Gas Chromatographic Analysis of Neodecanoic Acids in Onions

A method is described for determining residues of the desiccant, neodecanoic acid (NDA), in onion bulbs. Following isolation by selective methylation and removal of plant acids, NDA is converted to the pentafluorobenzyl derivative and determined by electron affinity gas chromatography. The method is capable of detecting about 0.08 ppm of NDA and has been applied to analysis of field samples.

Neodecanoic acid (NDA) is a mixture of di-α-substituted saturated decanoic acids sold by Agway, Inc. under the trade name Topper 5-E. Several recent publications have described the effective use of this chemical as a desiccating agent for onion foliage, prior to harvesting (Pendergrass et al., 1969, 1976; Isenberg and Abdel-Rahman, 1972). Previous reports have described the analysis of NDA using thin layer (Mizany, 1967; St. John and Lisk, 1969) and its possible translocation by liquid scintillation chromatography (Gilbert et al., 1974) with sensitivities of 1 and 0.01 ppm, respectively. The use of pentafluorobenzyl bromide (PFBB) in producing highly electron-capturing derivatives of carboxylic acids and phenols has found numerous applications in drug-screening programs. In the work reported, NDA is quantitatively determined by gas chromatography after derivitization with PFBB.

EXPERIMENTAL SECTION

Neodecanoic acid manufactured by the Exxon Chemical Co. as well as radioactive NDA (specific activity 0.77 μCi/mg; New England Nuclear, Boston, Mass.) were supplied by Agway, Inc. Radioactivity was determined by liquid scintillation counting using a Packard Tri-Carb Model 3310 instrument. Scintillation mixtures consisted of either *p*-dioxane and methyl Cellosolve (Wang and Willis, 1965) or of 2,5-diphenyloxazole (PPO) (0.6%) and 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) (0.005%) in 10 ml of toluene.

Onion bulbs were prepared for analysis by removing the outermost loose scales containing soil debris. A 40-g portion of chopped and mixed onion tissue was blended twice with 50 ml of acetone–orthophosphoric acid (80:1). The solution was filtered through S&S No. 595 paper and the volume adjusted to 100 ml. A 25-ml aliquot of the acid–acetone mixture was evaporated to about 10 ml at room temperature using a gentle stream of air and refluxed for 10–15 min with 5 ml of BF_3–methanol reagent (Mizany, 1967). Forty milliliters of water was added to the refluxed mixture which was then partitioned with two 5-ml portions of hexane. The hexane was extracted twice with 25 ml of 2% Na_2CO_3. The aqueous solution was acidified by adding 4 ml of HCl (5 *N*) and partitioned with two 5-ml portions of ethyl acetate.

NDA was next prepared for gas chromatography by reaction with PFBB. One hundred microliters of sequanol grade triethylamine (TEA) (Pierce Chemical Co., Rockford, Ill.) and 20 μl of PFBB (Pierce Chemical Co.) were added to the above combined ethyl acetate extracts contained in a screw-top test tube. The reaction mixture was held at 70°C for 15 min in the closed tube. After cooling, the tube cap was removed. The solution was evaporated to about 0.25 ml at 70°C with the aid of a gentle stream of air. The solution was finally evaporated to dryness with air at room temperature. The air stream was passed over the residue for an additional 5 min following dryness. The residue was dissolved in 10 ml of reagent grade cyclohexane and up to 1 μl of the resulting solution was analyzed by gas chromatography. The instrument was a Varian Aerograph Model 705 equipped with a ^{63}Ni detector. Nitrogen (26 cm^3/min) was the carrier gas. The column was 1.2 m long, 2 mm i.d. and packed with 3% OV-101 on 100–120 mesh Gas-Chrom Q. Operating temperatures were as follows: injector, 195°C, column, 125°C, and detector, 245°C.

Table I. Recovery of NDA from Onion Bulb Tissue

NDA concn, ppm	% recovery (recovery ± std dev)	No. of replicates
5	89 ± 10	12
1	84 ± 8	15
0.25	85 ± 13	3
0.1	66 ± 4	3

RESULTS AND DISCUSSION

In Figure 1 are illustrated gas chromatograms of field harvested onion bulbs which had been treated at 40 lb of NDA per acre and untreated (control) onions. As is evident in this figure, the partially resolved peaks labeled A, B, C, and D appeared at the same respective retention times and with approximately the same relative peak heights in gas chromatograms of derivatized NDA standards and NDA fortified (control) onion samples. Residues of NDA were calculated based on measurement of the peak height of peak D along its trailing edge. The sample shown in the upper chromatogram in Figure 1 was thereby calculated to contain 1.2 ppm of NDA. The results of analysis of NDA in three replicates of this field sample by this method were 1.0, 1.2, and 1.5 ppm. Somewhat greater separation of peaks A–D was achieved with a 1.8 m × 2 mm column containing the same type of packing material but the peaks were correspondingly broader and smaller. Poorer resolution was effected by columns containing 3% OV-17 on 100–120 mesh Gas-Chrom Q, 10% DC-200 on 80–100 mesh Gas-Chrom AW, 2% OV-210 and 1.5% OV-17 on 80–100 mesh Chromosorb W, or 0.25% EGS and 0.75% DEGS on 80–100 mesh Chromosorb W.

The recovery of NDA added to control onions is shown in Table I. Recovery losses of NDA were also investigated using radioactive NDA and liquid scintillation counting (lsc). Onion tissue was fortified with 1 ppm of [^{14}C]NDA and aliquots from each partition step as well as from the cyclohexane solution prepared after derivatization were assayed by lsc. Negligible losses of activity occurred in the extraction or partitioning steps. The overall recovery of labeled NDA was 81% and loss of radioactivity primarily occurred during evaporation of the ethyl acetate extract. Harvest residues of NDA on onions have been found to be in the range of 1 ppm when the compound is used at recommended rates of application (St. John and Lisk, 1969; Gilbert et al., 1974).

Figure 2 illustrates the effect of temperature on the derivatization reaction. The evaporation to dryness following the derivatization reaction was conducted at room temperature. As is evident, the optimum reaction temperature was in the range of 70°C. Figure 3 shows the effect of the quantity of PFBB reagent on the derivatization reaction. Maximum chromatographic peak response resulted when 20–30 μl of the reagent was used. Twenty microliters of PFBB was used since larger portions of the

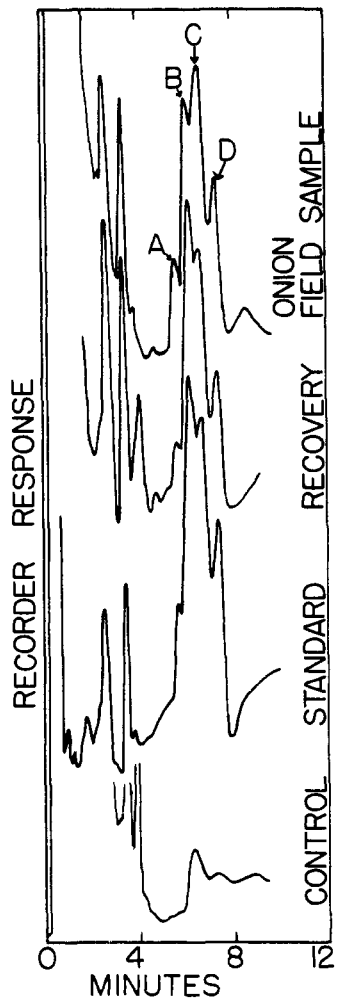

Figure 1. Chromatograms (top to bottom) of (1) onions field treated with 40 lb of NDA per acre, (2) 1 ppm of NDA added to control onions, (3) standard NDA (0.1 ng), and (4) control onions.

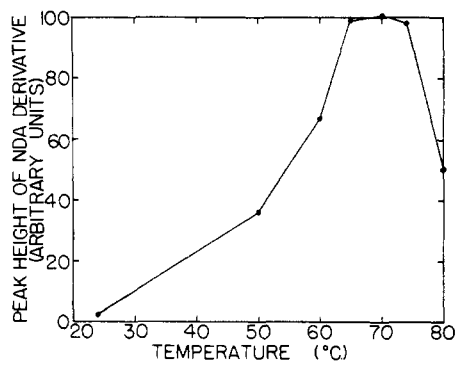

Figure 2. Effect of temperature on the derivatization reaction.

reagent resulted in larger extraneous peaks in the chromatograms. This quantity of reagent is several-fold greater than that routinely used for several phenols and carboxylic acids not having the neo configuration (Ehrsson, 1971; Maylin, 1975) and may be necessary since the carboxyl group of NDA is sterically hindered by the neo configuration. The derivatization reaction was also affected by

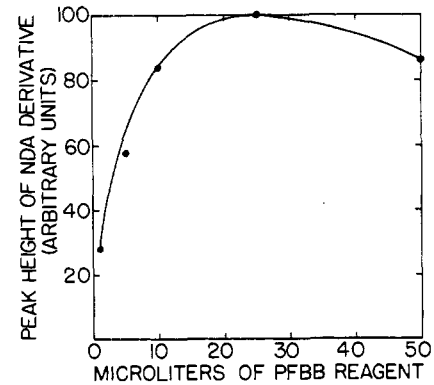

Figure 3. Effect of PFBB concentration on the derivatization reaction.

the level of TEA. For example, the use of 50 and 10 μl of TEA per sample resulted in 17 and 85% reduction, respectively, in NDA peak response as compared to that obtained with 100 μl of TEA per reaction.

Interfering materials are removed during evaporation of the ethyl acetate just prior to gas chromatography. Significant losses of product may occur by evaporation to dryness at temperatures greater than room temperature. The described analytical procedure is rapid and reproducible. The method has a limit of detection of about 0.08 ppm of NDA in onion bulb tissue which would correspond to a peak height of about 10% of full scale recorder deflection.

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