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THE EXISTENCE OF THE NON-INDOLIC CITRUS AUXIN IN SEVERAL PLANT FAMILIES*

L. N. LEWIS, R. A. KHALIFAH and C. W. COGGINS, Jr.

University of California, Citrus Research Center and Agricultural Experiment Station, Riverside, California

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Abstract—A second species of the genus Citrus and fruits from five other families were examined for their auxin content.

The non-indolic "citrus auxin" was detected in *Citrus limon* as well as *Citrus sinensis*, the original source of this compound. The fruits of date, fig, peach and Natal plum were also found to contain the "citrus auxin". This is the first report of the existence of this non-indolic auxin in fruit outside the family Rutaceae. Fruits of guava and strawberries contained a compound believed to be IAA, but they showed no traces of the "citrus auxin".

INTRODUCTION

YOUNG orange fruit (*Citrus sinensis*) contain a non-indolic auxin[1] which induces curvature of the Avena coleoptile and which has been named "citrus auxin". Work is in progress aimed at characterizing both the chemical structure of this compound and its biological activity. The work reported in this paper was done to answer two questions: (1) Is the compound specific to *Citrus sinensis*; and if not (2) is it present in the fruit of families other than the Rutaceae?

RESULTS AND DISCUSSION

An auxin-like substance which has the same spectrofluorometric, chromatographic, and biological properties as the "citrus auxin" of orange[1] has been extracted from the young fruit of lemon, date, Kadota fig, peach, and Natal plum (Table 1). This substance had an R_f value of 0·26–0·32 in butanol:ammonia:water and 0·33–0·40 in isopropanol:ammonia:water, in agreement with those previously reported for citrus auxin. The auxin from all the above fruit and that from *Citrus sinensis* had identical excitation and fluorescence spectra with maxima at 350 mμ, and 460 mμ, respectively. Regardless of the fruit used as a source of this auxin, the substance was active in the Avena curvature test. Therefore, it seems reasonable to conclude that the same non-indolic auxin exists in the orange, lemon, date, fig, peach, and Natal plum.

Citrus auxin is probably the same auxin Blommaert,[2] Hendershott,[3] and Nevins and Hemphill[4] observed in peach-bud extracts. These workers found a compound active in coleoptile straight-growth assays with an R_f similar to citrus auxin and which failed to

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[4] W. S. B. NEVINS and D. D. HEMPHILL, *Plant. Physiol.* 31, (Supp.) XXVIII (1956).

TABLE 1. THE DISTRIBUTION OF CITRUS AUXIN IN SIX PLANT FAMILIES

Common name	Family	Auxin
Date (*Phoenix dactylifera*)	Palmaceae	Citrus
Fig (*Ficus carica*)	Moraceae	Citrus
Peach (*Prunus persica*)	Rosaceae	Citrus
Strawberry (*Fragaria chiloensis*)	Rosaceae	IAA
Lemon (*Citrus limon*)	Rutaceae	Citrus
Orange (*Citrus sinensis*)	Rutaceae	Citrus
Guava (*Psidium guajava*)	Myrtaceae	IAA
Natal Plum (*Carissa grandiflora*)	Apocyanaceae	Citrus

develop color with Salkowski's reagent. Lombard,[5] and Stahly and Thompson[6] also reported the presence of a compound active in coleoptile straight-growth tests which chromatographed similarly to citrus auxin but did not give a positive color reaction to Ehrlich's reagent. Stahly and Thompson reported that any color formed with Ehrlich's and Salowski's reagents was transient. It is possible that too little auxin was present for reaction with the Salkowski or Ehrlich reagents but enough for the biological test. In this case, an indole compound could have been the active material. However, as citrus auxin is found in the young peach fruit, it is probable that it was the compound observed. The detection of non-indolic compounds in plants active in straight-growth assays has been fairly common, so the possibility that the auxin in peach was identical with citrus auxin was not noted by these workers. The Avena coleoptile curvature test, on the other hand, is a more specific assay, so the presence of a non-indolic compound in plants which is active in this test is noteworthy, and indicates the advantages of such tests.

Luckwill[7] reported the absence of indoleacetic acid and the presence of a widely distributed auxin (Malus auxin 2) in the apple fruit which was active in the oat coleoptile straight-growth assay. This auxin did not give the typical color reaction to Salkowski's or Ehrlich's reagents even though it was apparently present in sufficient quantity. Like citrus auxin, it had a higher R_f value than IAA (0·30–0·45 in butanol:ammonia), but we were unable to find either citrus auxin or IAA in a preliminary study of the Permain variety of apple. Wright[8] also found an auxin in the fruit of black currant (*Ribes nigrum*) which was active in the straight-growth test, chromatographed like citrus auxin, and gave a delayed reaction to Salkowski's reagent. No previous mention of the qualitative nature of auxins in dates, figs, or Natal plums was, however, noted in the literature. In view of the importance of synthetic growth regulators in fig,[9] further studies with this fruit should be of particular interest.

Although the citrus auxin was common to all these fruits, its relative concentration varied greatly. It was difficult to be precise in describing concentrations, as the extraction procedure was probably not equally efficient for all tissues. Generally, the peaches and dates had the lowest concentration, oranges and Natal plums contained about ten times more, and lemons and figs were another ten-fold higher. Such a wide range of concentrations emphasizes the importance of an overall balance of auxins, gibberellins, inhibitors, and perhaps kinins in

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[7] L. C. LUCKWILL and L. E. POWELL, JR., *Science* **123**, 225 (1956).
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plant growth and development. It is not adequate to think of the concentration of one compound in terms of fruit growth when a range of fruit in a similar stage of development has a 100-fold difference in concentration.

None of the "citrus auxin" was found in the strawberry (*Fragaria chiloensis*), nor the guava (*Psidium guajava*). Extracts of these fruits contained an auxin which appeared to be indole-3-acetic acid (IAA). The strawberry and guava auxin had identical R_f values with IAA in both solvent systems. They had the same excitation and fluorescent maxima as IAA, 290 mμ and 360 mμ, respectively, and the compound from both plants stimulated curvature in the bioassay. Nitsch[10] previously reported the presence of IAA in strawberry, based on chromatographic and positive reactions to Ehrlich's and Salkowski's reagents.

Although citrus auxin is not common to all fruits, it does appear to be present in a wide range of fruit types. Thus far, it seems that a fruit has either IAA or citrus auxin, since both compounds have not been found in any one fruit. The limited data now available suggest that non-indolic auxins are widespread in higher plants although not universal. It follows, however, that it is no longer adequate to consider IAA as the model for general auxin mediated studies.

The boundaries which define a fruit as containing indolic or non-indolic auxins are not clear. If the morphologically based phylogenetic rank given families by Bailey[11] is correct, the type of auxin is not related to the phylogenetic level. In fact, it cannot even be assumed that a family will contain one type auxin, as evidenced by the difference between strawberry and peach in Rosaceae. Again on morphologic criteria, the fig is primarily receptacular tissue, but so is the strawberry. Many more plants will have to be studied before such boundaries can be described and perhaps additional auxins will be added by then.

EXPERIMENTAL

The young fruit from eight plant species, representing six plant families (Table 1), were used in this experiment. The auxin was separated from two to three pounds of fruit from each species. The fruits were frozen immediately after harvest, lyophilized, and extracted with peroxide-free diethyl ether, as previously described by Khalifah *et al.*[1] The acidic compounds from this extraction were fractionated by two-dimensional descending paper chromatography using *n*-butanol:ammonia:water (4:1:1 v/v) and isopropanol:ammonia: water (10:1:1 v/v), respectively. After development, fluorescent and absorbing spots were detected by scanning the dried chromatogram with u.v. light (253 mμ, maximal intensity). These spots were cut out, eluted in water, and their excitation and fluorescence spectra were determined on an Aminco-Bowman Spectrophotofluorometer. The Avena coleoptile curvature test[12] was used to assay the biological activity of the extracted substances.

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