Figure 1. Threshold Concentration of Celery Juice vs. Total Ester Content.

The hydroxamic acid test for esters is applied to celery juice as a measure of total flavor potency. Before applying the test, it is necessary to concentrate the ester materials by adsorption on charcoal.Excellent correlation was obtained between total ester content and taste threshold of the juice.

Summary
The hydroxamic acid test for esters is applied to celery juice as a measure of total flavor potency. Before applying the test, it is necessary to concentrate the ester materials by adsorption on charcoal. Excellent correlation was obtained between total ester content and taste threshold of the juice.

Acknowledgment
The authors would like to thank Mr. Fred Schultz, Jr., of the Southern Regional Research Laboratory for his advice on the treatment of taste panel results.

LITERATURE CITED

AN EPHEDRA ALKALOID IN CITRUS JUICES

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Lake Alfred

Synephrine, an alkaloid belonging to the ephedrine group, has recently been isolated from citrus and identified by Stewart, Newhall and Edwards (7). This compound had not previously been found in plants. The purpose of this paper is to discuss some of the properties of synephrine, to report on its occurrence in citrus, and to propose it as a means of identification for certain citrus juices.

Syneprine was determined by paper chromatography following its separation from interfering substances in juice. The separation from concentrate was carried out by adding the contents of a six ounce can slowly with stirring to one liter of boiling 50:50 acetone:methanol, following which the mixture was cooled and allowed to settle overnight. In the case of single strength juice, one liter was first concentrated to about 300 ml using a rotary evaporator at 70° C. before adding it to the solvent mixture. Following the settling period, the mixture was filtered and washed with methanol in a Buchner funnel. The precipitate was discarded. The acetone and methanol were then removed on a rotary evapor-
ator, and the remaining solution was filtered. The filtrate was diluted to about one liter with water, the pH adjusted to 8.5 with ammonium hydroxide, and then passed through a resin column.

A column 2 cm in diameter filled with 10 cm of Dowex 50W X8, 200-400 mesh resin was used. The resin was prepared by washing with water, 4 per cent sodium hydroxide, and again with water until the effluent was neutral. An excess of 4 per cent hydrochloric acid was passed through the column followed by water until there was a negative test for chloride using silver nitrate. An excess of 2N ammonium hydroxide was passed through the column followed by water and finally methanol, following which the synephrine was eluted with approximately 200 ml of 2N ammonium hydroxide in methanol. The eluate was taken to dryness on a rotary evaporator and the residue dissolved in 2 ml of 10 per cent isopropyl alcohol.

Synephrine was separated from the other components of the citrus extract using the one dimensional paper chromatographic method of Stewart (6) (Figure 1). The alkaloid gave a reddish brown spot after treatment with the ninhydrin mixture and had an Rf value of 0.67. More consistent results have been obtained by increasing the ninhydrin concentration to twice that reported in the original paper. From 2 µl to 6 µl of the extracts were applied to each spot in 0.4 µl increments. The standards were prepared from l-synephrine isolated from tangerine leaves and purified by forming the oxalate salt and recovering the base. Purity was checked by infrared analysis. The synephrine standard was passed through a resin column of similar size to that used for the juice. The standards were then diluted so that 1 µl contained 0.1, 0.2, 0.3 and 0.4 µgm of nitrogen.

Properties

Synephrine is sometimes called Sympatol, and that which occurs in citrus is L-p-hydroxy α

Figure 1.—Paper chromatogram showing the separation of synephrine from basic amino acids in citrus juices. The row of spots on the right is synephrine. Note the absence of any in grapefruit.
(methylamino methyl) benzyl alcohol. Formula C₉H₁₃NO₂, molecular weight 167.2.

\[ \begin{array}{c}
\text{HO} \\
\text{CHOCH₂NHCH₃}
\end{array} \]

Synephrine forms small clear crystals which decompose at 162-164° C. It is soluble in methanol or water but only slightly soluble in acetone, ether or the higher alcohols. The best criteria for identification is by means of the infrared spectrophotometer which gives a different absorption spectrum for the levo and racemic forms. The compound is basic and probably occurs in citrus as a salt.

Synephrine was first synthesized in 1927 (5) and has been of interest because of its physiological properties and similarity to adrenaline. It can be transformed into adrenaline either by exposure to ultraviolet radiation (4) or by enzymatic activity (1). The levo isomer which occurs in citrus is reported to have 60 times the vasopressor activity of the dextro isomer (3). Synephrine has been recommended for the treatment of chronic hypotension (3) and as an antihistamine in the treatment of common colds (2).

**ANALYSES ON JUICES AND DRINKS**

Synephrine was determined on a variety of concentrated citrus juices and on samples of single strength orange, lemon and lime juice. The amount found was dependent on the variety, with the largest amounts present in Murcott and tangerine juice (Table 1). The lower amounts were in the juice of common orange varieties and in Temple juice. None was detected in grapefruit, lemon or lime juice. Synephrine was also found in canned single strength juice and in dried orange juice, indicating that the alkaloid is not decomposed by the treatments used in processing these products.

Analyses were also made on a number of commercial citrus drinks to which orange juice had been added. Synephrine was found in all of the drinks with only a trace showing in the soda (Table 2). As expected, synephrine was considerably lower in the drinks than in the undiluted juice.

**DISCUSSION**

The data presented in this paper show that synephrine is present in the juice of the common orange varieties grown in Florida. At the present there are no satisfactory procedures for identifying orange juice; for example, to determine the amount of natural juice that has been added to drinks, ales and sodas. It is believed that the synephrine content may be a useful guide for this purpose. Although tangerine and Murcott juices contained considerably higher amounts of the alkaloid than that from common orange varieties, this should not be a serious drawback to the procedure. Further studies will be required to determine the influence of fruit maturity on the synephrine content of the juice. Synthetic synephrine could not be

<table>
<thead>
<tr>
<th>Code</th>
<th>Product</th>
<th>mgs./6 oz.</th>
</tr>
</thead>
<tbody>
<tr>
<td>453</td>
<td>Hamlin concentrate</td>
<td>11.9</td>
</tr>
<tr>
<td>454</td>
<td>Hamlin concentrate</td>
<td>10.5</td>
</tr>
<tr>
<td>462</td>
<td>Valencia concentrate</td>
<td>9.9</td>
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<td>Valencia concentrate</td>
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</tr>
<tr>
<td>451</td>
<td>Pineapple concentrate</td>
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<tr>
<td>459</td>
<td>Pineapple concentrate</td>
<td>11.0</td>
</tr>
<tr>
<td>450</td>
<td>Temple concentrate</td>
<td>8.1</td>
</tr>
<tr>
<td>458</td>
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<td>10.1</td>
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<tr>
<td>452</td>
<td>Tangerine concentrate</td>
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<td>465</td>
<td>Tangerine concentrate</td>
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</tr>
<tr>
<td>449</td>
<td>Murcott concentrate</td>
<td>28.6</td>
</tr>
<tr>
<td>457</td>
<td>Murcott concentrate</td>
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</tr>
<tr>
<td>478</td>
<td>Orange, single strength</td>
<td>2.2</td>
</tr>
<tr>
<td>472</td>
<td>Instant orange juice*</td>
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</tr>
<tr>
<td>466</td>
<td>Marsh grapefruit concentrate</td>
<td>0</td>
</tr>
<tr>
<td>468</td>
<td>Duncan grapefruit concentrate</td>
<td>0</td>
</tr>
<tr>
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<td>Duncan grapefruit concentrate</td>
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</tr>
<tr>
<td>473</td>
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<tr>
<td>477</td>
<td>Lime juice</td>
<td>0</td>
</tr>
</tbody>
</table>

*USDA dried product, reconstituted to single strength.

**Table 1. Synephrine content of citrus juices.**

<table>
<thead>
<tr>
<th>Code</th>
<th>Product</th>
<th>mgs./6 oz.</th>
</tr>
</thead>
<tbody>
<tr>
<td>465</td>
<td>Soda</td>
<td>trace</td>
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<tr>
<td>464</td>
<td>Single strength drink</td>
<td>0.3</td>
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<td>Orange drink concentrate</td>
<td>1.7</td>
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<td>476</td>
<td>Orange drink concentrate</td>
<td>2.5</td>
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<tr>
<td>479</td>
<td>Orange drink concentrate</td>
<td>3.3</td>
</tr>
<tr>
<td>480</td>
<td>Orange drink concentrate</td>
<td>3.4</td>
</tr>
</tbody>
</table>

**Table 2. Synephrine content of orange drinks.**
readily added to drinks because it is a prescription type pharmaceutical and the levo isomer found in citrus would be expensive to manufacture.

The analytical data presented in this paper are preliminary and should serve only as a guide, as the values are limited in their quantitative accuracy to that of paper chromatography. However, there is little doubt that instrumental methods can be developed for determining synephrine that will be much more accurate and faster than those presented in this paper.

The interesting physiological properties of synephrine should not be overlooked. Its value as a stimulant and antihistamine undoubtedly contributes, along with vitamin C and other constituents, toward making orange juice a healthful drink. However, unlike vitamin C and possibly other constituents, it is unlikely that natural synephrine will be added to synthetic drinks.

**Summary**

Synephrine, \( l-p \)-hydroxy \( \alpha \) (methylamino methyl) benzyl alcohol, has been found in juice of the common varieties of oranges grown in Florida. The highest amounts were found in the juice of Murcots and tangerines and none was detected in grapefruit, limes, or lemons. The properties of this alkaloid are discussed and it was proposed that this compound be used as a means of identifying orange juice in diluted drinks.

**Acknowledgments**

The author wishes to express his thanks to Dr. F. W. Wenzel for supplying most of the juice samples used in this study and to Dr. Robert E. Berry, USDA Fruit and Vegetable Products Laboratory, Winter Haven, Florida, for the sample of instant orange juice.

**LITERATURE CITED**


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**DRIED CITRUS SEEDS—NUTRIENT COMPOSITION AND NUTRITIVE VALUE OF PROTEIN**

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Florida Agricultural Experiment Station

Gainesville

The state of Florida is considered a protein deficient area in terms of locally produced protein supplements for livestock feeding. Considerable quantities of protein supplements such as soybean meal and cottonseed meal are shipped into the state annually. In the production of dried citrus pulp, a by-product feedstuff of the citrus canning industry, dried citrus seeds are included. Twenty-five samples of dried citrus pulp examined by Ammerman and Arrington (1) contained 1.8 to 8.3% seeds with an average seed content of 4.8%. Citrus seeds are high in protein and thus may represent an important source of supplemental protein for livestock feeding.

The present study was conducted to determine the nutrient composition of whole dried citrus seeds, citrus seed hulls and citrus seed kernels and to determine the nutritive value of citrus seed protein in relation to soybean protein for lambs.

**Experimental Procedure**

Five samples of dried citrus seeds were obtained over an 8-day period in December 1962.