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The Isolation and Identification of *l*-Synephrine in the Leaves and Fruit of Citrus*

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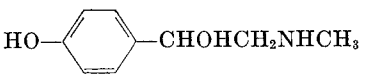
During a study of the relationship between trace element deficiencies and the free amino acids in citrus, an unknown basic nitrogen compound was detected in leaf extracts. In most samples there was more of the unknown constituent than of any other basic nitrogen compound. However, it was not present in leaf samples from trees with manganese deficiency. A comparison of its paper chromatographic R_F values with those of many known materials suggested that it was a compound not commonly found in plants. After extraction and isolation, it was identified by degradation studies and infrared analyses as *l*-*p*-hydroxy-α-(methylaminomethyl)benzyl alcohol, (synephrine, Sympatol),



an *Ephedra* alkaloid not previously known to occur in plants.

EXPERIMENTAL PROCEDURE

Extraction and Separation—Leaves collected from tangerine trees were frozen and extracted in a Lourdes mixer with either 80% aqueous ethanol or anhydrous methanol, the latter being a more suitable solvent. The extract was adjusted to pH 8.5 with ammonium hydroxide and passed through a Dowex 50-X4 ion exchange column. The column had first been treated with 1 N sodium hydroxide, followed by 4% hydrochloric acid, again with sodium hydroxide, and finally with water until the pH of the effluent was between 8 and 9. After passage of the extract, the column was washed with water and then with methanol. The *l*-synephrine was then eluted with 2 N ammonium hydroxide in methanol. The eluate was taken to dryness in a rotary vacuum evaporator at 50°. *l*-Synephrine crystals formed during the final stages of evaporation. These were dissolved in a small volume of hot methanol and recrystallized at 4°. This material could also be crystallized from water or from 80% acetone and water.

IDENTIFICATION

Properties of Base—The crystals were clear plates, soluble in methanol and water but only slightly soluble in acetone, ether, or the higher alcohols. The melting point is a decomposition point and is not a good criterion of purity since it varies with the rate of heating. Decomposition of *l*-synephrine, heated at approximately 1° per minute, took place at 162–164°.

$C_9H_{13}NO_2$

Calculated: C 64.65, H 7.84, N 8.38
Found: C 64.58, H 7.80, N 8.14

The synephrine extracted from citrus leaves was the levo isomer with an optical rotation in 0.5 N HCl, $[\alpha]_D^{25} = -55.60°$.

Derivatives

Oxalate Salt—This salt was readily prepared by adding slowly 159 mg (0.0018 mole) of dry oxalic acid to 400 mg (0.0024 mole) of synephrine in 25 ml of boiling methanol. Small crystals formed immediately, and after cooling, they were collected on a filter. When the salt was made from pure synephrine, an 86% yield was obtained. The oxalate salt was a stable derivative and was useful in the purification of *l*-synephrine. For purification, the salt was suspended in boiling methanol, and while the solution was still heating, water was added slowly until the crystals dissolved. A small amount of Darco G-60 activated carbon was added, and the hot mixture was filtered. Large, clear, thin plates formed when the filtrate was cooled to −18°. Infrared curves and elemental analysis before and after regeneration from this salt showed that the synephrine had not been altered. Elemental analysis of the salt suggested that a mole for mole combination did not take place but rather 2 moles of the base combined with 1 mole of the acid.

$(C_9H_{13}NO_2)_2(C_2H_2O_4)$

Calculated: C 56.32, H 7.09, O 30.01, N 6.57
Found: C 56.29, H 6.71, O 30.00, N 6.82

The melting point of the crystals was 221–222° (decomposition). Further evidence for the composition of the oxalate salt was obtained as follows. A minimal amount of 0.5 N NaOH was used to dissolve 500 mg of synephrine oxalate. Approximately 8 volumes of methanol were added, and a precipitate was formed. After settling overnight in a refrigerator, the precipitate was filtered and washed with methyl alcohol. It had a neutral reaction and was identified as sodium oxalate by infrared analysis. This decomposition yielded 132 mg of sodium oxalate, which is the approximate theoretical yield. The free base was recovered by adjusting the filtrate to pH 8.5 and passing it through a Dowex 50-X4 ion exchange column which had been prepared as described in "Extraction and Separation."

Degradations

Alkaline Fusion—The unknown was mixed with an excess of dry KOH in a tube and heated with an open flame in an atmosphere of nitrogen. A volatile amine distilled and was collected in a methanol solution of picric acid. A crystalline picrate salt was thus obtained which proved to have the same infrared absorption spectrum as an authentic sample of methylamine picrate.

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The residue from the fusion was made acid with hydrochloric acid, taken to dryness, and then triturated with methanol. After concentration, a crystalline product was isolated which was found to have an infrared absorption spectrum identical with that of an authentic sample of *p*-hydroxybenzoic acid.

Hofmann Degradation—Proof that the nitrogen atom was not in a ring was obtained by methylation of the base with methyl iodide. The resulting salt, m.p. 250° (decomposition), was subjected to Hofmann degradation by heating it with 30% aqueous sodium hydroxide. A volatile amine was collected and identified by infrared analysis as trimethylamine by preparation of its picrate salt.

Tests for Amines

A Van Slyke determination indicated that the unknown contained no significant amount of primary amino nitrogen. A test for tertiary amines which involved heating the unknown with acetic anhydride and citric acid, according to the method of Feigl (1), was negative. A positive test was obtained for a secondary amine, when the unknown was treated with 5% basic copper sulfate, and extracted with carbon disulfide and benzene (1).

Infrared Studies

Absorption curves were made with a Beckman model IR-4 double beam instrument with 1.5 mg of sample in a KBr pellet. From the results of the degradation studies, the tests for amines, the elemental analyses, and the negative tests for alkyl ether groups, it was considered likely that the unknown was *l*-synephrine. A synthetic sample of racemic synephrine tartrate was obtained, and the free base was regenerated from this salt. The infrared absorption curves obtained for racemic synephrine and the unknown were quite different, especially in the 3- to 4-μ and the 9- to 11-μ regions. There were also pronounced shifts in the 7- to 9-μ region (Fig. 1). However, when the oxalates were compared, the main differences were in the region from 11.8 to 12.8 μ, and these were primarily shifts. The tartrates also showed only slight differences. Purification procedures did not greatly change the differences in absorption peaks. These differences were suspected to be due to different crystal structures in the racemic and levo forms. Attempts to resolve the racemic form either as the tartrate, malate, or quinate or with amino acid oxidases were unsuccessful. Therefore, the unknown was racemized by refluxing a sample for 24 hours in an aqueous solution made slightly acid, approximately pH 1 to 2, with hydrochloric acid. The recovered base then gave an infrared curve identical with that of the racemic sample. The optical rotation of the racemized natural product was $[\alpha]_D^{25} = +5.80°$. The infrared absorption curves of the two racemic oxalates were also found to be identical.

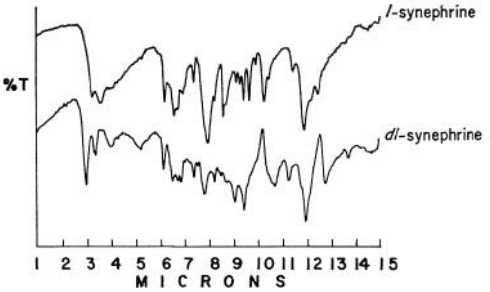

FIG. 1. Infrared absorption curves of *l*-synephrine and *dl*-synephrine.

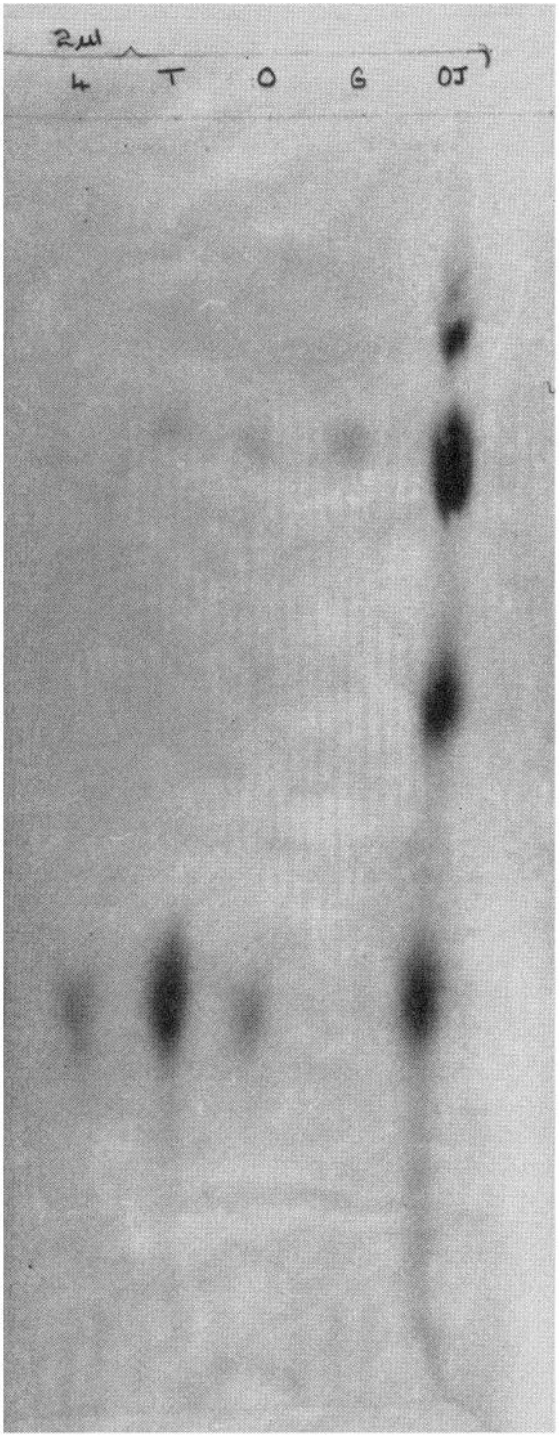

FIG. 2. Paper chromatogram showing synephrine in: *4*, known sample; *T*, tangerine leaves; *O*, orange leaves; *G*, grapefruit leaves; and *OJ*, orange juice. Spots in descending order are lysine, arginine, histidine, and synephrine.

Finally, synthetic samples of *l*-synephrine were obtained, and their infrared absorption curves were found to be identical in all respects with those run on samples extracted from citrus.

Chromatography

The best means found for separating *l*-synephrine in plant samples was by chromatography on EDTA-buffered paper with phenol-cresol as a solvent (Fig. 2) (2). By this procedure, synephrine has an R_F of 0.67. When a known sample of synephrine was compared with the unknown, identical R_F values were obtained. This was also true when two other solvent combinations were used.

OCCURRENCE

l-Synephrine has been observed in several hundred samples of citrus leaves with the greatest amounts occurring in mandarin varieties. Sufficient quantitative determinations have not been made to establish the range of concentration. Single analyses on tangerine and orange leaves showed them to contain 3.1 and 0.5 mg per g (green weight), respectively. Up to this time, none has been found in the leaves of grapefruit, pumelo, or shaddock (Fig. 2). However, in tangelos, which are hybrids of mandarin-pumelo crosses, the alkaloid is present. *l*-Synephrine has not been found in the roots of any variety of citrus. One sample of Valencia orange juice from a commercial extractor contained 4.8 mg per liter. Further studies will be reported elsewhere on the range of concentration in which synephrine occurs in citrus fruit juices.

DISCUSSION

Synephrine was first reported as a synthetic compound by Legerlotz (3) in Germany in 1927. It is used as a sympathomimetic agent, as a vasopressor in hypotension (4), and as an antihistamine in the treatment of common colds (5). Recently, synephrine has been reported to occur in human urine (6–8). The amounts present in urine fluctuate considerably, which led Kakimoto and Armstrong (7) to suggest that, rather than being a normal metabolite, synephrine probably comes from dietary sources. The presence of *l*-synephrine in citrus would tend to further substantiate this. Recently, Axelrod (9) demonstrated that a nonspecific enzyme in rabbit liver would form adrenaline from synephrine. This would suggest that citrus juice may be an indirect dietary source of adrenaline in man.

l-Synephrine is the only alkaloid known to occur in citrus. However, other nitrogen bases have been reported, and these have been reviewed by Sinclair (10). According to this review, betaine and stachydrine were isolated by the Japanese worker, Yoshima, in 1918. These are the methylated derivatives of glycine and proline, respectively. Choline was reported as a constituent of the juice in 1935 by Nelson *et al.* and later confirmed by Swift and Veldhuis. Putrescine was found by Herbst and Swell. The lack of structural similarity between synephrine and these bases, known to occur in citrus, makes it unlikely that their biosyntheses are related. Pisano *et al.* (6) have speculated that the biosynthesis of synephrine in animals may follow the route of β-hydroxylation of tyramine to form norsynephrine followed by the N-methylation of norsynephrine to synephrine by the same enzyme which converts norepinephrine to epinephrine. It will be interesting to determine if the same pathway is followed in plants.

SUMMARY

l-p-Hydroxy-α-(methylaminomethyl)benzyl alcohol (synephrine, Sympatol) has been isolated and identified from citrus leaves and juice. Identification was by means of degradations, derivatives, chromatography, and comparison of infrared absorption curves with those of samples from synthetic origin. Synephrine is not previously known to occur in plants.

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