Determination of *N-m*-Tolyl Phthalamic Acid Residues in Food Crops

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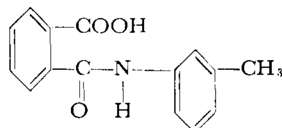
A method is described for the determination of *N-m*-tolylphthalamic acid residues at the 0.1-p.p.m. level. A caustic distillation hydrolyzes the chemical liberating *m*-toluidine which distills into aqueous acid, forming an acid salt. The *m*-toluidine is then diazotized with nitrous acid and coupled with *N*-naphthylethylenediamine. The azo compound formed appears as a magenta coloration and is measured at 560 mμ in 10-cm. cells. The determination has been applied to tomatoes, Lima beans, strawberries, and cherries.

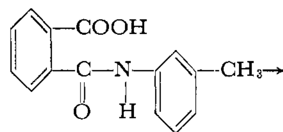
Phthalamic acid derivatives have been shown to produce varying degrees of growth response in tomatoes and other crops. In particular, *N-m*-tolylphthalamic acid (Duraset) shows promise for increasing yields of Lima beans and tomatoes (*3*, *5*). It also increases flower production and prevents abscission of the flower and young fruit. To determine whether residues were present at harvest time, a very sensitive analytical method was necessary.

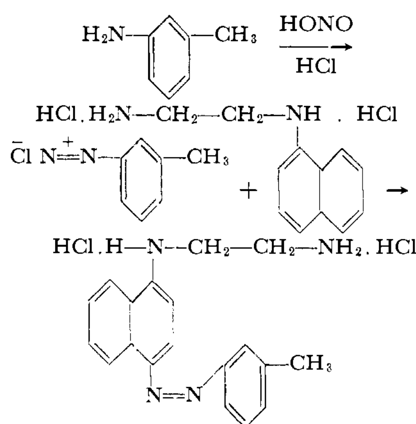
As the chemical structure of Duraset is



the most favorable approach seemed to be hydrolysis of the amide linkage and determination of the amine. Coupling with diazotized sulfanilic acid was tried first, as this had been very effective for the microdetermination of 1-naphthylamine in *N*-1-naphthylphthalamic acid (Alanap) (*4*). However, when applied to Duraset the color formed was not sufficiently sensitive at the desired level of 0.1 p.p.m.

Reported in the literature for parathion (*1*, *2*) is a method in which an aromatic amine is diazotized and then coupled with *N*-1-naphthylethylenediamine dihydrochloride. The color is extremely sensitive and the method seemed suited to the Duraset problem. When applied to Duraset, the chemistry of the reaction was as follows:





After separation and concentration steps, Duraset was found recoverable from untreated samples at the 0.1-p.p.m. level, and this method was adopted.

Analytical Method

Apparatus and Special Reagents. A 250-ml. double-thickness distillation flask with side arm and condenser equipped with ball and socket joints. The apparatus is described fully in the method for *N*-1-naphthylphthalamic acid residues (*4*).

N - 1 - Naphthylethylenediamine dihydrochloride, 1%, stored in a brown bottle. This reagent should be made fresh every day.

Procedure. Add 100 grams of sample (homogenized in a Waring Blendor or like device) to a double-thickness distillation flask. (Fresh Lima beans require 1 part of water to 3 parts of beans to facilitate homogenization.) Add approximately 1 gram of 20- or 30-mesh granular zinc and approximately 1 gram of household paraffin wax. For tomatoes, add 50 grams of sodium hydroxide pellets, and for Lima beans, 70 ml. of 40% sodium hydroxide solution. Immediately attach the flask to the distilling apparatus and carefully bring the contents to a boil with swirling. Preheating at a low temperature greatly reduces frothing and bumping. The heating may then be increased and the distillation should proceed smoothly.

Collect 35 ml. of distillate in a 50-ml. graduated mixing cylinder containing 5 ml. of 1*N* hydrochloric acid. Disconnect the condenser and rinse with 10 ml. of diethyl ether (Baker's purified). Pour the rinsing and distillate into a 125-ml. separatory funnel. Rinse the graduate with 20 ml. of ether and add to the separatory funnel. Shake the contents of the separatory funnel well and let separate for 3 to 5 minutes; then return the aqueous phase from the separatory funnel to the cylinder. Add 4 ml. of 0.125% sodium nitrite solution and shake well. Allow to stand for 5 minutes. Add 2 ml. of 2.5% ammonium sulfamate and shake well. After allowing the solution to stand for 10 minutes, add 4 ml. of 1% *N*-1-naphthylethylenediamine dihydrochloride. Shake the solution well and allow it to stand at least 45 minutes. After the color formation is complete, transfer the solution to a 125-ml. separatory funnel and add 20 ml. of saturated disodium phosphate solution.

Extract vigorously with 30 ml. of ether and discard the aqueous layer. Then extract the ether remaining in the separatory funnel with three 10-ml. portions of 1*N* hydrochloric acid. Dilute the aqueous acid extracts to 40 ml. in a graduated mixing cylinder with 1*N* hydrochloric acid. Measure the absorbance in a Beckman DU spectrophotometer in 10-cm. cells at 560 mμ using distilled water as the reference. The color is stable for several hours.

Calculation.

$$\text{P.p.m.} = \frac{\text{absorbance at 560 m}\mu \times 33}{\text{sample weight}}$$

The calculation is derived by taking purified Duraset through the described analytical procedure and assuming the color obtained is equal to 100%. Using this factor, good recoveries were obtained from untreated samples to which known amounts of Duraset were added.

Discussion

Reagents Used in Distillation. Thirty to 35% sodium hydroxide is necessary to effect quantitative distillation of the

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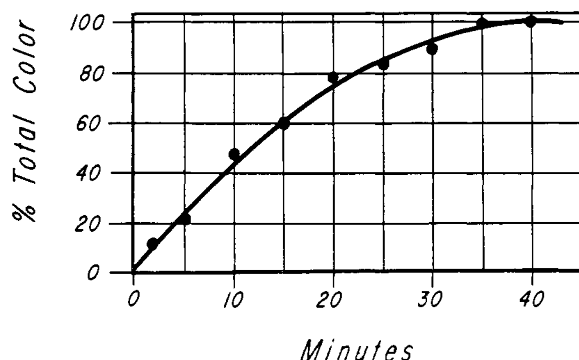

Figure 1. Rate of increase of Duraset color vs. time

m-toluidine. As tomatoes contain a high percentage of water, solid sodium hydroxide is used to keep the concentration at this high level. Lima beans, on the other hand, contain little water so aqueous sodium hydroxide solution can be used. Zinc apparently acts as a reducing agent to prevent oxidation of the amine during digestion and distillation. The wax is added to lessen foaming of the sample.

Treatment of Distillate before Color Formation. Hydrochloric acid is used to react with *m*-toluidine to form the water-soluble hydrochloride. Much of the plant material which is present in the distillate can be removed by an ether extraction. Many of these materials appear to have some reaction with the color-forming reagents. After color formation, their chemical properties follow closely those of the purple dye formed by the *m*-toluidine. Removal is most easily effected before the color reaction.

Color Formation. Sodium nitrite is added to diazotize the *m*-toluidine and the reaction is complete in 5 minutes. The ammonium sulfamate destroys any excess nitrous acid which must be removed because it also forms a purple dye with *N*-naphthylethylenediamine. Ten minutes are sufficient for this step. The *N*-1-naphthylethylenediamine dihydrochloride is then added. Figure 1 shows the time curve of color formation. The spectrophotometric curve has a maximum at 560 mμ (*1*). Although the color of the dye is stable for several days, the color of the interference increases on long standing. The absorbance measurements must be made within 3 hours after color formation is complete.

Extraction after Color Formation. Spectrophotometric curves were run of the purple dye obtained in various pH solutions. Above pH 6, all the dye was present in the basic form as a yellow color. It was hoped that, at a pH close to this transition pH for the desired constituent, the best separation could be obtained between the desired dye and other colored materials still present from the vegetable. By using disodium phosphate it was possible to obtain a pH at which the desired dye was extracted quantitatively into ether, leaving much of the undesired interfering color in the aqueous phase. Then an acidic extraction of the ether layer gave quantitative recovery of the desired constituent.

With any series of treated samples, two untreated samples are also analyzed, one of which contains a known amount of added Duraset. As levels of Duraset residues are usually very low or nonexistent, 0.1 p.p.m. of Duraset was added to the untreated samples. Table I shows typical recovery data obtained from tomatoes, cherries, and strawberries.

The reaction, although sensitive, is relatively nonspecific, and background interferences, unless removed, are large—i.e., the equivalent of several parts per million—compared to the small amount of Duraset being sought. Consequently, a series of separation steps is necessary to keep the background color sufficiently low to determine the Duraset residue accurately. Using the described method, background interferences in untreated samples have absorbance values equivalent to 0.05 to 0.08 p.p.m. of Duraset.

Table I. Recoveries of Knowns

(0.1 p.p.m. of Duraset added in all cases)

Duraset Recovered, P.P.M.[a]	Recovery, %	Duraset Recovery, P.P.M.[a]	Recovery, %
Tomatoes		*Cherries*	
0.078	78	0.098	98
0.092	92	0.079	79
0.107	107	0.090	90
0.088	88	0.070	70
0.080	80	0.080	80
0.096	96	0.073	73
0.099	99	0.062	62
0.087	87	Av.	79 ± 12
0.090	90		
0.073	73	*Strawberries*	
0.099	99	0.065	65
0.086	86	0.088	88
0.084	84	0.085	85
Av.	89 ± 9	0.101	101
		0.085	85
		Av.	85 ± 13

[a] After correction for background interference from untreated sample.

Acknowledgment

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FOOD DISCOLORATION

Reddening of White Onion Bulb Purees

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Purees prepared from succulent inner scales of onion bulbs, when acidified with acetic acid, turn pink to red on storage at room temperature. Joslyn (*31*) observed that this reddening occurred more rapidly and the color was more intense at higher concentrations of acetic acid (5%) than at lower levels (2%), and that addition of salt, even in amounts up to 25%, did not prevent reddening. This reddening was more intense in macerates of white onions than in those prepared from red onion bulbs. In onions susceptible to reddening, pigment formation occurred in mechanically bruised or finely cut tissue and did not occur in carefully cut sliced onions. Sliced onions steamed before crushing yielded purees which did not redden. This pigmentation was confirmed by Cruess (*14*) and more recently investi-