was placed on a hot plate at 105 °C for 2 min. Long wavelength UV light was used to locate the standard position, which then was used to locate DON in the channels with sample extracts. The DON was removed from the TLC plate with an Eluchrom apparatus (Camag Applied Analytical Industries, Wilmington, NC) by using 2.5 mL of chloroform–acetone–2-propanol (70:15:15) as the eluting solvent. The eluate was evaporated to dryness under a stream of nitrogen and redissolved in acetone before analysis by MS.

MS. Low-resolution spectra (55–455 u in 1.0 s) were obtained by on-column capillary GC introduction with a modified Finnigan-MAT 3300F CI quadrupole mass spectrometer (Brumley et al., 1982). Primary ionization was achieved by a 140-eV electron beam from a heated rhenium filament with a 0.50-mA emission current. Reagent gas was methane (99.7% purity, Matheson Gas

Division of Chemical Technology (W.C.B., S.H.A., K.D.W., and J.A.S.) and Division of Chemistry and Physics (M.W.T. and C.K.C.), Food and Drug Administration, Washington, DC 20204.

[1]American University Summer Research Participation Program, 1983.

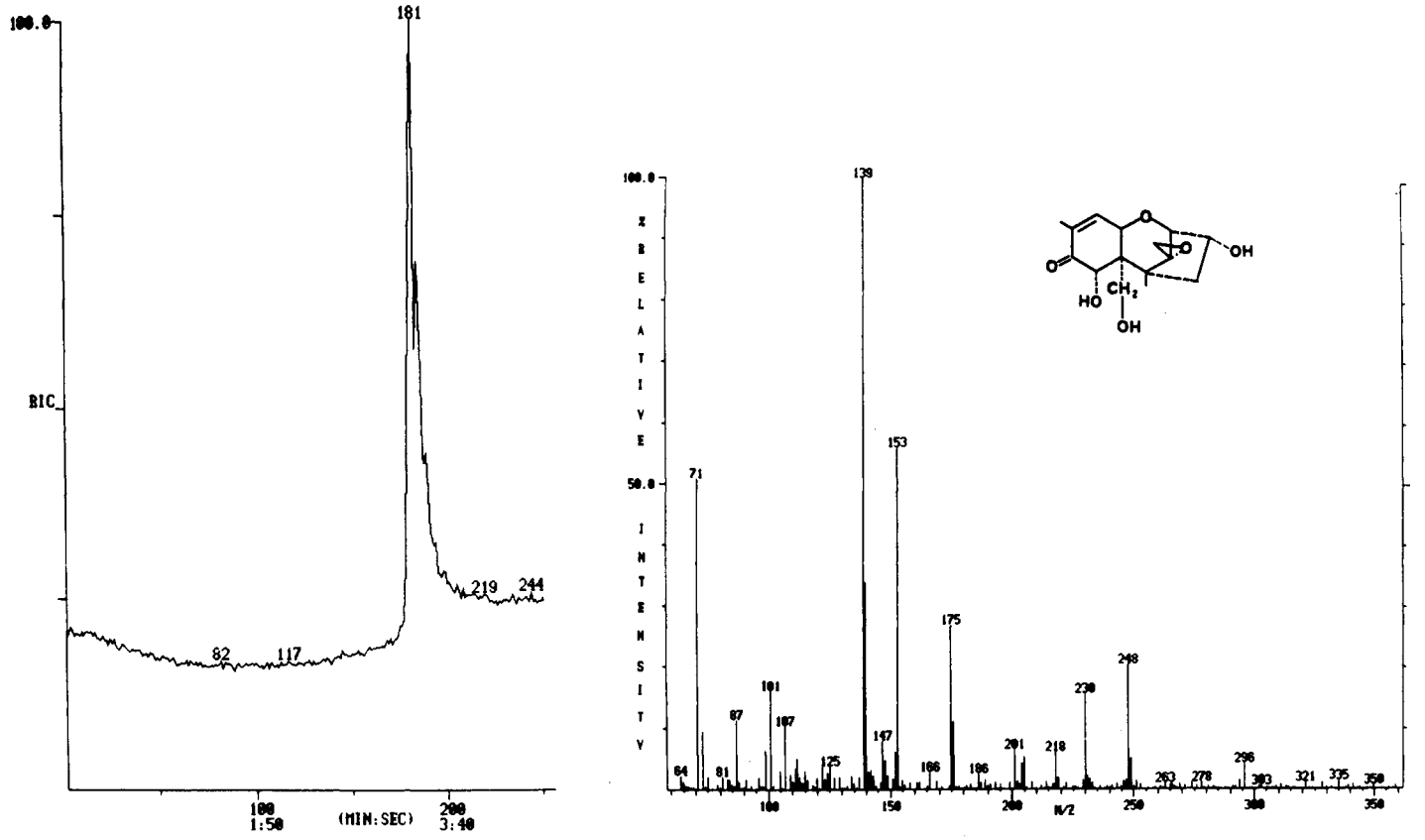


Figure 1. Total ion chromatogram (55–455 u) and resonance EC NICI mass spectrum of ca. 26 ng of a DON standard by on-column capillary GC. (RIC = reconstructed total ion chromatogram; numbers over peaks refer to the number of scans.)

Products, Inc., East Rutherford, NJ) at a source pressure of 0.2 torr measured with a McCloud gage. The gas was introduced via the GC makeup line. A 6-m fused (0.2 mm i.d.) silica capillary column coated with methyl silicone (no film thickness specified) (Hewlett-Packard, Avondale, PA) was joined directly to the ion source. A 5-μL Hamilton syringe was used for on-column injection, and the column was ballistically heated from 40 to 250 °C after injection. Other conditions were He carrier gas at ca. 20 cm/s at 40 °C, 220 °C transfer line, and an ion source at 100–130 °C.

CID/MIKES experiments were performed on a VG ZAB-2F instrument under resonance EC conditions with instrument parameters as previously described (Brumley et al., 1982).

Reagents. DON standards maintained by the Food and Drug Administration were dissolved in acetone (Burdick & Jackson Laboratories, distilled-in-glass). Purity of DON (≥ 95%) was judged by TLC, HPLC, and GC/MS.

RESULTS AND DISCUSSION

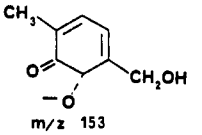
Our NICI GC/MS technique differs from previously reported methods for the identification of DON in grains primarily in the use of TLC isolation and screening, no derivatization to facilitate GC and cleanup, and resonance EC for the mass spectral ionization conditions. The advantages of the TLC isolation and quantitative screening procedure include its rapidity, multiple sample processing, and inexpensive equipment and its compatibility with the GC/MS approach described here. First we describe the NI mass spectrum of DON in detail since this relates to the specificity of this technique.

Resonance EC Spectrum of DON. The NICI GC/MS spectrum of a DON standard is shown in Figure 1 along with the total ion chromatogram. Several features of the spectrum parallel behavior described under OH^- ionization conditions (Brumley et al., 1982). Under resonance EC conditions, a relatively low abundance $M^{-\cdot}$ ion at m/z 296 is produced. An ion at m/z 294 represented as $(M - H_2)^{-\cdot}$ is also observed.

The ions above m/z 201 occur predominantly at even masses and result from rearrangement processes and fragmentation. Thus, ions at m/z 278, 266, 248, 230, 218, and 204 may be postulated as $(M - H_2O)^{-\cdot}$, $(M - CH_2O)^{-\cdot}$, $(M - CH_2O - H_2O)^{-\cdot}$, $(M - CH_2O - 2H_2O)^{-\cdot}$, $(M - 30 - CH_2O - H_2O)^{-\cdot}$, and $(M - 44 - H_2O - CH_2O)^{-\cdot}$. We have hypothesized that CH_2O is lost at C_{15} (Brumley et al., 1982). We also propose that 44 u can be lost as C_3–C_4 with substituents. Previous CID/MIKES experiments (Brumley et al., 1982) have suggested that ions at m/z 278, 266, and 230 can arise as a result of fragmentations from $M^{-\cdot}$. These ions can also be formed as a result of dissociative EC (Hunt and Crow, 1978).

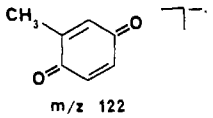
Ions at and below m/z 201 occur predominantly at odd masses and include m/z 175, 153, 147, 139, 123, 107, and 71, although a limited number of ions at even masses also appear. We offer a speculative rationale for the occurrence of some of these ions. The ion at m/z 175 can be represented as $(M - 73 - 18 - 30)^-$ as a result of the combined losses of H_2O, CH_2O, and the structural unit composed of O_1–C_2–C_3–C_4 with substituents. Fragmentation involving the rings on the epoxide side of the molecule is apparently extensive under NICI conditions.

The ion at m/z 153 was shown previously (Brumley et al., 1982) to be consistent with cleavage and rearrangement to an ion we represent here as



Ions at m/z 122 and 139 (140 predominates in the OH^- spectrum with solid probe introduction) also presumably involve elements of the same ring structure. Additional

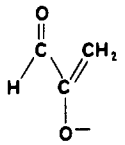
CID/MIKES experiments show that m/z 153 can give rise to ions at m/z 123 and 122 as well as m/z 135 and 107. We represent m/z 122 as



m/z 122

if it originates from m/z 140 or as the ortho isomer if it arises from m/z 153.

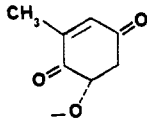
The ion at m/z 71 is produced by an unknown pathway and may arise from O_1–C_2–C_3–C_4 to yield



m/z 71

An ion at m/z 71 has previously been observed in the collisionally activated spectra of polyhydroxy compounds such as glucose under triple quadrupole (Hunt et al., 1980) and CID/MIKES conditions (McClusky et al., 1978).

The ion at m/z 139 is also produced by an unknown pathway and is analogous to m/z 140 in our previous work (Brumley et al., 1982).



m/z 139

We offer these ion structures and fragmentation correlations as a rationalization of the spectrum without suggesting that we have definitively determined actual ion structures or mechanisms of fragmentation. Additional CID/MIKES experiments have shown that m/z 230 gives rise to several ions including m/z 201 and 186, m/z 218 to m/z 175, m/z 205 also to m/z 175, and m/z 175 to m/z 147. These data suggest that some of the observed fragmentation pathways are interrelated. Observed decompositions are also consistent with the recently reported MS/MS spectrum of m/z 248 of DON (Plattner and Bennett, 1983) with a triple quadrupole instrument. Molecular factors favoring the extensive fragmentation observed in DON are not entirely clear. From previous work we know that the conjugated carbonyl function is stabilizing toward resonance EC (Brumley et al., 1981) and the role of the hydroxyl group at C_7 appears to be important as well. The overall result is that extensive fragmentation of the molecule is observed.

None of the experiments in this or previously cited work has revealed precursors for m/z 153 and 139. One possible explanation for this nonobservance is that both ions are produced by dissociative EC. In this circumstance, the electron interaction occurs initially at the equilibrium geometry of the neutral molecule and this initial condition is not accessible (low probability and competing processes) by collisional activation of $M^{-\cdot}$ at its equilibrium geometry. A second, related explanation postulates that the collisionally stabilized $M^{-\cdot}$ ion no longer has the same molecular structure as the neutral molecule as a result of rearrangements, and therefore neither it nor its daughters can give rise to these fragment ions (m/z 139 and 153) upon collisional activation.

The spectrum produced by resonance EC provides a specific indication of the presence of DON, as may be seen by comparing it with the spectra of similar trichothecenes (Brumley et al., 1982). Fragmentation is not as extensive as in EI spectra, but the fragment ions produced are more structurally significant than those observed with heptafluorobutyrate (Rothberg et al., 1983) and Me_3Si ether derivatives of DON. Resonance EC conditions appear to be about an order of magnitude more sensitive than OH^- conditions.

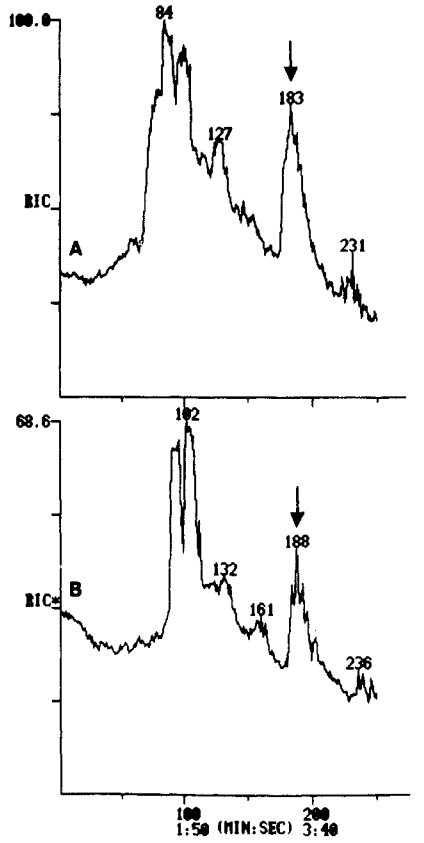


Figure 2. Total ion chromatograms (55–455 u) obtained from (A) snack food (130 ppb) and (B) flour (200 ppb); ca. 0.2 g equiv injected. (RIC = reconstructed total ion chromatogram; arrow denotes response attributed to DON.)

Analysis of Samples. The total ion chromatograms (m/z 55 to 455) obtained from samples of snack foods (chips or biscuits made from corn or wheat) and flour are given in Figure 2. About 0.2-g equiv of each sample was injected on-column and contained about 30–40 ng of DON by TLC quantitation. Almost all of the coextractives from the matrix elute before DON. Figure 3 shows the background-subtracted spectrum of DON obtained from the response of the snack food sample. The spectrum compares favorably with the spectrum of the standard (Figure 1). The molecular ion and fragment ions at m/z 248, 230, 175, 153, 139, and 71 are of primary consideration. In addition, ions of low relative abundance at m/z 218, 201, 147, and 107 are also present. An examination of the individual ion current chromatograms reveals that all ions common to DON are not subject to interference from the matrix. We illustrate this finding for six ions in Figure 4. The large, early responses in the total ion chromatogram are due primarily to three ions, m/z 79, 81, and 144.

The compatibility of on-column GC/MS with TLC isolation is evident from the variety of samples that were successfully analyzed for DON during various surveys (Table I). Representative samples from each of the product categories were submitted for MS analysis (15 of 228 total number) and in each case the presence of DON indicated by TLC was confirmed by MS. The advantages of this approach include the simplicity of eliminating derivatization and associated stability problems (Scott et al.,

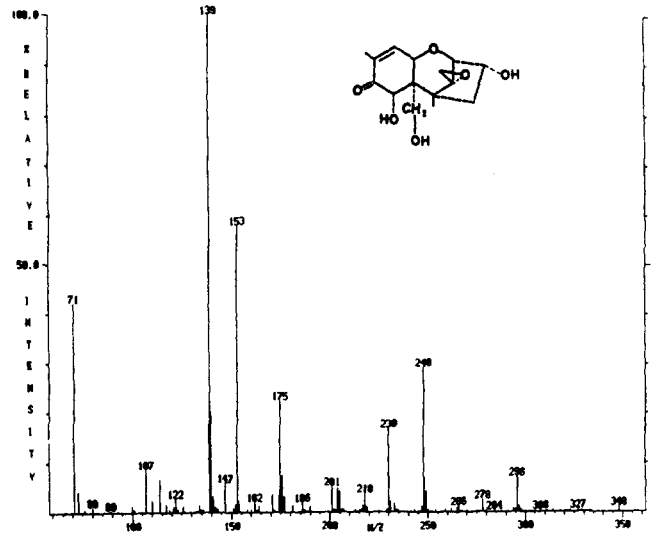


Figure 3. Resonance EC NICI mass spectrum of DON identified in the snack food sample in Figure 2 part A (about 35 ng).

Table I. DON in Grains and Snack Foods Determined by TLC

product	no. of samples	range DON detected,[a] μg/g	av level detected, μg/g	no. of samples DON positive
wheat[b]	57	ND–9.0	3.6	54
corn	50	ND–2.1	0.34	48
flour	50	ND–0.5	0.11	44
corn meal	50	ND–0.3	0.08	45
snack foods[c]	21	ND–0.5	0.11	14

[a] ND = not detected. [b] Data for wheat have been reported by Eppley et al. (1984). [c] Chips or biscuits made mainly from corn or wheat.

1981) because samples may be stored and analyzed in batches.

The survey used wheat samples obtained through the Federal Grain Inspection Service (FGIS) of the U.S. Department of Agriculture (USDA) from grader stations in selected areas of four midwestern states where contamination of 1982 winter wheat with Fusaria was reported. The corn samples were also obtained from grader stations throughout the corn belt during 1983 through FGIS of USDA. Flour and corn meal samples were obtained from the Agricultural Marketing Service Grain Laboratory, USDA, Beltsville, MD. Snack food samples were purchased in local grocery stores during 1983.

TLC quantitation of DON in the grains and food products ranged from not detected to 9.0 μg/g. The limit of determination was 0.04 μg/g and the average coefficient of variation for quantitations above 0.1 μg/g was about 8%. Recoveries of DON averaged about 80% and quantitative results obtained by TLC were comparable to those from a GC quantitative method performed in another laboratory (Eppley et al., 1984; Trucksess et al., 1984). The average level found in the wheat was about 3.6 μg/g compared to an average level of about 0.11 μg/g in snack foods. The results indicate that DON contamination was widespread in grains sampled (more than 80% of all samples were positive) and that DON was also detectable in foods derived from grains.

The specificity of the full scan technique and its compatibility with low cost screening procedures are obvious from the data presented. The demonstrated level of confirmation is about 130 ppb DON; however, analysis of standards suggests that lower levels are attainable. Modification of the procedure to use multiple ion detection

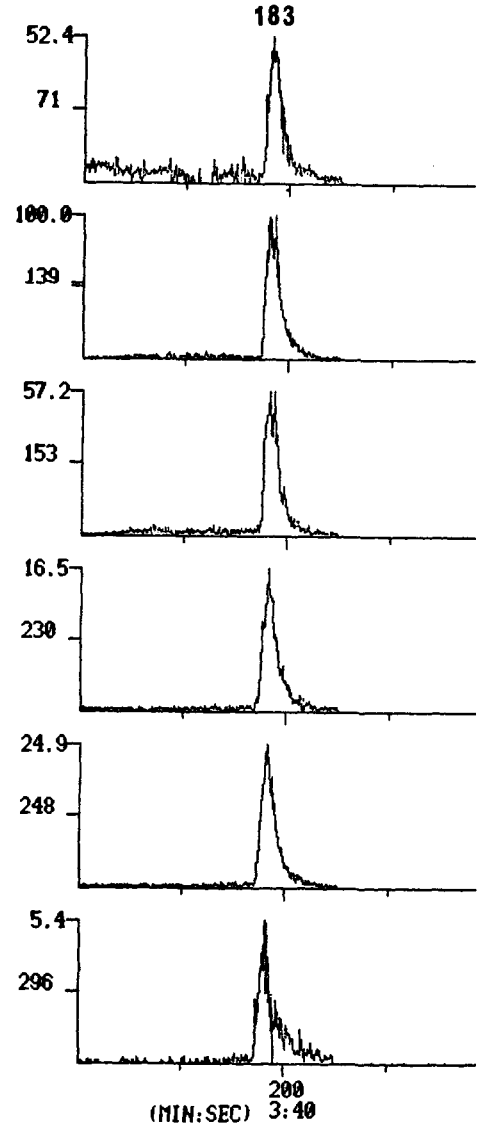


Figure 4. Six individual ion current chromatograms obtained from analysis of the snack food sample (Figure 2 part A).

in place of full scans is also feasible and may be used to reach lower levels. However, in view of the relatively high levels encountered in samples and the lack of toxicity data for human exposure, the full scan technique we describe is appropriate for present monitoring activities because it retains higher specificity than ion monitoring approaches.

Registry No. DON, 51481-10-8.

LITERATURE CITED

Bennett, G. A.; Peterson, R. E.; Plattner, R. D.; Shotwell, O. L. *J. Am. Oil Chem. Soc.* **1981**, *58*, 1002A–1005A.

Bennett, G. A.; Stubblefield, R. D.; Shannon, G. M.; Shotwell, O. L. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1478–1480.

Brumley, W. C.; Andrzejewski, D.; Trucksess, E. W.; Dreifuss, P. A.; Roach, J. A. G.; Eppley, R. M.; Thomas, F. S.; Thorpe, C. W.; Sphon, J. A. *Biomed. Mass Spectrom.* **1982**, *9*, 451–458.

Brumley, W. C.; Nesheim, S.; Trucksess, M. W.; Trucksess, E. W.; Dreifuss, P. A.; Roach, J. A. G.; Andrzejewski, D.; Eppley, R. M.; Pohland, A. E.; Thorpe, C. W.; Sphon, J. A. *Anal. Chem.* **1981**, *53*, 2003–2006.

Chang, H. L.; DeVries, J. W.; Larson, P. A.; Patel, H. H. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 52–54.

Eppley, R. M.; Trucksess, M. W.; Nesheim, S.; Thorpe, C. W.; Wood, G. E.; Pohland, A. E. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 43–45.

Hunt, D. F.; Crow, F. W. *Anal. Chem.* **1978**, *50*, 1781–1784.

Hunt, D. F.; Shabanowitz, J.; Giordani, A. B. *Anal. Chem.* **1980**, *52*, 386–390.

Knauss, K.; Fullemann, J.; Turner, M. P. *High Resolut. Chromatogr. Chromatogr. Commun.* **1981**, *4*, 641-643.
Kuroda, H.; Mori, T.; Nishioka, C.; Okasaki, H.; Takagi, M. *J. Food Hyg. Soc. Jpn.* **1979**, *20*, 137-142.
McClusky, G. A.; Kondrat, R. W.; Cooks, R. G. *J. Am. Chem. Soc.* **1978**, *100*, 6045-6051.
Mirocha, C. J.; Pathre, S. V.; Schauerhamer, B.; Christensen, C. M. *Appl. Environ. Microbiol.* **1976**, *32*, 553-556.
Mirocha, C. J.; Pawlosky, R. A.; Chatterjee, K.; Watson, S.; Hayes, W. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1485-1499.
Morooka, N.; Uratsuji, N.; Yoshizawa, T.; Yamamoto, H. *J. Food Hyg. Soc. Jpn.* **1972**, *13*, 368-375.
Pathre, S. V.; Mirocha, C. J. *Appl. Environ. Microbiol.* **1978**, *35*, 992-994.
Pathre, S. V.; Mirocha, C. J. *J. Am. Oil Chem. Soc.* **1979**, *56*, 820-823.
Plattner, R. D.; Bennett, G. A. *J. Assoc. Off. Anal. Chem.* **1983**, *66*, 1470-1477.
Rosen, R. T.; Rosen, J. D. *Biomed. Mass Spectrom.* **1982**, *9*, 443-450.
Rothberg, J. M.; MacDonald, J. L.; Swims, J. C. "Xenobiotics in Foods and Feeds"; Finley, J. W., Schwass, D. E., Eds.; American Chemical Society: Washington, DC, 1983; pp 271-281.
Sano, A.; Asabe, Y.; Takitani, S.; Ueno, Y. *J. Chromatogr.* **1982**, *235*, 257-265.
Sato, N.; Ueno, Y. "Mycotoxins in Human and Animal Health"; Rodricks, J. V., Hesseltine, C. W., Mehlman, M. A., Eds.; Pathotox Publishers, Inc.: Park Forest South, IL, 1977; pp 295-307.
Scott, P. M.; Lau, P.-Y.; Kanhere, S. R. *J. Assoc. Off. Anal. Chem.* **1981**, *64*, 1364-1371.
Takitani, S.; Asabe, Y.; Kato, T.; Suzuki, M.; Ueno, Y. *J. Chromatogr.* **1979**, *172*, 335-342.
Tatsuno, T.; Ohtsubo, K.; Saito, M. *Pure Appl. Chem.* **1973**, *35*, 309-313.
Trenholm, H. L.; Cochrane, W. P.; Cohen, H.; Elliot, J. I.; Farnworth, E. R.; Friend, D. W.; Hamilton, R. M. G.; Neish, G. A.; Standish, J. F. *J. Am. Oil Chem. Soc.* **1981**, *58*, 992A-994A.
Trucksess, M. W.; Nesheim, S.; Eppley, R. M. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 40-43.
Ueno, Y., Ed. "Trichothecenes, Chemical, Biological and Toxicological Aspects"; Kodansha Ltd.: Tokyo and Elsevier: Amsterdam, 1983.
Vesonder, R. F.; Ciegler, A.; Jensen, A. H. *Appl. Microbiol.* **1973**, *26*, 1008-1010.
Vesonder, R. F.; Ciegler, A.; Rogers, R. F.; Burbridge, K. A.; Bothast, R. J.; Jensen, A. H. *Appl. Environ. Microbiol.* **1978**, *36*, 885-889.
Yoshizawa, T.; Morooka, N. *Agric. Biol. Chem.* **1973**, *37*, 2933-2934.

Received for review August 23, 1984. Revised manuscript received January 17, 1985. Accepted February 25, 1985.

Inhibition of the Formation of α-*p*-Dimethylstyrene and *p*-Cymen-8-ol in a Carbonated Citral-Containing Beverage System

Val E. Peacock*[1] and David W. Kuneman[1]

The addition of isoascorbic acid to a carbonated beverage system containing 3 ppm citral strongly inhibits the formation of α-*p*-dimethylstyrene (II) and *p*-cymen-8-ol (VI), which contribute to off-flavors in lemon juice. Previous attempts to inhibit this reaction of citral to II and VI with antioxidants have been unsuccessful. Increasing the pH and reducing storage temperature also significantly retard the formation of II and VI. Citric acid degradation of a carbonated beverage containing only citral resulted in the formation of *cis*- and *trans*-*p*-menth-2-ene-1,8-diols, *cis*- and *trans*-*p*-menth-1-ene-3,8-diols, *cis*- and *trans*-*p*-mentha-2,8-dien-1-ols, 2,3-dehydro-1,8-cineole, α-*p*-dimethylstyrene, *p*-cymen-8-ol, *p*-mentha-1,5-dien-8-ol, and *p*-mentha-1(7),2-dien-8-ol but not the disproportionation product α-terpineol as previously reported. In addition, two compounds suspected to be *cis*- and *trans*-isopiperitenols were observed in the above citral beverage. Lithium aluminum hydride reduction of a mixture of isopiperitenone and piperitenone yielded the same two compounds suspected to be the isopiperitenols but no piperitenol.

The extent of the food industry's interest in the acid-catalyzed cyclization of citral, a major component of lemon flavor, in aqueous citric acid is evidenced by the number of independent groups publishing results on this reaction (Clark et al., 1977; McHale et al., 1977; Kimura et al., 1983). This reaction not only reduces the intensity of the lemon flavor in a product due to decreased levels of citral, but also, as observed by Kimura et al., 1983, results in the formation of undesirable off-flavors in the product. Kimura et al., 1983, have blamed α-*p*-dimethylstyrene (II) and *p*-cymen-8-ol (VI), which are oxidation products of the citral cyclization products *p*-mentha-1(7),2-dien-8-ol (VII) and *p*-mentha-1,5-dien-8-ol (V), as being responsible for the undesirable flavor formed in aged lemon juice. Their attempts to inhibit the formation of these undesirable oxidation products by the addition of the antioxidants BHT, BHA, *n*-propyl gallate, α-tocopherol, nordihydroguaiaretic acid, or *n*-tritriacontane-16,18-dione to aqueous citral mixtures proved unsuccessful.

Philip Morris Inc., Beverage Research Center, Richmond, Virginia 23261.
Current address: The Seven-Up Company, St. Louis, MO 63114.

RESULTS AND DISCUSSION

The mechanism for the transformation of citral to V and VII, and then to II and VI is shown in Figure 1. In this scheme citral is cyclized to the menthadienols V and VII (Clark et al., 1977; McHale et al., 1977; Kimura et al., 1983), and these are then oxidized by what has been reported to be a disproportionation mechanism to the equilibrium mixture of II and VI (Kimura et al., 1983).

Effect of Temperature on the Formation of α-*p*-Dimethylstyrene (II) and *p*-Cymen-8-ol (VI). In Fig-

0021-8561/85/1433-0330$01.50/0