Total Residues of 2-Naphthoxyacetic Acid, 2-(6-Hydroxynaphthoxy)acetic Acid, and 2-(7-Hydroxynaphthoxy)acetic Acid in Tomato Plants and Fruit by High-Performance Liquid Chromatography

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A study was undertaken to determine the total residues including the sugar- and protein-bound residues of 2-naphthoxyacetic acid (BNOA), 2-(6-hydroxynaphthoxy)acetic acid (6-OH-BNOA), and 2-(7-hydroxynaphthoxy)acetic acid (7-OH-BNOA) in tomato fruit, blossoms, and leaves from plants treated with BNOA. Analyses were by high-performance liquid chromatography. There were no detectable residues of 7-OH-BNOA at a method sensitivity of 0.05 ppm on any of the samples and <0.05 ppm of BNOA and 6-OH-BNOA on the tomato fruit. Tomato plant leaves had small amounts of BNOA and 6-OH-BNOA.

Various chemicals of the plant growth regulating hormone types such as BNOA, 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid have been investigated for their effects on tomatoes (Mann and Minges, 1949; Wittwer and Reath, 1952). Emphasis in these studies was on tomatoes grown for early market production rather than on those grown for processing.

Rathore et al. (1983) separated standard mixtures of organic acids including 2-naphthoxyacetic acid (BNOA) by paper chromatography. Thin-layer chromatography has also been used for separations of similar compounds (Gupta et al., 1984; Rathore et al., 1985). Baldi et al. (1985) homogenized apples and tomatoes in acidified acetone for extraction and analyzed 1-naphthaleneacetic acid and BNOA as their pentafluorobenzyl derivatives by gas–liquid chromatography. Fechner (1981) analyzed BNOA as its methyl ester and 2-naphthol after separation by high-performance liquid chromatography. Archer and Stokes (1980) determined the levels of BNOA and 2-naphthol on field-sprayed tomato blossoms, leaves, and fruits by high-performance liquid chromatography and an ultraviolet light detector.

An inclusive computer search of the literature from 1967 to 1986 did not show any information about the two possible metabolites of 2-naphthoxyacetic acid (BNOA), the plant growth regulator, namely 2-(6-hydroxynaphthoxy)acetic acid (6-OH-BNOA) and 2-(7-hydroxynaphthoxy)acetic acid (7-OH-BNOA) (CAS, STN, 1967–1986).

The present study was undertaken to determine the total residues including the sugar- and protein-bound residues of BNOA, 6-OH-BNOA, and 7-OH-BNOA in tomato fruit, blossoms, and leaves from plants treated with BNOA. No reports are in the current literature concerning the detection or levels of 6-OH-BNOA or 7-OH-BNOA, which are possible metabolites of BNOA when applied to materials such as tomato blossoms or plants.

MATERIALS AND METHODS

Reagents. Pesticide-grade acetone and acetonitrile, reagent-grade anhydrous ethyl ether, and HPLC-grade methanol were the purified solvents used. Silica gel, grade 923, was used as received from Davison Chemicals, Baltimore, MD.

BNOA was provided by Chevron Chemical, Ortho Division, San Francisco, CA. 6-OH-BNOA and 7-OH-BNOA were prepared by reacting equimolar amounts of the corresponding naphthalenediol with chloroacetic acid, and they were purified by liquid chromatography.

The 2,6-naphthalenediol was acquired from Aldrich and purified in the following manner: A mixture of 5.0 g of technical-grade material, 5.8 mL of acetic acid anhydride, and 8.4 g of sodium acetate was heated under reflux for 3 h. While still warm, the mixture was poured into 100 mL of water with stirring. The precipitate that formed, after drying in air, weighed 6.73 g (88%); mp 172–174 °C [lit. (Chatt and Wynne, 1943) mp 173 °C]. The crude 2,6-diacetoxynaphthalene was recrystallized from ether–methylene chloride to give 4.14 g of shimmering white needles, mp 176–177.5 °C. A mixture of 50 mL of degassed (N_2) 1 N aqueous sodium hydroxide and 2.02 g of the diester was heated with hot water until it became homogeneous. About 30 min was required. The solution was cooled and acidified to pH 5.3 with 3 N hydrochloric acid to precipitate the diol. The yield of dried product was 1.07 g; mp 222–223 °C [lit. mp 222 °C].

6-OH-BNOA was prepared by adding 0.78 g of purified 2,6-naphthalenediol to 4.0 mL of 5.0 N aqueous sodium hydroxide that had been well flushed with nitrogen. The mixture, under nitrogen, was stirred and heated in a boiling water bath for about 10 min to dissolve the diol, and 0.46 g of chloroacetic acid was added in one portion. Heating and stirring were continued for 45 min, during which time a small amount of precipitate formed. After the mixture was allowed to cool to room temperature, it was acidified with 6 N hydrochloric acid and stirred for an additional 10 min to coprecipitate the product and untreated diol. When dry, the light brown solid weighed 0.85 g. A portion was dissolved in 50% aqueous methanol containing 0.1% phosphoric acid and analyzed by HPLC on a Whatman Partisil M9 ODS 3 column with a flow rate of 6.0 mL/min. At 260 nm, relative responses of 43, 51, and 5 were observed for the diol, 6-OH-BNOA, and 2,6-naphthalenedioxyacetic acid. TLC (SiO_2, acetone) gave R_f values of 0.68, 0.37, and 0.03 for the diol, 6-OH-BNOA, and the diacid. Column chromatography (SiO_2, acetone) of a 170-mg portion of the crude product gave 78 mg of 6-OH-BNOA as a light gray solid, mp 164–166 °C [lit. (Byrde et al., 1956) mp 165–166 °C]. ^{1}H NMR (360 MHz, acetone-d_6): δ 4.8 (s, CH_2), 7.11 (d, J = 9 Hz, C_3-H or C_7-H), 7.21 (br s, C_1-H or C_5-H), 7.62 (d, J = 8.0 Hz, C_4-H or C_8-H), 7.66 (d, J = 10.09 Hz, C_4-H or C_8-H). MS (70 eV) m/z (rel intens): 218 $[M]^+$ (100), 160 $(C_{10}H_8O_2)^+$ (32), 159 $(C_{10}H_7O_2)^+$ (91), 143 $(C_{10}H_7O)^+$ (12), 131 $(C_9H_7O)^+$ (80),

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Table I. Residues of BNOA and 6-OH-BNOA on Field-Treated Tomato Leaves and Blossoms[a]

spray treatment no.	size of fruit, g	expt day no.	residues,[b] ppm: leaves BNOA	leaves 6-OH-BNOA	blossoms BNOA	blossoms 6-OH-BNOA
1		0	2.40 ± 0.18	<0.050	4.80 ± 0.23	<0.050
	23	5	0.255 ± 0.019	0.140 ± 0.010		<0.050
		10	<0.050	0.238 ± 0.017		<0.050
	53					
2		10	1.62 ± 0.12	0.223 ± 0.016	2.54 ± 0.12	<0.050
	84	15				<0.050
		20	<0.050	0.261 ± 0.018		<0.050
	136					
3		20			1.88 ± 0.09	<0.050
	161	25				<0.050
		35	0.079 ± 0.006	0.132 ± 0.009		
		42	<0.050	0.072 ± 0.005		
	248	49	<0.050	<0.050		

[a] Whole plant sprayed with 84 ppm BNOA. [b] There were no detectable residues of 7-OH-BNOA at a method sensitivity of 0.05 ppm on any of the samples and <0.05 ppm of BNOA and 6-OH-BNOA on the tomato fruit. Analysis of untreated samples showed no interference.

115 $(C_9H_7)^+$ (14), 77 $(C_6H_5)^+$ (16).

The same procedure was used to convert 5.00 g of commercial 97% 2,7-naphthalenediol (Aldrich) and 2.95 g of chloroacetic acid to 6.22 g of crude 7-OH-BNOA. TLC (SiO_2, 2:1:0.1 ethyl acetate–methanol–acetic acid) gave R_f values of 0.75, 0.59, and 0.36 for the diol, 7-OH-BNOA, and 2,7-naphthalenedioxyacetic acid. A portion was dissolved in 45% aqueous methanol containing 0.1% phosphoric acid and analyzed by HPLC as described above. At 260 nm relative responses of 10, 80, and 10 were observed for the diol, 7-OH-BNOA, and the diacid; HPLC of 0.50 g of the crude product gave 0.33 of light gray solid, mp 209.5–210.5 °C; the literature melting point (Zhang et al., 1979) was 208–210 °C. ^1H NMR (360 MHz, acetone-d_6): δ 4.82 (s, CH_2), 7.01 (br app d, J = 9 Hz, C_3-H and C_6-H), 7.10 (d, J = 3.6 Hz, C_1-H or C_8-H), 7.14 (d, J = 2.2 Hz, C_1-H or C_8-H), 7.70 (d, J = 9 Hz, C_4-H or C_5-H), 7.72 (d, J = 9 Hz, C_4-H or C_5-H. MS (70 eV) m/z (rel intens) 218 $[M]^+$ (100), 160 $(C_{10}H_8O_2)^+$ (31), 159 $(C_{10}H_7O_2)^+$ (7), 143 $(C_{10}H_7O)^+$ (23), 131 $(C_9H_7O)^+$ (82), 115 $(C_9H_7)^+$ (15), 77 $(C_6H_5)^+$ (16).

High-Performance Liquid Chromatography. The HPLC system consisted of a Waters 6000A pump delivering 1 mL/min of mobile phase through a 3.9 mm (i.d.) × 15 cm stainless steel column packed with μ-Bondapak C18 to a Kratos FS 970 fluorescence detector with excitation set at 223 nm and a 330-nm cutoff filter on the emission side. The mobile phase was prepared by mixing 15 mL of acetonitrile, 85 mL of water, and 0.1 mL of glacial acetic acid. Samples were introduced through a Waters Model 710B automatic sample injector, and data processing was done by a Digital Equipment Professional Model 350 computer with a 10-Mbyte Winchester hard disk and Waters 840 software.

Application of BNOA to Plants. Tomatoes (variety UCT-175) were planted in two rows 10 m long and 0.5 m wide in field plots at the University of California at Davis. An 84 ppm solution of BNOA was prepared by dissolving 3.14 g of Berry-Set (Science Products, Chicago, IL) in 1 L of water. The mixture was applied with a Hudson Sprayer, 8.5-L capacity, fitted with 0.95-cm-i.d. Teflon-lined, flexible neoprene rubber tubing between the pressure tank and the spray nozzle. A continuous fine spray covered the entire plant.

There were three applications to the same tomato plants in duplicate plots, spaced 10 days apart (Table I). Blossoms were sampled every 5 days for 25 days; leaves were sampled on days 0, 5, 10, 20, 35, 42, and 49; and tomato fruit was sampled on days 5, 10, 15, 20, 25, and 49. No tomatoes were on the plants at the initial spraying.

Extraction of Samples. Duplicate samplings of 25 g of tomato fruit, 6 g of leaves, or 3 g of blossoms were weighed into a 500-mL stainless steel blender cup. Untreated controls were fortified at this point for recovery studies with analytical-grade standards. Fifty milliliters of 1 N HCl was added to the fruit, 25 mL to the leaves and to the blossoms. After the mixture was blended at medium speed for 2 min and the contents poured into a clean 500-mL round-bottom flask, the blender jar was rinsed with one extraction volume of 1 N HCl. The sample was then placed on a heating mantle, attached to a condenser, and boiled under reflux for 30 min. After being cooled in an ice bath, the sample was filtered through glass wool, and solids were returned to the round-bottom flask along with one volume of 1 N HCl and refluxed another 30 min. Refluxing a third time for 30 min completed the extraction step.

The volume of the combined filtrates was measured, and an aliquot equivalent to 5 g of tomato fruit, 2 g of leaves, or 1 g of blossoms was placed in a 100-mL beaker. The pH was raised to 9.0 by dropwise addition of concentrated ammonium hydroxide. In a 250-mL separatory funnel, the now-alkaline extract was partitioned twice with 40 mL of ethyl ether, and then the combined ether phases were shaken with 10 mL of water. The aqueous phases were pooled in a 500-mL round-bottom flask to which was added 25 mL of methanol to prevent foaming. This solution was evaporated to dryness in a rotary evaporator at 40 °C and reduced pressure, beginning with 200 mbars and finishing with approximately 10 mbar. Twenty-five milliliters of methanol was added to the flask, which was again evaporated to dryness. Ten milliliters of acetone was placed in the flask, and the salts were loosened from the sides with a spatula. The sample was acidified with 0.2 mL of concentrated phosphoric acid, mixed well, and filtered through a 7-cm Whatman No. 1 filter paper into a 19 × 150 mm glass-stoppered test tube. The flask and filter were rinsed with four 5-mL portions of acetone and the combined filtrates concentrated to 1 mL in a 30 °C water bath under a gentle stream of nitrogen. Two milliliters of ethyl ether was added, and the sample was cleaned up on a silica gel column.

Cleanup. A glass wool plug was placed in a 40 × 1 cm (i.d.) column, followed by 5 g of silica gel in an acetone slurry. When the silica gel had settled, it was topped with glass wool and the acetone allowed to drain to the top of the adsorbent, which was then rinsed with 10 mL of ether. When the ether had drained to the top of the silica gel, the sample was added followed immediately by 5 mL of

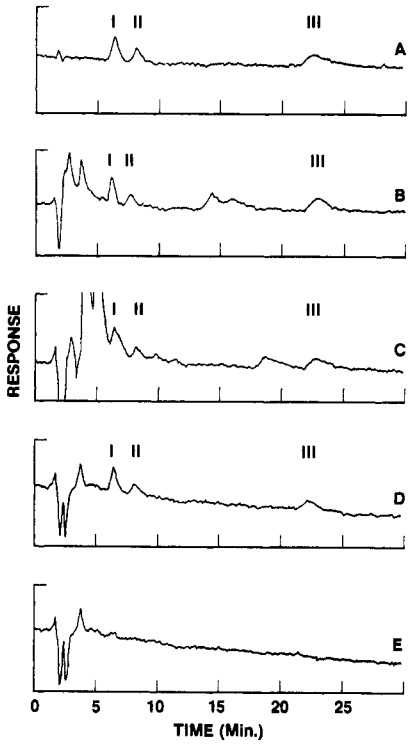

Figure 1. Typical high-performance liquid chromatograms of (A) 0.2 ng of 6-OH-BNOA (I), 0.2 ng of 7-OH-BNOA (II), and 0.2 ng of BNOA (III); (B) 4 mg of untreated tomato leaves fortified with 0.05 ppm of I–III; (C) 4 mg of untreated tomato blossoms fortified with 0.05 ppm of I–III; (D) 4 mg of untreated tomatoes fortified with 0.05 ppm of I–III; (E) 4 mg of treated tomatoes sampled 49 days after initial application.

mixed hexanes. A 100-mL round-bottom flask was placed under the column and the test tube rinsed with 2 mL of acetone. The sample was eluted with 50 mL of 1% v/v acetic acid in ethyl ether. The eluate was concentrated at 30 °C and 460 mbar to 1 mL and then transferred to a 15-mL graduated centrifuge tube. The flask was rinsed with 1-mL portions of mobile phase, the total volume not exceeding 5 mL. Any remaining ether was evaporated over the mobile phase under a gentle stream of nitrogen. The sample was diluted with mobile phase to an appropriate volume for analysis by HPLC.

RESULTS AND DISCUSSION

Typical chromatograms for a field-treated tomato sample harvested 49 days after initial application for standards of 6-OH-BNOA, 7-OH-BNOA, and BNOA and for untreated tomato fruit, leaves, and blossoms fortified with 0.05 ppm of each standard are shown in Figure 1. The retention time for 6-OH-BNOA was 6 min, for 7-OH-BNOA, 8 min, and for BNOA, 23.5 min. Recoveries at fortification levels of 0.05, 0.1, and 0.25 ppm on untreated tomato fruit were 76.0–92.0% for 6-OH-BNOA, 76.0–93.0% for 7-OH-BNOA, and 76.0–84.0% for BNOA. On untreated leaves the corresponding recovery ranges were 71.0–78.0%, 79.0–96.0%, and 80.0–88.0%. On untreated blossoms the corresponding recovery ranges were 77.0–90.0%, 72.0–85.0%, and 79.0–90.0%. The tomato fruit chromatogram is obviously a straight base line since no residues were detected.

The samples were refluxed with 1.0 N HCl to attempt to release any glucoside and protein-bound conjugates of BNOA. Table I shows that on the blossoms and tomatoes after refluxing there were no 6-OH-BNOA or 7-OH-BNOA residues detected (<0.05 ppm). On the leaves no 7-OH-BNOA residues were detected but 6-OH-BNOA residues were measured to day 49 after the experiment was initiated. No residues of BNOA, 6-OH-BNOA, and 7-OH-BNOA were detected (<0.05 ppm) on the tomato fruit at any sampling.

From the time buds appear on the tomato plant until they set, the usual time required is about 5–10 days. The time required from full blossom to fruit set is about 5 days. Therefore, when blossoms are sprayed at the recommended time interval of 10 days, no blossom could be sprayed more than once. When the full blossoms were sampled and analyzed according to the spray treatment schedule in Table I up to day 20, no 6-OH-BNOA or 7-OH-BNOA was found within the method sensitivity level (0.05 ppm). No interferences were observed in the control samples.

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Registry No. BNOA, 120-23-0; 6-OH-BNOA, 10441-36-8; 7-OH-BNOA, 63037-96-7; chloroacetic acid, 79-11-8; 2,6-naphthalenediol, 581-43-1; 2,7-naphthalenediol, 582-17-2.

LITERATURE CITED

Archer, T. E.; Stokes, J. D. "Residue Analysis of β-Naphthoxyacetic Acid and β-Naphthol on Field-Sprayed Tomatoes by High-Pressure Liquid Chromatography". *J. Agric. Food Chem.* **1985**, *28*, 877–880.

Baldi, M.; Bovolenta, A.; Penazzi, L.; Pederzini, C.; Buriani, S.; Pacaterra, F. "GLC-ECD Determination of α-Naphthaleneacetic and β-Naphthoxyacetic Acids after Derivatization with Pentafluorobenzyl Bromide and Confirmation by HRGC-M.S.-E.I.". *Ind. Conserv.* **1985**, *60*, 304–309.

Byrde, J. R. W.; Harris, J. F.; Woodcock, N. "Fungal Detoxication. The Metabolism of ω-(Naphthyloxy)-*n*-alkylcarboxylic Acids by *Aspergillus niger*". *Biochem. J.* **1956**, *64*, 154–160.

Chatt, J.; Wynne, W. P. "1,3,5,7-Tetranitronaphthalene and the Isomeric Tetranitro-Derivatives Obtained from 2,6-Dinitronaphthalene by Nitration". *J. Chem. Soc.* **1943**, 33–36.

Chemical Abstracts Service, STN International Online Computer Search, 1967–1986, Columbus, OH 43210.

Fechner, R. "Liquid Chromatographic Determination of Beta-naphthoxy-acetic Acid and Beta-naphthol, Its Metabolite". *Tag. Akad. Landwirtschaftswiss. DDR* **1981**, *187*, 159–165.

Gupta, S.; Rathore, H.; Ali, I.; Ahmed, S. "Thin Layer Chromatographic Studies of 30 Organic Acids on Calcium Sulfate". *J. Liq. Chromatogr.* **1984**, *7*, 1321–1340.

Mann, L.; Minges, P. "Experiments on Setting Fruit with Growth-regulating Substances on Field-Grown Tomatoes in California". *Hilgardia* **1949**, *19*, 309–337.

Rathore, H.; Kumari, K.; Garg, M. "Chromatographic Studies of 34 Organic Acids on Papers Impregnated with Hydroxides of Aluminum and Cadmium". *J. Liq. Chromatogr.* **1983**, *6*, 973–981.

Rathore, H. S.; Kumari, K.; Agrawal, M. "Quantitative Separation of Citric Acid from Trichloroacetic Acid on Plates Coated with Calcium Sulfate Containing Zinc Oxide". *J. Liq. Chromatogr.* **1985**, *8*, 1299–1317.

Straub, J.; Widmer, W.; Schneider, H. U.S. Patent 2 452 171, Oct 26, 1948; *Chem. Abstr.* **1949**, *43*, 7698a.

Wittwer, S. H.; Reath, A. N. "Early Tomato Production in Michigan". *Mich., State Coll. Agric. Appl. Sci., Exp. Stn. Circ. Bull.* **1952**, *No. 220*, 35 pp.

Zhang, H.-L.; Qu, C.-J.; Chen, R.-L.; Gao, Y.-S. "Tumor Chemotherapy. XXXV. Synthesis of Derivatives of Some Plant Growth Regulators and Their Antitumor Activity". *Yao Hsueh Hsueh Pao* **1979**, *14*, 302–308.

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