

Figure 1. Absorbance spectra of titanium complexes with sinapine and rapeseed extract.

which confirmed no losses occurred during the extraction procedure. Unlike the titanium reagent the Reinecke salt can be added directly to the final extract. However, the time required for preparing the Reinecke salt, adjusting the dilution of the extract, plus the hour needed for the Reinecke salt-sinapine complex to form followed by centrifugation and solubilization of the complex in slightly acidified methanol is a far lengthier procedure than the 1-h period to dry the aliquot prior to the addition of $TiCl_4$. This is necessary as the reaction between sinapine and $TiCl_4$ proceeds best in concentrated HCl. This is distinct from the phenolic compounds where the reaction pro-

ceeded best in acetone. This study establishes the titanium method as a new and reliable procedure for the determination of sinapine. Studies are currently underway to establish the nature of the complex formed.

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A New Procedure for Extraction and Determination of Biphenyl in Citrus Fruit

Biphenyl is used extensively to protect overseas shipments of fresh citrus fruits. Low residue tolerances imposed by some importing countries have necessitated further research to insure protection by the fungicide without exceeding allowable levels. Biphenyl is commonly extracted by steam distillation, which requires 1-3 h. A method of direct solvent extraction, requiring only a few minutes, is described herein. This method consists of blending peel samples with ethyl acetate, filtering the extract, and analyzing aliquots of the extract by gas chromatography.

The fungistatic agent biphenyl has been used to prevent decay of fresh citrus shipments for many years. One or more kraft paper pads impregnated with biphenyl are enclosed in each box of fruit. The amount of biphenyl absorbed into the oils of the peel depends on variety, duration of exposure, temperature, pad placement, amount of biphenyl per pad, and fruit maturity (Norman et al., 1969, 1971; Rygg et al., 1964; Rajzman, 1965). Low tolerance levels have been imposed by importers, such as Japan and Belgium (70 ppm), but in a few instances the amounts of adsorbed biphenyl have exceeded those levels (Wardowski et al., 1979). Therefore, further research is needed to determine more precisely application procedures and residue levels so that the fungicide insures protection without exceeding tolerance limits.

As noted by Beernaert (1973), many papers have been published on the analysis of biphenyl, and in almost all instances, steam distillation was used for isolation of the material. One exception was ether extraction by Soxhlet of peel that had been air-dried 12 h, but no data showing recovery of biphenyl from fresh fruit were given (McCarthy and Winefordner, 1965). An automated procedure was proposed by Gunther and Ott (1966) based on a steam distillation method previously described (Gunther et al., 1963). In Japan, the present method of analysis involves a distillation procedure similar to that described by Newhall et al. (1954) and the collection of the distillate in a solvent such as cyclohexane as used by Hayashi et al.

(1972). We considered this procedure, which is also recommended by the Pharmaceutical Society of Japan (1971) and by the AOAC (1970), rather time consuming. To expedite our research, we developed the direct solvent extraction procedure described herein.

MATERIALS AND METHODS

Grapefruit from simulated shipping experiments were used as test material. Results of various treatments relating to factors affecting the rate of adsorption of biphenyl are presented elsewhere (Wardowski et al., 1979). Rinds of five fruit were cut into six equal sections, and one section of rind from each fruit was combined for analysis. The sections were cut into smaller pieces, placed in a blender (Waring explosion-proof), and ground 4 min with 300 mL of reagent grade ethyl acetate. The slurry was filtered with cheesecloth, and a small portion was centrifuged at 2000 rpm for 5 min. A 2- μ L portion of the supernatant was injected without further cleanup into a gas chromatograph. The instrument used was a Microtek GC-2000R with a 15% silicone gum rubber column, 80/100 mesh, SE-30 (methyl), 3 m \times 6 mm. Temperatures were as follows: oven, 175 $^{\circ}$ C; inlet, 200 $^{\circ}$ C; and flame ionization detector, 250 $^{\circ}$ C. The carrier gas was N_2 at 100 mL/min. Concentrations were determined by measurement of peak heights. For calculations, no adjustment of volume due to presence of water in the peel was considered necessary. Analysis by liquid chromatography (Davis and Munroe,

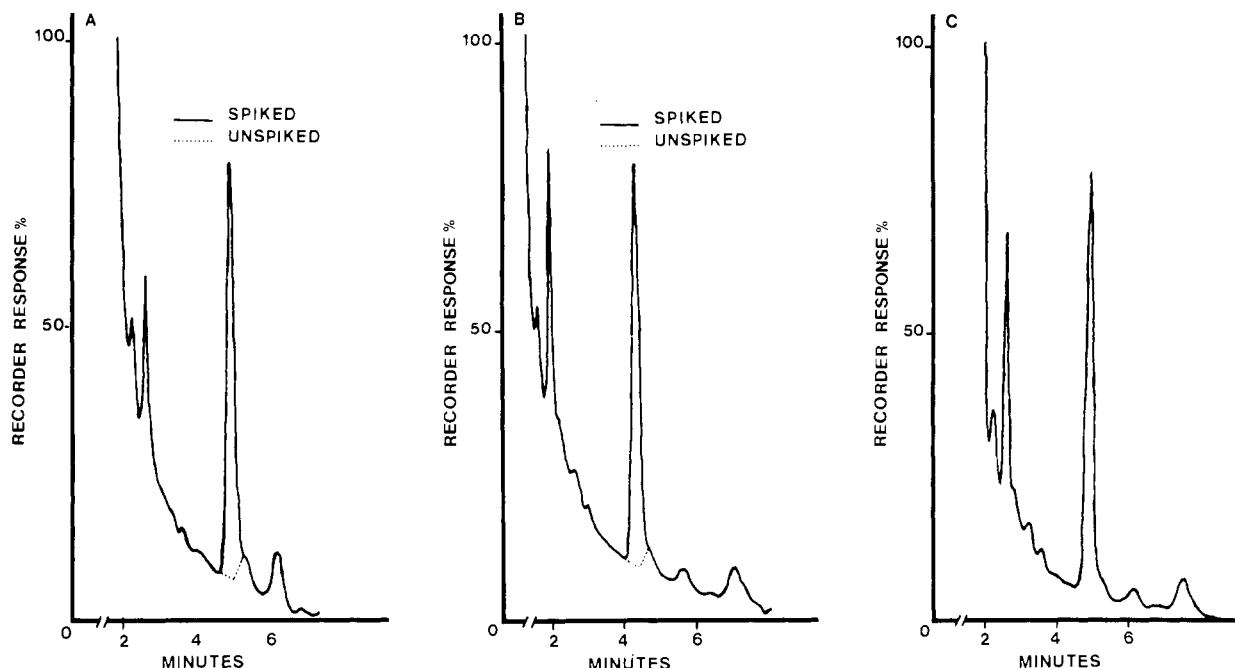


Figure 1. Chromatograms of (A) 40 ppm biphenyl in 'Marsh' grapefruit extract, attenuation $\times 32$; (B) 40 ppm biphenyl in 'Valencia' orange extract, attenuation $\times 32$; and (C) extract of (B) reduced 50% in volume by distillation, attenuation $\times 64$.

Table I. Solubility of Biphenyl in Ethyl Acetate and Ethyl Alcohol^a

solvent	g/100 mL
ethyl acetate	42.7
ethyl acetate sat. with water	41.8
95% ethanol	5.1
76% ethanol	1.3
67% ethanol	0.6
57% ethanol	0.3

^a Determined by GC of saturated solutions at 26.5 °C.

1977) should have been equally satisfactory.

RESULTS AND DISCUSSION

Extraction. When peel was ground with ethyl acetate plus water to increase liquid volume, a formidable emulsion developed. Ethyl acetate alone gave a slightly cloudy solution, easily clarified by short-term, low-speed centrifugation. A ratio of about 300 mL of ethyl acetate to 200 g of peel afforded good blending. Ethanol was tried as the extracting solvent, but since it is miscible with the water of the rind, the volume of extract was increased. This required an additional analysis for ethanol content of the extract to determine the final volume. In addition, biphenyl is less soluble in ethanol than in ethyl acetate (Table I). Other solvents, such as cyclohexane or petroleum ether, could likely serve equally as well as ethyl acetate since they are good solvents for biphenyl and have low water solubility. The recovery of biphenyl added as dry crystals to and blended with peel tissue and ethyl acetate averaged 99.0% (Table II).

Comparison with Distillation Method. For the simulated shipping tests, we analyzed a total of 16 comparable lots of fruit from ten separate biphenyl treatments in common with two other laboratories (lab A: J. O. Craig, Florida Department of Agriculture, Division of Fruit and Vegetables Insp., Winter Haven; lab B: S. V. Ting, Florida Department of Citrus, Lake Alfred), with both using the same steam distillation procedure. The mean parts per million results were as follows: this lab, 15.7; lab A, 14.1; and lab B, 15.4. Analysis of variance (Table III) showed differences among treatments, but no differences among

Table II. Recovery of Biphenyl Added to Grapefruit Peel and then Extracted with Ethyl Acetate

sample ^a	g added	g recov.	% recov.
1	0.0118	0.0118	100.0
2	0.0256	0.0246	96.1
3	0.0332	0.0320	96.4
4	0.0367	0.0365	99.5
5	0.0394	0.0393	99.8
6	0.0516	0.0520	100.8
7	0.0556	0.0543	97.7
8	0.0621	0.0618	99.5
9	0.0710	0.0715	100.7
10	0.0864	0.0863	99.9
mean			99.031
SD			1.689
SE of mean			0.537
coeff. of var.			0.017

^a Fruit weight average, 453 g; peel weight average, 158 g; 250 mL of ethyl acetate for extraction.

Table III. Analysis of Variance of Results from Three Methods and Ten Treatments

sources of variation	df	F	
treatments (T)	9	8.50	<i>a</i>
methods (M)	2	1.55	NS
this lab vs. A-B	1	1.40	NS
A vs. B	1	1.70	NS
T \times M	18	7.05	NS
error	18		

^a Significant at the 0.1% level.

the three labs, between the results of our method and the distillation procedure, between labs A and B, or between methods irrespective of treatment.

We analyzed a total of 31 lots of fruit from 16 treatments in either duplicate or triplicate. There was significant difference between treatments and lots of the same treatment, but no differences among samples from the same lot.

Our assay method does not take into account the biphenyl in the pulp. However, when we separately analyzed the pulps of 20 samples of this test, results ranged from 0.14 to only 1.28 ppm and averaged 0.53 ppm. Thus, the

Table IV. Reduction in Volume of Biphenyl Solutions

sample ^a	residue		distillate	
	mL	g of biphenyl	mL	g of biphenyl
1	55	0.0404	45	ND ^b
2	45	0.0383	55	ND
3	32	0.0406	68	ND
4	14	0.0408	86	<0.1

^a Each sample contained 0.040 g of biphenyl in 100 mL.

^b ND, not detectable.

contribution of biphenyl residues in pulp was regarded to be of minor importance compared to the time-saving factor, 5 min vs. 1–3 h. The presence of biphenyl in the pulp could have been due to contamination during the cutting process.

Other Considerations. Despite the high vapor pressure of biphenyl ($\times 10^3$; 1.38 cmHg at 35 °C) (Davis and Munroe, 1977), ethyl acetate solutions could be reduced in volume without loss of biphenyl. Ethyl acetate solutions of biphenyl, 40 mg in 100 mL, were distilled in a Soxhlet equipped with a drain; and no loss was detectable even when the volume was reduced to as low as 32 mL (Table IV).

In addition, biphenyl was added to extracts of non-treated orange and grapefruit peels in amounts equivalent to 40 ppm; and analyses showed that interference from extracted peel components was minimal (Figure 1A,B). Under our conditions of gas chromatography, limonene, the dominant volatile oil, had a retention time of less than 2 min. Linalool, another common volatile component was eluted in just over 2 min. Other components were eluted after the biphenyl, so we allowed the chromatogram to run for about 8 min to clear the column. The orange extract was reduced in volume to one-half the original by distillation, but showed no loss of biphenyl and no increase in interference from the volatile components (Figure 1C). Thus, if the amount of biphenyl residue in fruit is very low, the volume of extract can be safely reduced for better chromatographic analysis.

Analyzing composites of whole peel gave no more accurate results than analyzing composites comprising $1/6$

or $1/8$ portions of peel from different fruit. The dominant source of error in the analysis of biphenyl residues in citrus fruit is apparently the fruit sampling (de Vos, 1969). Thus, analyzing the largest peel-segment composites that can be conveniently handled seems to be the best procedure at this time.

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