γ) have been added, the maximum is fairly broad and still displaced to 440 to 470 mμ.

The absorption spectrum of the Allen reaction (Figure 1, upper right; lower left) in the presence of dehydroepiandrosterone (65 γ) demonstrates a remarkably distinct and prominent maximum at 600 mμ and a much less prominent absorption at 380 mμ. In the presence of neutral extracts of bovine urine, this reaction demonstrates absorption maximum at 370, 465, and 540 mμ, respectively (Figure 1, upper right, lower left). These same effects are noted when neutral extracts of wether and goat urine are employed (Figure 1, lower left). When the reaction is performed in the presence of a neutral extract of bovine urine to which has been added 50 γ of dehydroepiandrosterone (Figure 1, upper right), the urinary chromogens completely obscure the maxima characteristic of the steroid alone, but are still consistent with the urinary, neutral extract spectra.

The absorption spectrum of the Pincus reaction (Figure 1, lower right) in the presence of androsterone (150 γ) demonstrates a prominent double peak, the first wave of which is at 610 mμ and the second, a slightly higher wave, at 660 mμ. In addition, there is a slight, although definite, indication of absorption occurring at 545 mμ. When this reaction is performed in the presence of a neutral extract of bovine urine, two obvious maxima located at 470 and 540 mμ are discernible. When a mixture of a neutral extract of bovine urine and known 17-KS is subjected to this reaction, three distinct maxima at 470, 545, and 590 mμ, respectively, become obvious. In addition, there is a faint, but again a definite, peak at about 650 mμ.

Biological assays (Table I), employing the chick comb test indicate that neutral extracts of wether urine contain few, if any, androgenic ketones, despite the characteristic, albeit displaced, maxima demonstrable by spectrophotometric methods.

Discussion

In the case of the Zimmerman reaction, interfering materials in the neutral extracts of urine largely displace the absorption maximum characteristic of a known 17-KS (dehydroepiandrosterone) in the presence of this reaction. In the case of the Allen reaction, the prominent maximum occurring at 600 mμ, when pure steroid (dehydroepiandrosterone) is subjected to the reaction conditions, completely disappears when the neutral urine extract containing steroid is treated likewise. In the case of the Pincus reaction, not only is there apparent displacement of the maxima which are characteristic of the pure steroid (androsterone) in the presence of this reaction, but also virtually complete disappearance of the second prominent wave at 660 mμ.

These data indicate that the characteristic absorption spectra may be obtained employing the Allen, Pincus, and Zimmerman reactions in the presence of pure 17-KS, neutral extracts of urine, and neutral extracts of urine to which have been added known amounts of pure 17-KS. In each case, however, the absorption spectra, although characteristic, are different. Moreover, unidentified compounds, normally present in bovine, ovine, and goat urine, not only may be responsible for the shifts in maxima which have been demonstrated, but also may serve to enhance or otherwise confound the effect of small amounts of 17-KS present either normally or as a consequence of intentional addition. Thus, it would appear that small amounts of endogenous 17-KS would not be detectable in neutral extracts of ruminant urine which also contain these interfering substances, when subjected to the reaction conditions of any of these methods.

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FUNGICIDE EVALUATION

Fungicidal Activity of Some New Amino Alcohols Synthesized from Citrus (+)-Limonene

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The synthesis of several new amino alcohols from (+)-limonene has been reported (*2*). Partial hydrogenation involving the exocyclic double bond of (+)-limonene followed by oxidation of the endocyclic double bond with peracetic acid afforded *p*-menthane-1,2-epoxide. The epoxide ring was readily cleaved by ammonia to give the mixed trans isomers of 2-amino-1-*p*-menthanol (I). Cleavage of the epoxide by methylamine and dimethylamine afforded the mixed trans isomers of 2-methylamino-1-*p*-menthanol (II) and 2-dimethylamino-1-*p*-menthanol (III), respectively. Since this work was reported, an additional new amino alcohol, 2-dimethylamino-$\Delta^{8(10)}$-*p*-menthen-1-ol (IV), has been prepared by cleavage of $\Delta^{8(10)}$-*p*-menthene-1,-2-epoxide by dimethylamine. This series of amino alcohols was originally prepared for animal testing with the hope that some of the compounds would show physiological activity. No such activity was observed for any member of this series.

A preliminary investigation of these compounds as fungicides, for sanitizing of food processing plants, was investigated after becoming familiar with the properties of another derivative of (+)-limonene, tetrahydrocarvone (*1, 3*). It bore a menthollike odor and, in dilution, the odor resembled other compounds used for sanitizing purposes. The amino alcohols—I, II, III, and IV—although not as pleasant in odor, were also investigated as possible fungicides.

Experimental Procedure

Fungicidal Tests on Gauze Pads. Emulsions of the compounds to be tested were prepared in a 1 to 10 dilution with 2 drops of Tween 80 to ensure

Several derivatives of 2-amino-1-*p*-menthanol synthesized from (+)-limonene have been found to be fungicidal when tested as antimildew agents on gauze pads and as chemicals for the control of decay in Valencia oranges.

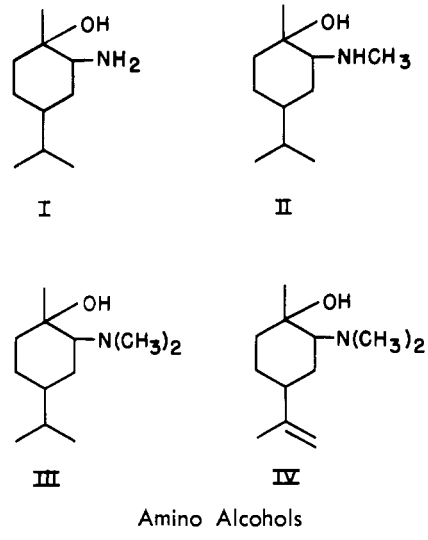
complete dispersion. The total volume in all cases was 100 ml. Further serial dilutions of 1 to 100, 1 to 1000, and 1 to 10,000 were then made. Gauze was cut in strips about 3 inches wide and folded into squares six layers thick. These were dipped into the emulsions and thoroughly wetted. Six pads were treated with each dilution of each compound tested, beginning with the greatest dilution. The pads were wrung free of excess emulsion by hand and inoculated by pressing one surface on the moldy wall of a storage room. They were then folded with the inoculated surface inside, placed in Petri dishes, and incubated in a dark space at room temperature. Control pads were treated individually with water, water and Tween 80, and salicylanilide (put into solution using anhydrous sodium carbonate) at dilutions of 1 to 1000, 1 to 10,000, 1 to 100,000, and 1 to 1,000,000. These pads were inoculated and incubated with the pads treated with the test compounds. All the pads were kept moist by adding, when needed, a mildew test solution of nutrient salts comprising 3 grams of sodium nitrate, 1 gram of dipotassium phosphate, 0.25 gram of magnesium sulfate, 0.25 gram of potassium chloride, and sufficient water to give a total volume of 1000 ml.

Fungicidal Test on Fresh Fruit. Freshly picked Valencia oranges were randomized into six lots of 50 each. The oranges were not washed prior to treatment and one untreated lot was used as a check. Aqueous emulsions containing 0.0001, 0.001, 0.1, and 1.0% of 2-dimethylamino-1-*p*-menthanol (III) were prepared using a few drops of Tween 80 as the emulsifying agent. Each emulsion was applied by a small brush to the stem-end portion of the oranges in one 50-fruit lot. The stem end was treated because most of the decay organisms are at this location. The treated fruit was stored with the untreated check lot at 21° and 85 to 90% relative humidity for 4 weeks. Each lot was examined at weekly intervals for decay. Decay in citrus fruits is caused by the molds, *Penicillium digitatum* Sacc., *Penicillium italicum* Wehmer, and the rot-inducing *Diplodia natalensis* Pole-Evans and *Phomopsis citri* Fawc.

The food-plant "cleanup" tests were made using specimens and rinse water from a citrus processing pilot plant. The sanitizing tests employed inoculations of raw sewage, specimens collected from the facilities and tile floors of the men's wash room, and strains of unidentified enteric streptococci. Beef yeast–extract agar was melted, cooled, and inoculated with the above-mentioned materials. After the inoculum was blended with the liquid agar, plates were poured and permitted to solidify. The chemicals to be tested were spotted on the surfaces of the agar with a standard loop inoculation needle. The plates were incubated at 30° C. Salicylanilide was not used in the sanitizing tests.

Table I. Fungicidal Effect of (+)-Limonene Derivatives Tested Using Inoculated Gauze Pads

Chemicals	Incubation Period, Days	Highest Dilution Showing No Growth	Results for Next Higher Dilution
Tetrahydrocarvone	57	1:100	1:1000 slightly musty, scant growth on all pads
2-Amino-1-*p*-menthanol (I)	53	1:1000	1:10,000 slightly musty, spotted. Less than 50% were damaged
2-Methylamino-1-*p*-methanol (II)	43	1:100	1:1000 inconsistent, occasionally musty, moldy, and spotted
2-Dimethylamino-1-*p*-menthanol (III)	43	1:1000	1:10,000 musty, moldy, and spotted
2-Dimethylamino-$\Delta^{8(10)}$-*p*-menthen-1-ol (IV)	31	1:1000	1:10,000 moldy and spotted
Salicylanilide control	43	1:10,000	1:100,000 musty and spotted



Amino Alcohols

Results and Discussion

The preliminary investigation of compounds I, III, and IV as fungicides is encouraging (Table I). Only tetrahydrocarvone and compound II do not justify further testing as fungicides. The gauze pads treated with tetrahydrocarvone showed no inhibition after 57 days of incubation. At a dilution of 1 to 1000, all pads were musty and showed growth of mold. After repeated tests, compound II was inconsistent in preventing growth at dilutions of 1 to 1000. When inhibition occurred at 1 to 1000, the next higher dilution permitted slight mustiness, moldiness, and spotting in a number of pads.

The greatest fungicidal activity was noted for the three remaining compounds —I, III, and IV. In tests on I growth was completely inhibited at a dilution of 1 to 1000. At the next higher dilution there was no visible growth of mold and only a slight mustiness and spots on less than half of the pads. The other two amino alcohols, III and IV, were about equal in their mold-inhibiting properties. Dilutions of 1 to 1000 completely prevented growth, while at the next higher dilution a very few pads remained undamaged.

Salicylanilide was used as a fungicide control. It prevented mold growth on gauze pads at a dilution of 1 to 10,000; the next higher dilution, 1 to 100,000, prevented growth in more than half of the pads.

The nutrient solution added to the inoculated gauze pads supplied adequate growth conditions for the microorganisms; therefore it is felt that these amino alcohols have been given a severe test.

The amino alcohols were not effective for cleanup and sanitizing purposes at any dilution, as determined by inoculated beef yeast–extract agar plates. Neither did any dilution prevent the growth of enteric streptococci on the surface of the same medium.

The fungicidal tests on Valencia oranges using various concentrations of III are summarized in Table II. Compound III was selected because the first tests on gauze pads indicated that it might be more fungicidal than the other amino alcohols prepared. These results show that some reduction of decay

Table II. Valencia Oranges Given Stem-End Treatment of 2-Dimethylamino-1-p-menthanol (III)

III, Concn., %	Decay, %			
	1 wk.	2 wk.	3 wk.	4 wk.
0.0 (check)	4	8	10	14
0.0001	4	10	10	16
0.001	2	4	6	14
0.01	0	6	8	16
0.1	0	4	4	8
1.0	0	0	2	6

is provided after 3 weeks' storage by the emulsion containing only 0.001% of III. The emulsion containing 1.0% of III provides very effective decay control (80%) after 3 weeks' storage at 21° and moderate control (57%) after 4 weeks' storage. Decay control refers to per cent reduction of decay based on the untreated check. This decay control is of the same order of magnitude as that provided by citrus fruit fungicides such as Dowicide A. However, results comparing the effectiveness of compounds III with Dowicide A are not complete. It is likely that compounds I and IV will show a similar degree of decay control, but they have not yet been tested on citrus fruits.

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PESTICIDE RESIDUE ANALYSIS

Microcoulometric Gas Chromatography of Pesticides

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A new rapid screening method of pesticide residue analysis has been developed. Quantitative analysis for several pesticides, such as γ-BHC, aldrin, dieldrin, DDT, chlordan, endrin, toxaphene, and other chlorinated organic pesticides, can be made in a single determination requiring only 1 hour. The method, based on gas chromatography and coulometric detection, can also be used for thiophosphates.

THE PROBLEM of identifying and quantitatively measuring pesticide residues on agricultural products has become increasingly difficult owing to the wide variety of chemicals used for controlling pests. The number of chemicals in use increases annually, which continually increases the number of combinations of pesticides and plant materials. In order to make the task of the analyst somewhat less difficult, a program of development of rapid screening tests for pesticide residues on fresh vegetables was undertaken. The main objective was to develop a rapid, yet sensitive, technique that would be useful for a wide variety of plant materials. In addition to good sensitivity, the method must be reasonably simple to perform, and yet it should give quantitative information concerning a wide variety of materials. At the beginning of this study, a complete survey of available equipment and methods was made, and it was decided that a new and fresh approach to the problem was needed. Such techniques as paper chromatography were considered, along with column chromatography and various classical colorimetric methods. At that time there was no published work on gas chromatography as a tool for rapid screening pesticide residue analyses. After a cursory laboratory evaluation of the potentiality of gas chromatography, it was decided that this technique, if properly developed, showed great promise (*3*). Commercially available equipment was surveyed and found to be lacking in certain necessary features for this particular problem. Therefore, a method and instrument development program was undertaken. This resulted in development of new rapid screening procedures for pesticide residues on leafy vegetables and other food products.

In order to accomplish a rapid screening procedure for a variety of pesticides, it was necessary to develop new equipment. This new equipment had to be consistent with the concept that a single catchall type of extraction would be used, followed by a rapid step in which the extract would be concentrated to a small volume, with or without additional cleanup procedures. If a cleanup procedure was necessary, it could be relatively crude.

Equipment

Figure 1 shows a block diagram demonstrating the principles on which the new instrument operates. The instrument consists of three major modules and a titration cell. The first module contains a gas chromatographic column and microcombustion furnace. The second, not shown in Figure 1, is a power supply for the gas chromatograph. The third module is the coulometer.

It was found that such chlorinated pesticides as lindane (γ-BHC), aldrin, dieldrin, endrin, DDT, toxaphene, chlordan, gamma-chlordan, heptachlor, heptachlor epoxide, ronnel, and DD could be successfully gas chromatographed, only if materials of construction were carefully selected. After considerable research it was found that a $^1/_4$-inch in outside diameter by 6-feet long aluminum column packed with 30-to-60-mesh Chromosorb (Johns-Manville Co.) coated with 15 to 30% by weight of Dow-Corning high vacuum silicone grease is a satisfactory gas chromatographic column. In order to avoid chemical effects it was necessary to acid-wash the Chromosorb with hot 6*N* hydrochloric acid, followed by a thorough

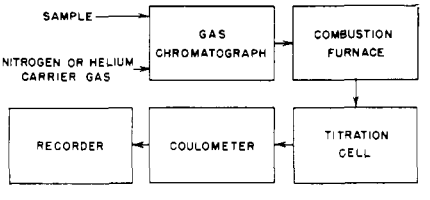

Figure 1. Block diagram of pesticide analyzer