Spectrophotometric Microdetermination of 1-Naphthaleneacetic Acid in Pineapple

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The growth regulator 1-naphthaleneacetic acid is estimated in pineapple by solvent extraction and ultraviolet spectrophotometry after removal of interferences by permanganate acidified with phosphoric acid. This sensitive procedure can determine 0.03 p.p.m. of the growth regulator.

SINCE CLARK AND KERNS (*2*) found that 1-naphthaleneacetic acid (α-naphthaleneacetic acid, ANA, NAA, or SNA, the sodium salt) was effective for flower induction in pineapple, this growth regulator has been used commercially to bring about uniform flowering of pineapple.

With the advent of amendments to existing laws governing chemical residues in food, fruit must be analyzed to ascertain the presence or absence of microquantities of ANA. An accurate quantitative procedure for determining ANA in agricultural products does not appear to be available. Sen and Leopold (*5*) employed a paper chromatographic technique in which the separated ANA was detected with bromocresol green indicator. This method was tried but failed to have the required sensitivity. Schwartz *et al.* (*4*), in studying the hydrolysis of naphazoline, determined ANA by ultraviolet spectrophotometry following silicic acid chromatography.[1]

In the absence of a colorimetric method for ANA, the ultraviolet absorption procedure appeared to be best suited to its estimation in pineapple provided that interferences could be completely removed. The high degree of absorption by ANA particularly at 223 mμ should afford adequate sensitivity (Figure 1).

The method involves solvent extraction, isolation of extracted acids, removal of interferences by acid permanganate oxidation, and then ultraviolet spectrophotometry of the neutral isolate.

[1] Since this paper was submitted for publication, two methods for determining ANA have appeared in this Journal. Zweig *et al.* (*8*) employed gas chromatography followed by ultraviolet spectrophotometry, and Bache *et al.* (*1*) used silicic acid chromatography followed by either ultraviolet spectrophotometry or colorimetry.

Experimental

Reagents. Ether, U.S.P., peroxide-free. Keep in the presence of added ferrous sulfate acidified with a small quantity of dilute sulfuric acid.

Petroleum ether–ethyl ether mixture. Mix 1 volume petroleum ether (60° to 110° C.) and 2 volumes of ethyl ether. Keep the mixture acid-free by adding a small amount of 0.3N NaOH and shaking. Decant the solvent layer for use.

Sodium sulfate–sodium hydroxide, 45% (w./v.). Dissolve 450 grams of sodium sulfate decahydrate in hot water and cool. Add 5 ml. of 6N NaOH and dilute to 1 liter with water.

Potassium permanganate, 0.05N.

ANA standards. Weigh 25 mg. of ANA, transfer to a 100-ml. volumetric flask with 0.1N ammonium hydroxide, and dilute to volume with the ammonium hydroxide. Dilute this 250 p.p.m. solution to prepare a 5 p.p.m. solution.

Procedure. Place 200 grams of fruit in a Waring Blendor, add 200 ml. petroleum ether–ethyl ether solvent, and blend 30 seconds or until well homogenized. Pour into two 250-ml. centrifuge bottles, stopper, and centrifuge at 2500 r.p.m. for 5 minutes. Decant or siphon off 130 ml. of extract which contains all fatty substances as shown by the complete absence of pigment in the aqueous layer.

Place the extract in a 250-ml. centrifuge bottle and add 65 ml. of the sodium sulfate–sodium hydroxide reagent. Stopper with a thin polyethylene- or Saran-covered rubber stopper and shake moderately for 2 minutes. Avoid vigorous shaking as this will result in tight emulsions. Centrifuge at 2500 r.p.m. for 10 minutes. This should result in a clear aqueous layer. If some emulsion is present, siphon off most of the solvent layer and add 25 ml. of ether. Mix to eliminate emulsions.

Table I. Standard Absorbance Values Read on Beckman DK2 Spectrophotometer

ANA, μG./10 Ml.	Absorbance[a]	Absorbance/μG.
5	0.213	0.0426
10	0.418	0.0418
20	0.835	0.0418
30	1.253	0.0418
		Av. 0.0420

[a] Difference of 1-cm. cell readings at 223 and 243 mμ.

Pipet 50 ml. of the clear aqueous layer (equivalent to 100 grams of fruit) into a 125-ml. separatory funnel and add 2 ml. of 22N phosphoric acid. Titrate the easily oxidizable substances with 0.05N potassium permanganate until a light pink color persists and then add 2 ml. of the permanganate. Shake and allow to stand 10 minutes. Extract with a 15-, and two 10-ml. portions of ether, combine extracts, and wash twice with water.

Extract the acidic components in the ether extract with 15- and 10-ml. portions of 0.1N NH_4OH followed by two washings with water and run the aqueous extracts into a 200-ml. tall form beaker. Evaporate to about 5 ml. and transfer to a 10-ml. volumetric flask. Dilute to volume with water, and run the absorption spectrum on the Beckman DK-2 spectrophotometer from 335 to 215 mμ, using a 1-cm. cuvette against a reagent

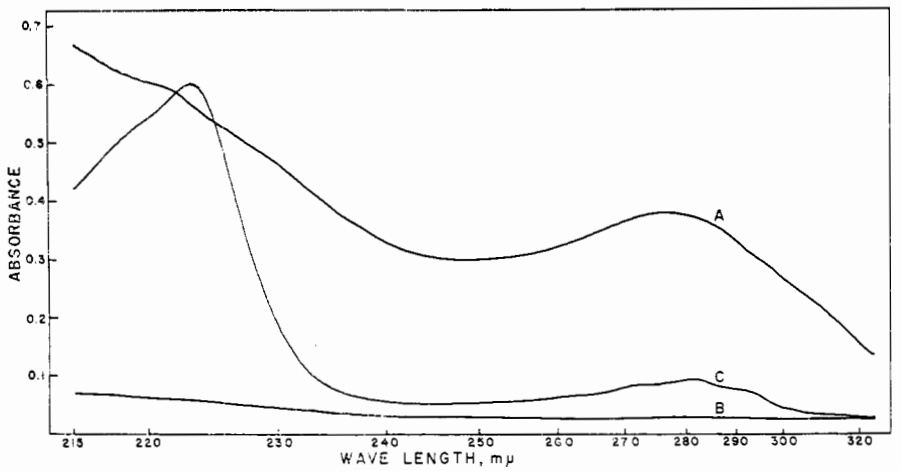

Figure 1. Effect of phosphoric acid–permanganate on ultraviolet absorbance in canned pineapple extracts with and without addition of 0.125 p.p.m. ANA

(A) Not treated with phosphoric acid–permanganate
(B) Treated with phosphoric acid–permanganate
(C) ANA and treated with phosphoric acid–permanganate

Table II. Recovery of ANA Added to Fresh and Canned Pineapple[a]

Added, P.P.M.	Recovered,[b] P.P.M.	Recovery, %
	Fresh	
0.125	0.110	88
0.125	0.117	94
	Canned	
0.050	0.045	90
0.100	0.089	89
0.200	0.176	88
0.062	0.056	90
0.125	0.107	86
0.250	0.212	85
0.125	0.110	88
0.250	0.231	92
0.250	0.221	88
0.125	0.118	94

[a] 100-gram sample.
[b] Corrected for apparent values of 0.013 p.p.m.

blank prepared by starting with 130 ml. of the petroleum ether–ethyl ether solvent itself rather than the fruit extract.

In the presence of ANA, maxima will be evident at 223, 272, 282, and 292 mμ (Figure 1). Read the absorbance at 223 mμ, and also the minimum absorbance at 243 mμ. Obtain the difference and refer to the standard curve prepared below for micrograms ANA in the sample represented in the final 10-ml. volume. Divide this by 100 to obtain p.p.m. ANA in the fruit. The least amount determinable is 0.03 p.p.m. Less than this value is considered zero. Report any true ANA as the difference between the value found in the sample minus that found in control, non-ANA treated fruit.

To prepare the standard curve, transfer 0, 1, 2, and 4 ml. of the 5 p.p.m. standard to 10-ml. volumetric flasks, dilute to volume with 0.1*N* ammonium hydroxide, and run the spectra from 250 to 215 mμ. Read the absorbance at 223 and 243 mμ, and plot the curve with the differences between these readings. Standard readings are linear over a wide range (Table I).

Instead of using a recording spectrophotometer, an instrument such as the Beckman DU may be employed. For small amounts of ANA, however, a few points above and below 223 mμ should be taken to be certain that a definite peak exists.

Remarks and Discussion

Extraction. Inasmuch as ANA is readily soluble in ethyl ether or petroleum ether–ethyl ether mixtures, the initial extraction was carried out with the latter solvent which was appreciably less volatile. At the pH of pineapple fruit tissue of 3 to 4, ANA is readily extracted when applied as the sodium salt.

Subsequent alkaline extraction of the petroleum ether–ethyl ether extract removes the ANA and leaves any nonacidic components in the solvent layer. Nonacidic substances which absorb in the ultraviolet are thus eliminated.

Removal of Interferences. Since many organic and inorganic acids absorb light in the low ultraviolet region, such absorbers must be completely removed. While most inorganic compounds are not extracted by the petroleum ether–ethyl ether mixture, many acidic organic compounds may be extracted in the same manner as ANA.

Extracts of fresh pineapple appear to contain relatively small amounts of such substances. Canned pineapple extracts, however, have appreciable quantities which, if not removed, would render the determination unfeasible (Figure 1). Among the compounds formed in canned pineapple, *p*-coumaric acid appears to be present in amounts highly absorbing in the ultraviolet region (*7*).

Attempts at removal of interferences by absorption with Attapulgus clay–celite mixtures or by ion exchange chromatography (*6*) were not successful. Nitration of isolated residues followed by colorimetry also failed. Interferences could not be removed by evaporation.

Oxidation of interferences, as suggested by Gunther and Blynn (*3*), was tried. While hydrogen peroxide appeared to decompose ANA seriously, permanganate acidified with phosphoric acid proved to be completely effective in removing all interferences without degrading ANA, as shown in Figure 1. The three absorption spectra were prepared by the above method. Curve *C* is identical to that of ANA itself. That permanganate with phosphoric acid had no effect on ANA was further demonstrated by using a 10-fold larger quantity (20 ml.) of 0.05*N* permanganate.

Volatile interferences present in the sample or introduced by any reagent is conveniently removed during the evaporation of the ammonium hydroxide extract.

Recovery and Sensitivity. Recovery of 85 to 94% of microquantities of ANA added to fresh and canned pineapple and determined by the above method is shown in Table II. Measurement of such small amounts of ANA by ultraviolet spectrophotometry is evidently quite feasible as long as interfering absorbers are adequately removed. For a 100-gram sample, the lower limit of sensitivity is approximately 0.03 p.p.m. At this level, the maximum absorption at 223 mμ less that at 243 mμ measures about 1.2 inches (0.12 absorbance) on our Beckman DK-2 recording spectrophotometer.

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