Kirksey, J. W., Cole, R. J., *Mycopathol. Mycol. Appl.* **54**, 291 (1974).
Kurtz, T. E., Link, R. F., Tukey, J. W., Wallace, D. L., *Technometrics* **7**, 95 (1965).
Marchelli, R., Vining, L. C., *J. Antibiot.* **28**, 328 (1975).
Nitsch, J. P., Nitsch, C., *Plant Physiol.* **31**, 94 (1956).
Pouchert, C. J., Campbell, J. R., "The Aldrich Library of NMR Spectra", Vol. IV, Aldrich Chemical Co., Inc., Milwaukee, Wis., 1974.
Takahashi, C., Yoshishira, K., Natori, S., Umeda, M., *Chem. Pharm. Bull.* **24**, 613 (1976).

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Gas Chromatographic–Mass Spectral Analyses of *s*-Triazine Metabolites

William R. Lusby* and Philip C. Kearney

Trimethylsilyl (Me_3Si) derivatives of six suspected metabolites of 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) were prepared by reaction with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 150 °C for 15 min. Three of the suspected metabolites, 2,4,6-trihydroxy-*s*-triazine (cyanuric acid), 2,4-dihydroxy-6-amino-*s*-triazine (ammelide), and 2,4-diamino-6-hydroxy-*s*-triazine (ammeline), yielded $(Me_3Si)_3$ derivatives. 2-Amino-4-ethylamino-6-hydroxy-*s*-triazine (*N*-ethylammeline) and 2,4-dihydroxy-6-ethylamino-*s*-triazine (*N*-ethylammelide) gave predominately the $(Me_3Si)_2$ derivitives. 2-Hydroxy-4,6-bis(ethylamino)-*s*-triazine (hydroxysimazine) yielded the Me_3Si product plus a lesser amount of the $(Me_3Si)_2$ derivative. The derivatives were analyzed by gas chromatography–mass spectrometry, with electron impact ionization (70 eV) and chemical ionization (isobutane). Peaks for the parent M, M – 15 (minus methyl), and trimethylsilyl (*m/e* 73) ions were among the largest in the electron impact spectra for all derivatives. The chemical ionization spectra confirmed the molecular weights of the derivatives.

s-Triazine herbicides rank number one in production of all herbicides. Their principal use is on corn, sorghum, and sugar cane. In 1974, 95 million pounds of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) were produced in the U.S., and 75 million pounds were used domestically (von Rumker et al., (1974).

Metabolites of *s*-triazine herbicides have been isolated from many sources. In 1969, Montgomery et al. isolated *s*-triazine metabolites from corn, and hydroxysimazine [2-hydroxy-4,6-bis(ethylamino)-*s*-triazine] was identified as a major product from simazine. Dehalogenated, dealkylated, and deaminated derivatives of the *s*-triazine herbicides have been isolated from several microbial systems (Kaufman and Kearney, 1970). In 1972, Bakke et al. isolated metabolites from the rat. Many other investigators have isolated *s*-triazine metabolites from other sources. Most *s*-triazine metabolites are highly polar and often cannot be analyzed directly by gas chromatography. Their polarity usually renders mass spectral analysis difficult, even by direct probe introduction.

For our investigation of the gas chromatographic–mass spectral analysis (GC–MS) of trimethylsilyl derivatives of *s*-triazine metabolites, we chose six compounds reported to be *s*-triazine herbicide metabolites or likely to be isolated in metabolic studies. The compounds were 2-hydroxy-4,6-bis(ethylamino)-*s*-triazine(hydroxysimazine), 2-amino-4-ethylamino-6-hydroxy-*s*-triazine (*N*-ethylammeline), 2,4-dihydroxy-6-ethylamino-*s*-triazine (*N*-ethylammelide), 2,4-diamino-6-hydroxy-*s*-triazine (ammeline), 2,4-dihydroxy-6-amino-*s*-triazine (ammelide), and 2,4,6-trihydroxy-*s*-triazine (cyanuric acid). The compounds were either supplied by CIBA-Geigy Corporation or obtained from commercial chemical supply houses.

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METHODS AND MATERIALS

The trimethylsilyl (Me_3Si) derivatives were prepared essentially by the method of Flint and Aue (1970). About 100 μg of the compound to be derivatized was placed into a 1-mL micro-reaction vial, and while the vial was swept with dry nitrogen, 100 μL of bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added. The vial was capped and heated at 150 °C for 15 min; excess BSTFA reagent was evaporated with a stream of dry nitrogen; then 100 μL of hexane was added. A portion of the hexane solution was analyzed by use of a Dupont 491B gas chromatograph–mass spectrometer which was fitted with a 6-ft by 2 mm i.d. 3% OV-17 column, temperature programmed from 155 to 235 °C at 4 °C/min. For electron impact spectra an ionizing voltage of 70 eV and a source temperature of 220 °C were used. For chemical ionization spectra, isobutane was used as the reagent gas and the source temperature was 170 °C.

RESULTS AND DISCUSSION

A gas chromatogram of Me_3Si derivatives of cyanuric acid, ammelide, ammeline, *N*-ethylammelide, *N*-ethylammeline, and hydroxysimazine is shown in Figure 1. Under our reaction conditions both the Me_3Si and $(Me_3Si)_2$ derivatives of hydroxysimazine were produced. Two derivatives, $(Me_3Si)_3$ and $(Me_3Si)_2$ were also formed from *N*-ethylammelide. For cyanuric acid, ammeline, and ammelide, the $(Me_3Si)_3$ derivatives were the predominant products. The reaction yielded the $(Me_3Si)_2$ derivative of

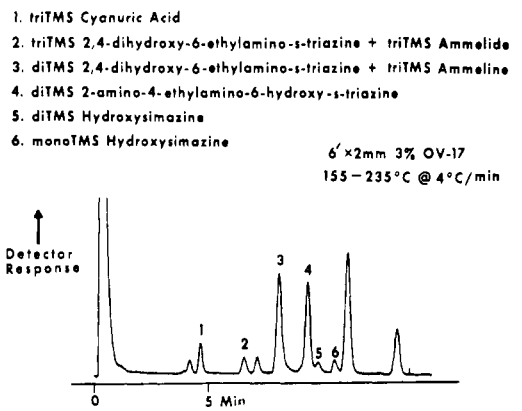

Figure 1. Gas chromatogram of trimethylsilyl derivatives of cyanuric acid, ammelide, *N*-ethylammelide, *N*-ethylammeline, and hydroxysimazine.

N-ethylammeline. The $(Me_3Si)_2$ derivative of ammelide coeluted with the $(Me_3Si)_3$ derivative of *N*-ethylammelide, and the $(Me_3Si)_2$ derivative of *N*-ethylammelide coeluted with the $(Me_3Si)_3$ derivative of ammeline.

The $(Me_3Si)_3$ derivative of cyanuric acid (Figure 2) yielded relatively few ions under electron impact conditions; the relative abundances of only five fragments exceeded 10%. The molecular ion (*m/e* 345) had a relative abundance of 75% while the fragment due to the loss of a methyl group (*m/e* 330) had a relative abundance of 23%. The base peak, at *m/e* 73, was due to the trimethylsilyl group. The fragment at *m/e* 100 was presumably due to $Me_2Si{=}O^+{-}CN$, which would be produced by a methyl loss from a trimethylsilyl group followed by detrimerization of the *s*-triazine ring. A likely structure for the fragment at *m/e* 147 is $Me_2Si{=}O^+{-}SiMe_3$, which would result from the successive formation of four-member cyclic intermediates in a manner similar to that reported by McCloskey et al. (1968).

Ammelide and ammeline derivatives fragmented by processes analogous to those of the silylated cyanuric acid (Figure 2). The additional fragment at *m/e* 285 in the spectrum for ammeline was probably due to the loss of a methyl radical, followed by the loss of a neutral molecule of isocyanic acid. Also present in the ammelide and ammeline spectra was a fragment at *m/e* 214 (13%, 34%); it was formed by the loss of a methyl radical, followed by the loss of the elements of trimethylsilyl cyanate or trimethylsilylcyanamide. The ammeline derivative gave a prominent fragment of *m/e* 99 due to $Me_2Si{=}N^+H{-}CH$, analogous to the $Me_2Si{=}O^+{-}CN$ from the cyanuric acid derivative.

The introduction of an ethylamino moiety into the remaining compounds permitted the facile loss of a neutral molecule of ethylene (Figure 3). This loss, preceded or followed by the loss of a methyl radical, accounted for the four prominent fragments of highest *m/e* for derivatives of *N*-ethylammeline, *N*-ethylammelide, and hydroxysimazine. These derivatives also underwent decomposition via the loss of elements of trimethylsilyl cyanate, trimethylsilylcyanamide, dimethylmethylenesilane, ethylcyanamide, hydrogen cyanide, and carbon monoxide from the ring.

A detailed examination of the fragmentation of the $(Me_3Si)_2$ derivative of *N*-ethylammeline indicated the participation of the side chains. We therefore propose the fragmentation scheme in Figure 4. For many of the ions shown (Figure 4), noncyclic structures are possible. The molecular ion I (*m/e* 299) can lose a methyl radical to form ion II (*m/e* 284), which can then lose a neutral molecule

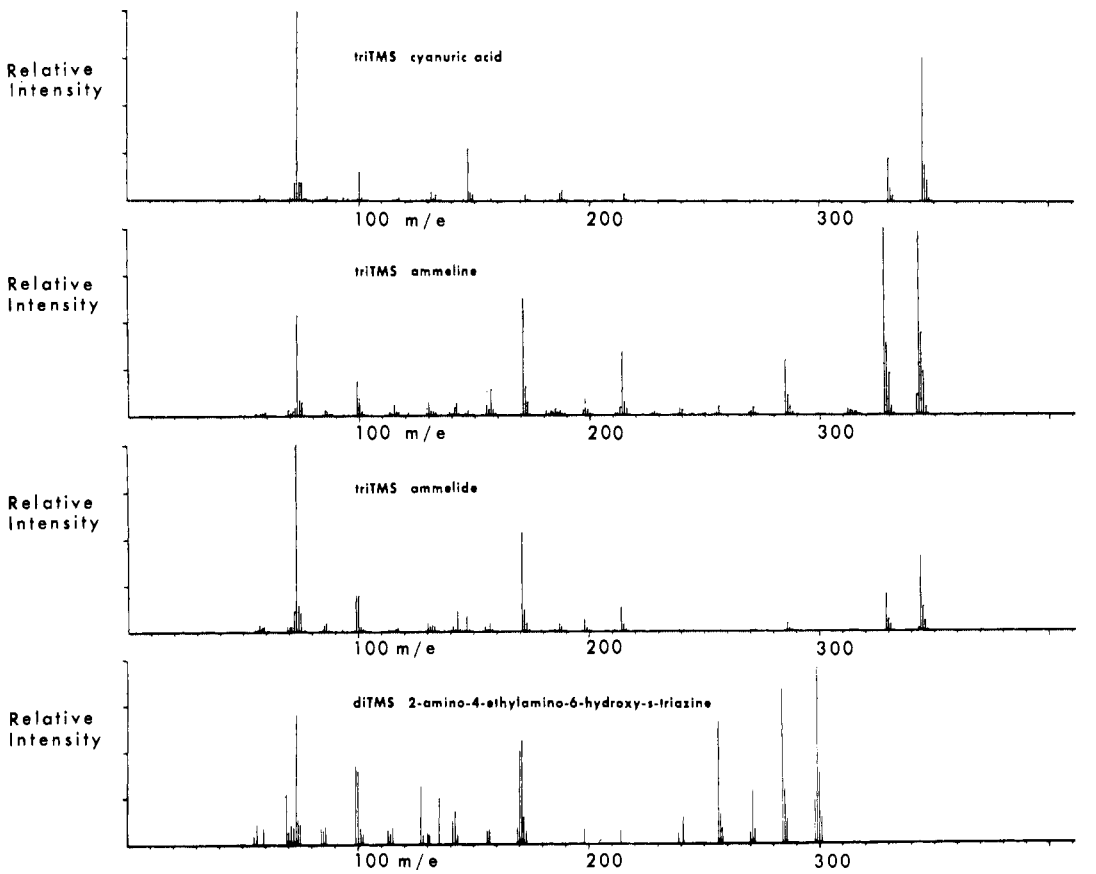

Figure 2. Mass spectra of tris(trimethylsilyl)cyanuric acid, tris(trimethylsilyl)ammeline, tris(trimethylsilyl)ammelide, and bis(trimethylsilyl)-2-amino-4-ethylamino-6-hydroxy-*s*-triazine (*N*-ethylammeline).

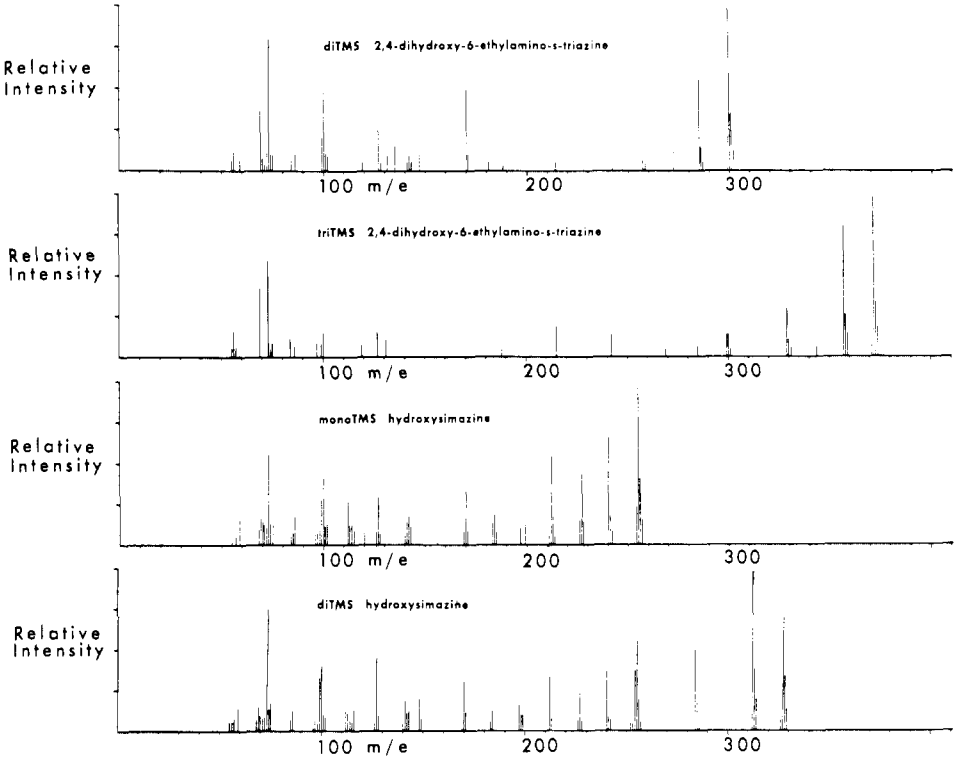

Figure 3. Mass spectra of bis(trimethylsilyl)-2,4-dihydroxy-6-ethylamino-*s*-triazine (*N*-ethylammelide), tris(trimethylsilyl)-2,4-dihydroxy-6-ethylamino-*s*-triazine(ammelide), (trimethylsilyl)hydroxysimazine, and bis(trimethylsilyl)hydroxysimazine.

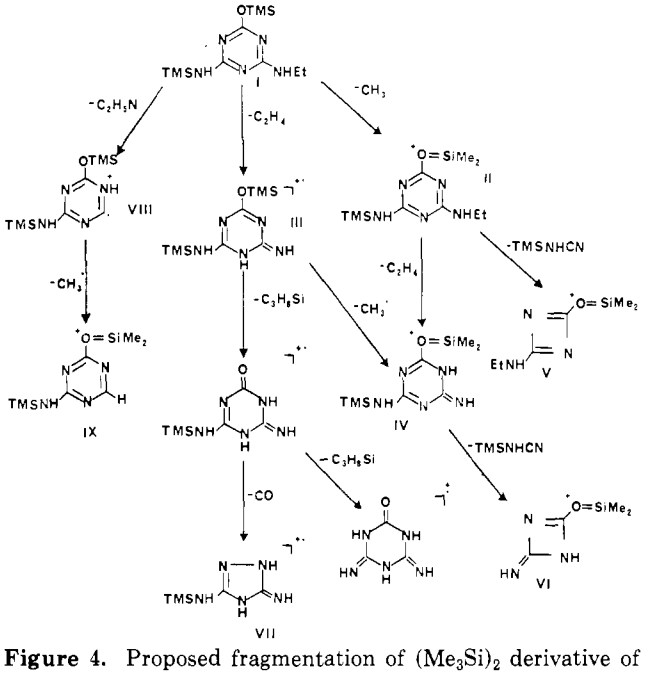

Figure 4. Proposed fragmentation of $(Me_3Si)_2$ derivative of *N*-ethylammeline.

of ethylene via a McLafferty type rearrangement to form ion IV (*m/e* 256). Alternatively the ethylene molecule can be lost first to give ion III (*m/e* 271): and then a methyl radical can be eliminated to yield ion IV. This quartet of intense fragments at the high *m/e* end of the spectrum characterized the trimethylsilyl derivatives of *s*-triazines having an ethylamine side chain. The loss of the elements of trimethylsilylcyanamide from ion II gave the intense fragment V at *m/e* 170. A similar loss from ion IV yielded fragment VI at *m/e* 142. By way of a McLafferty type rearrangement, the elements of dimethylmethylenesilane can be lost from ion III to give an ion at *m/e* 199, which can then lose a neutral molecule of carbon monoxide to give the five-member cyclic ion VII (*m/e* 171). Ion III can also lose the elements of dimethylmethylenesilane from the aminotrimethylsilyl group instead of the hydroxytrimethylsilyl group to give an ion at *m/e* 199, which can then lose a neutral molecule of hydrogen cyanide to give an ion at *m/e* 172 (pathway not shown). From the molecular ion, the ethylamine portion can be lost with a transfer of hydrogen to the ring, to give ion VIII (*m/e* 256), which has the same nominal mass as ion IV. Other investigators (Ross and Tweedy, 1970) using high-resolution mass spectrometry have reported elemental compositions for ions from both (1) the sequential loss of methyl group and ethylene and for (2) the loss via a McLafferty rearrangement of the ethylamino group from ethylamino-*s*-triazine compounds. From ion VIII a methyl radical can be given up to yield fragment IX at *m/e* 241. The fragments at *m/e* 100 and 99 in the mass spectrum of *N*-ethylammeline are probably due to $Me_2Si{=}O^+{-}CN$ and $Me_2Si{=}N^+H{-}CN$, respectively. The fragment at *m/e* 73 is due to the trimethylsilyl group. We believe that many of the remaining fragments result from losses involving trimethylsilyl cyanate in a manner analogous to the losses, shown in Figure 4, involving trimethylsilylcyanamide.

Chemical ionization gave the quasi-molecular ions as the base peaks for all derivatives. Silicon isotopic satellite peaks were observed at M + 2 and M + 3.

The techniques we used should be applicable to the analysis of the six suspected metabolites of simazine and related *s*-triazine herbicides. Progress on the identification of some of these metabolites in soils has been hampered by the lack of a sensitive, specific method. Provided that these suspected metabolites can be sufficiently purified, trimethylsilylation affords an excellent opportunity for verifying their presence.

LITERATURE CITED

Bakke, J. E., Larson, J. D., Price, *J. Agric. Food Chem.* **20**, 602 (1972).

Flint, G. T., Aue, W. A., *J. Chromatogr.* **52**, 478 (1970).

Kaufman, D. D., Kearney, P. C., *Residue Rev.* **32**, 235 (1970).
McCloskey, J. A., Stillwell, R. N., Lawson, A. M., *Anal. Chem.* **40**, 233 (1968).
Montgomery, M. L., Botsford, D. L., Freed, V. H., *J. Agric. Food Chem.* **17**, 1241 (1969).
Ross, J. A., Tweedy, B. G., *Org. Mass Spectrom.* **3**, 219 (1970).
von Rumker, R., Lawless, E. W., Meiners, A. F., "Production, Distribution, Use and Environmental Impact Potential of Selected Pesticides"; EPA 540/1-74-001, 1974.

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A Method for the Determination of 2-Imidazoline Residues in Food Crops

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A method was developed for the determination of 2-imidazoline in food crops at levels of from 0.1 to 1 ppm. The procedure involves adsorption on a cation-exchange resin, derivatization with *p*-nitrobenzoyl chloride, purification of the derivative on silica gel, and quantitation by ultraviolet absorption after high-pressure liquid chromatography. Mean recoveries ranged from 87.3 to 101% and were not affected by the presence of zineb, ethylenediamine, or ethylenethiuram monosulfide (DIDT).

2-Imidazoline has been identified in plant extracts as a degradation product of ethylenethiourea, an intermediate in the decomposition of ethylenebis(dithiocarbamate) fungicides (Vonk and Kaars Sijpesteijn, 1971). Plants grown outdoors and treated with labeled mancozeb have been reported to accumulate imidazoline in an amount equivalent to 8% of the applied radioactivity 2 weeks after treatment (Lyman, 1971). More recently, Vonk (1976) has examined the fate of [^{14}C]zineb on lettuce grown in a greenhouse and found a similar accumulation 3 weeks after application. The transformation of ethylenethiourea to 2-imidazoline can be carried out photochemically in the presence of a sensitizer (Vonk, 1975), although it has been concluded that while plant constituents are involved, the reaction in plants is probably not photochemical and may be nonenzymic. In view of the probable occurrence of 2-imidazoline in crops treated with ethylenebis(dithiocarbamate) fungicides, the following method was developed to determine such levels of this compound as may exist in foods.

EXPERIMENTAL SECTION

Materials. 2-Imidazoline was synthesized by the oxidation of ethylenethiourea with hydrogen peroxide as described by Vonk (1975). A solution of 30% hydrogen peroxide (32 g) was added dropwise to a stirred solution of ethylenethiourea (10 g) in water (200 mL). The temperature was maintained at 20–30 °C by cooling in an ice bath. After the addition of the peroxide, the solution was stirred for 2 h, then concentrated to approximately 50 mL on a rotary evaporator. The imidazoline was adsorbed on a 3.3 × 10 cm column of Dowex 50W × 8 (50–100 mesh) and the column washed with distilled water (150 mL). Imidazoline was eluted with 4 M HCl (100 ml) and the eluate taken to dryness on a rotary evaporator. The resulting syrup was placed in dessicator over NaOH pellets and evacuated. Crystallization occurred within 1–2 days.

The picrate of 2-imidazoline hydrochloride was prepared and melted at 165–166 °C (lit. 164–165 °C; Jentzsch and Seefelder, 1965). Thin-layer chromatography on cellulose with acetonitrile–water–ammonium hydroxide (80:18:2) as developing solvent gave a single purple spot (R_f 0.50) when sprayed with ferricyanide–nitroprusside reagent. A mass spectrum of the solid introduced on a probe at 85 °C showed a strong (base peak) molecular ion at m/e 70.

Solutions of imidazoline hydrochloride used for fortifying samples were prepared in distilled water and added to the samples in volumes of 0.5 mL or less before extraction.

Nitrobenzoylimidazoline standard was prepared by adding *p*-nitrobenzoyl chloride (1.86 g, 10 mmol) in acetone (10 mL) to 2-imidazoline hydrochloride (214 mg, 2 mmol) in 1 M aqueous sodium carbonate (15 mL). After stirring for 15 min, further 1 M sodium carbonate (25 mL) was added and the mixture was extracted with dichloromethane (25 mL). The crude product obtained by evaporation of the solvent was purified by chromatography on a 1.5 × 15 cm column of silicic acid (Woelm, activity I, 100–200 μm) in dichloromethane using 2% isopropyl alcohol in dichloromethane as eluting solvent. The purified material ran as a single spot on thin-layer chromatography using silica gel plates (Eastman) impregnated with fluorescent indicator and 2% isopropyl alcohol in dichloromethane as developing solvent. The compound had a melting point of 86–88 °C and gave a weak molecular ion at m/e 386 by electron impact mass spectrometry. Chemical ionization resulted in a molecular ion at m/e 387.

Cation-exchange resin (Dowex 50W × 8, 100–200 mesh) resin was purchased from Sigma Chemical Co., St. Louis, Mo., and was washed in bulk as described previously (Newsome, 1974). Ion-exchange columns containing 3.0 mL settled volume of resin were prepared exactly as described earlier (Newsome, 1974). The columns were eluted before use with 1 M NaOH (20 mL), 5 M NaCl (20 mL), 1 M HCl (20 mL), and finally distilled water until the effluent was neutral.

Silica gel for adsorption chromatography, 100–200 μm particle size, was manufactured by Woelm and was used without washing or deactivation.

p-Nitrobenzoyl chloride, 98% pure, was purchased from Aldrich Chemical Co. (Canada) Ltd., Montreal, Quebec. The reagent for nitrobenzoylation consisted of a 0.1% solution in dichloromethane and was prepared afresh before use.

Analytical Procedure. Samples (10.0 g) of previously blended crop were extracted with 0.1 M HCl (25 mL) by

Food Research Laboratories, Bureau of Chemical Safety, Foods Directorate, Health Protection Branch, Health and Welfare Canada, Tunney's Pasture, Ottawa, K1A 0L2, Ontario, Canada.

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