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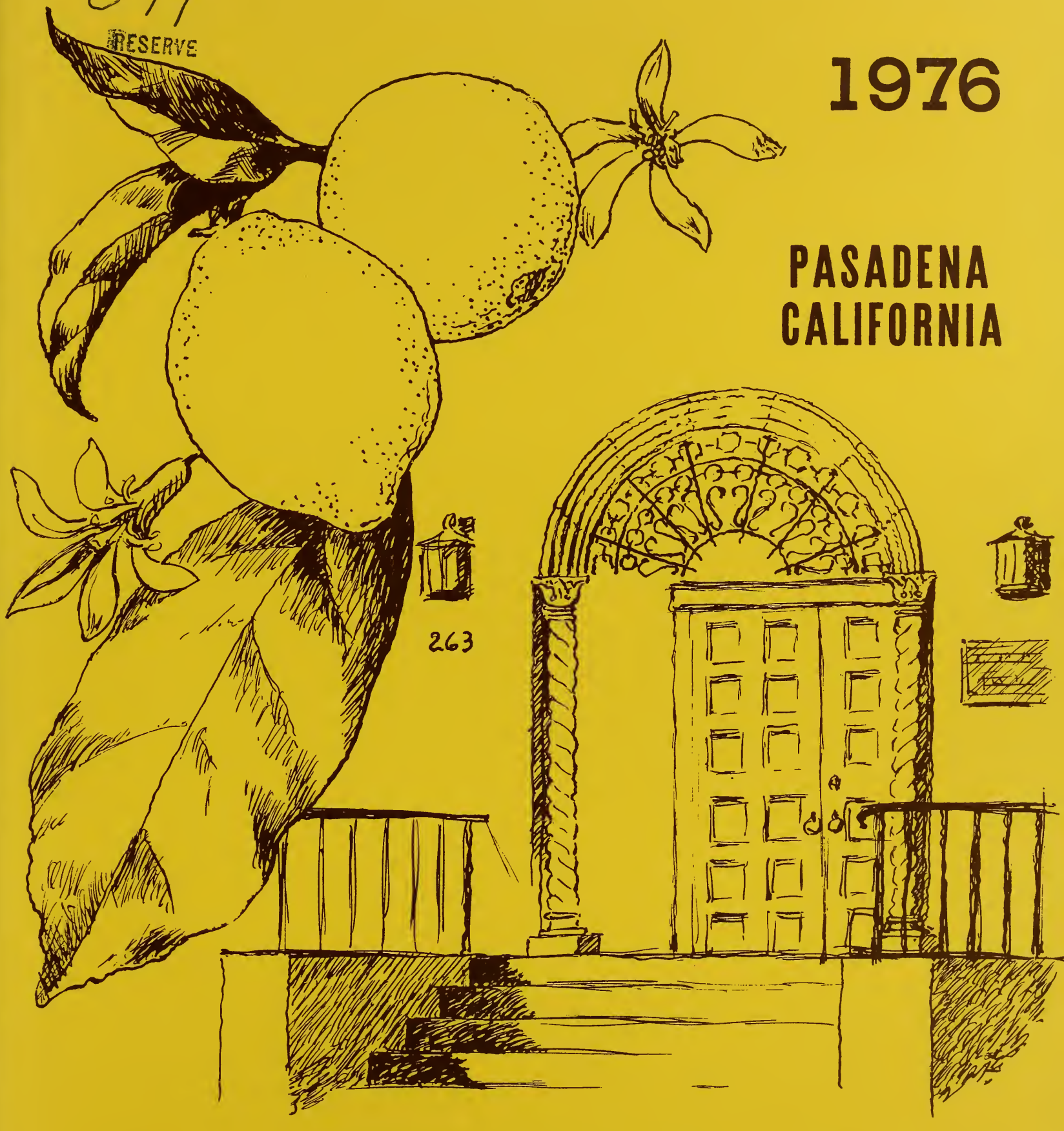

CITRUS RESEARCH CONFERENCE

CITRUS RESEARCH CONFERENCE

December 8, 1976

Pasadena, California

ABSTRACTS OF PAPERS

Sponsored By:

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue
Pasadena, California 91106

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

FOREWORD

This Citrus Research Conference is being held to bring to members of the citrus and allied industries in southern California and Arizona the latest results of research on the chemistry, pharmacology, and technology of citrus fruits and their products carried on by the Agricultural Research Service, U.S. Department of Agriculture. The following are participating in this year's conference:

Western Region

Fruit and Vegetable Chemistry Laboratory
263 South Chester Avenue, Pasadena, California 91106

Western Regional Research Center
Berkeley, California 94710

Southern Region

U.S. Citrus and Subtropical Products Laboratory
600 Avenue S, N.W., Winter Haven, Florida 33880

U.S. Horticultural Research Laboratory
2120 Camden Road, Orlando, Florida 32803

Conference headquarters:

Huntington-Sheraton Hotel
1401 South Oak Knoll Avenue
Pasadena, California 91109

P R O G R A M

CITRUS RESEARCH CONFERENCE

Wednesday, December 8, 1976

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WELCOMING REMARKS: W. D. McClellan, Area Director
California-Hawaii-Nevada Area
ARS/USDA, Fresno, California

INTRODUCTORY REMARKS: Russell L. Hanlin
Vice President, Products Group
Sunkist Growers,Inc.
Sherman Oaks, California

CHAIRMAN: Vincent P. Maier, Director
Fruit and Vegetable Chemistry Laboratory
Pasadena, California

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BIOSYNTHESIS OF LIMONOIDS IN CITRUS AND A PREHARVEST APPROACH TO THE REDUCTION OF LIMONIN BITTERNESS*

Shin Hasegawa, Henry Yokoyama and John E. Hoagland
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In dealing with the problem of limonin bitterness of citrus juices, we have initiated recently, in addition to an enzymic process, a new approach, which deals with preharvest treatments of citrus to reduce the limonoid content of fruit tissues. In this regard, there is a need for knowledge about how, where, and when limonoids are synthesized in citrus.

Last year at this meeting we suggested briefly that limonoids in citrus are synthesized in leaves and translocated to fruit tissues. Recent radioactive tracer work showed that limonoids accumulated in citrus seeds are also translocated from leaves. Quantitative analyses of limonin and nomilin, and changes in ratios of the two in leaves, fruit tissues and seeds during the growth of lemon confirmed that leaves are the major site of limonoid biosynthesis. No evidence as to the presence of limonoid synthetic systems in fruit tissues and seeds was found.

We chose two compounds for studies on the inhibition of limonoid biosynthesis in lemon leaves. Based on data accumulated it was postulated that these compounds would inhibit an enzyme(s) involved in the biosynthesis of limonoids. As expected, we observed a profound inhibitory effect on the biosynthesis of limonoate A-ring lactone (LARL) when lemon leaves were sprayed with either of these compounds.

Leaves sprayed with 300 or 500 ppm of the first compound contained 3- or 12.7-fold less LARL, respectively, than the control 8 days after treatment. The second compound gave a lesser but significant effect; the difference in LARL content between the treated (300 ppm) and the control was about 2.5-fold. Also, the formation of nomilin was inhibited to a similar extent by both compounds.

The above results suggested also that limonoids are turning over continuously in leaves. Radioactive tracer work confirmed the above. More important, the work showed that both compounds had no effect on the biodegradation of limonoids in leaves.

It has been shown previously that limonoids are metabolized via 17-dehydrolimonoids in citrus. Recently, our radioactive tracer work showed that limonoids are metabolized also via deoxylimonoids.

*Work supported in part by the Citrus Products Technical Committee.

Since citrus leaves, particularly young ones, are the active site of limonoid biosynthesis, the preharvest application of compounds that influence limonoid biosynthesis in citrus leaves may have potential as an approach to reducing the limonoid content of citrus fruit.

RECENT STUDIES OF LEMON JUICE CLOUD CHEMISTRY*

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Lemon juice cloud is a complex system, both in terms of its overall chemical constitution and the variety of types of particles present. In our studies of cloud chemistry we have pursued several approaches toward our ultimate goal of relating the behavior of cloud under various conditions to its chemical properties. Here we report the current status of three lines of investigation.

Recent work on the origin of the hesperidin crystals found in lemon juice has shown that they arise by crystallization of a soluble form of hesperidin present in the albedo of the fruit. Microscopic observations indicate that albedo normally contains no visible crystalline hesperidin, but upon disruption of the tissue crystals quickly form. When the commercial juice process was simulated in the laboratory, a rapid increase in the concentration of insoluble hesperidin was observed during the first 5 hours after extraction. However, if the juice was immediately passed through a filter with a pore size of 0.5 μm, no hesperidin crystallized from the filtrate during this time period, but it came out of solution during the next few days. Apparently the formation of crystals is much faster in the presence of the cloud. These results suggested that an enzyme might be responsible for the conversion of the soluble form of hesperidin to crystals. However, crystallization was not prevented by autoclaving the fruit or by soaking albedo tissue in 3N HCl, conditions which would be expected to destroy any enzyme activity.

Treatment of cloud with purified enzymes can provide information about the surface chemistry of the particles. To insure that the effects observed were due to the cloud rather than soluble material, the juice samples to be discussed were diafiltered, i.e., soluble material was removed while the volume was kept constant by adding water. The resulting suspension of cloud in water was then treated with an enzyme. Pectinase caused a rapid aggregation of the cloud particles in diafiltered reconstituted commercial concentrates. This suggests that pectin may play an important role in the surface chemistry of the particles. However, cloud of diafiltered fresh juice, either prepared by hand reaming or taken from a commercial processing line, was stable to pectinase, as was cloud of diafiltered reconstituted concentrate produced by reverse osmosis. Apparently the normal commercial concentration processes change the surface properties of the cloud particles.

*Work supported in part by the Citrus Products Technical Committee.

Insoluble complexes of pectin and protein are being studied for two reasons: (1) such complexes may be present in cloud; (2) the complexes might be useful in formulating clouding agents for beverages. For a given pectin-protein combination, the properties of the complex (solubility, particle size, and tendency to aggregate) are dependent upon the manner in which the pectin and protein solutions are mixed, the pH, and the ionic strength of the medium. Changing either the pectin or the protein can also have a large effect upon the properties of a complex. Photomicrographs of various types of complexes will be shown.

NONVOLATILE COMPONENTS IN CITRUS TAXONOMY

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A review of published and unpublished work on some of the nonvolatile components in: (*C. sinensis*), (*C. aurantium*), (*C. grandis*), (*C. reticulata*), (*C. paradisi*, Macf.) and crosses between several of these species is presented.

We have found that methoxyl flavonoids, flavanones and coumarins make good taxonomic marker compounds. These compounds can be used to separate nucellar from zygotic seedlings. Leaf extracts of the following cvs. 'Dancy', 'Valencia', 'Parson Brown', 'Pineapple', 'Marsh' and 'Ruby Red' were prepared from six different root stocks (seedling, rough lemon, sour orange, sweet lime, Cleopatra and *Poncirus trifoliata*) for each cv., a total of 36 samples. The six 'Dancy' extracts, compared by tlc, revealed identical methoxyflavonoid patterns. Comparison of the 18 'Valencia', 'Parson Brown', and 'Pineapple' samples showed their methoxyflavonoid pattern to be identical. The three orange cvs. could not be separated or identified by this specific solvent system, but the various root stocks had no effect on the flavonoid patterns. Comparison of the 'Marsh' and 'Ruby Red' samples showed their coumarin patterns to be identical and not affected by the root stocks.

Nine known hybrids produced by a Clementine x Orlando cross were examined. They each give a distinctive pattern and could be separated from each other either by difference in concentration or lack of certain methoxyflavonoids. In eight of the nine hybrids the methoxyflavonoids were derived from a single parent. One hybrid appeared to contain a combination of flavonoids from both parents. No coumarins appeared to be present even though the 'Orlando' has a grapefruit parent. A cross between a 'Mediterranean Sweet' x 'Pineapple' orange produced hybrids with distinctive tlc patterns and they will be discussed.

We tested a number of different crosses: (*C. sinensis* x *C. sinensis*), (*C. paradisi* x *C. paradisi*), (*C. sinensis* x *C. paradisi*) and (*C. sinensis* x *C. grandis*). The findings will be discussed.

This tlc system should provide a useful procedure for identifying zygotic plants and can probably be used in determining the parentage of some unknown hybrids, as well. It should be of assistance to plant breeders, geneticists and horticulturists.

The method used was as follows: Plates were of Silica Gel GF (20 x 20 cm, 250 μ, Analtech, Inc., Wilmington, Delaware) and Baker-Flex Polyamide 6 (20 x 20 cm, J. T. Baker Chemical Co., Phillipsburg, New Jersey). Solvent Systems were (A) chloroform-acetic acid, 99-1 by volume; (B) benzene-acetone-acetic acid, 43-5-2; (C) hexane-benzene-acetone-methanol, 6-3-1-0.5, (D) hexane-benzene-acetone-methanol, 6-3-1-0.05, (E) benzene-acetic acid-water-nitromethane, 34-32-5-18; and (F) nitromethane-methanol, 5-2. Solvent systems A-E were used with the Silica Gel GF plates and F was used with the polyamide plates. Solvent systems A-D were used for the "nonpolar" portion and solvent systems E and F were used on the polar portion of the leaf extract (which contained the flavanone glycosides).

SAFETY EVALUATION OF THE SWEETENER NEOHESPERIDIN DIHYDROCHALCONE: 2-YEAR STUDY IN DOGS

M. R. Gumbmann, D. H. Gould, D. J. Robbins
and A. N. Booth
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With the uncertainties regarding the availability of low-calorie sweeteners for general food uses still unresolved, neohesperidin dihydrochalcone (NDHC) continues to promise considerable potential for filling part, at least, of the need in this area. Previously reported long-term studies of NDHC in rats were quite favorable and these have been followed by a now completed, 2-year evaluation in dogs.

Twenty-four pure bred beagle dogs, in groups of three for each sex, were fed NDHC added to a commercial dog chow to provide 0, 0.2, 1.0 and 2.0 g/per Kg body weight. Feed consumption, body weight and general condition were carefully monitored for the 2-year period. At 6-month intervals, blood samples were obtained for hematology and biochemical analysis, and urinalyses were performed. Complete necropsies were conducted at the end of 2 years, at which time all organ systems were examined grossly, followed by microscopic examination of approximately 30 tissues from each dog. As with the study in rats, the present work with dogs indicates NDHC possesses a very low order of toxicity.

Metabolic fate studies with ^{14}C-labeled NDHC in rats show that 90% of orally administered NDHC may be excreted in the urine and the remainder in the feces in 24 hours with no accumulation in tissues.

STUDIES OF THE METABOLIC FATE OF NEOHESPERIDIN DIHYDROCHALCONE

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An important aspect of the toxicological evaluation of compounds is the identification of metabolites. To obtain this information for the dihydrochalcone sweeteners, isovanillin labelled with carbon-14 at the aldehyde group has been prepared and converted to [^{14}C]-neohesperidin. The following reactions were used:

Naringin ⟶ Phloracetophenone 4'-β-neohesperidoside

Phloracetophenone 4'-β-neohesperidoside + [^{14}C]-Isovanillin ⟶ [^{14}C]-Neohesperidin chalcone ⟶

[^{14}C]-Neohesperidin chalcone ⟶ [^{14}C]-Neohesperidin ⟶ [^{14}C]-Neohesperidin dihydrochalcone

In experiments being carried out in the Pharmacology Laboratory of the Western Regional Research Center [^{14}C]-neohesperidin and [^{14}C]-neohesperidin dihydrochalcone were administered orally to rats and the urine collected over a 24 hour period. Work on the identification of metabolites is now underway at the Fruit and Vegetable Chemistry Laboratory.

Chromatographic analysis of the urine or of extracts of the urine indicates that [^{14}C]-neohesperidin dihydrochalcone fed at a level of 1 mg/kg body weight yields only one major metabolite. At a dose level of 100 mg/kg a second, minor metabolite begins to appear. Cochromatography with the labelled starting material shows that neither the major nor minor product is unchanged neohesperidin dihydrochalcone. Furthermore, neither the major nor minor product appears to be the aglycone, hesperetin dihydrochalcone. R_f values and extraction data suggest that the major metabolite is a rather polar substance and the minor metabolite non-polar. Incubation with the enzyme β-glucuronidase appears to have no effect.

Analysis of an extract of the urine of a rat receiving 1 mg/kg of [^{14}C]-neohesperidin showed the presence of one major and two minor metabolites. None of the compounds appeared to be affected by the enzymes sulfatase or β-glucuronidase. Cochromatography with neohesperidin and neohesperidin dihydrochalcone showed no identity. It has not been determined yet whether the major metabolites from the flavanone and dihydrochalcone are identical, although their R_f values show close correspondence.

Our results will be compared with those that Booth and co-workers obtained earlier using unlabelled substrates.

We wish to thank Givaudan Research Company, Ltd. for preparation of the labelled compounds.

ENHANCEMENT OF COLOR AND PROVITAMIN A QUALITY OF CITRUS FRUIT: SOME ASPECTS OF THE CURRENT RESEARCH ON BIOREGULATORS*

Henry Yokoyama, Wan-Jean Hsu, Stephen M. Poling,
Ernest Hayman and Charles DeBenedict
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In past studies we have directed a great deal of our research efforts toward development of more effective bioregulators for improving the color and provitamin A content of oranges and other citrus fruits. These studies have resulted in improved bioregulators that elicit more appropriate color and provitamin A response patterns in citrus fruits. In this past year we reached a stage in our overall developmental work where more emphasis could be placed on studying the factors that are essential for putting the bioregulators into commercial use. Studies were initiated on methods of applying the bioregulators to citrus cultivars for external and internal color enhancement from both the preharvest and postharvest bases. In this connection, preliminary field test and pilot-plant studies were carried out. Investigation of improved formulations to facilitate the penetration of the applied bioregulator into the fruit is being actively pursued; the problem of penetration of bioregulator into the endocarp of fruit having a thicker, tighter peel is also being studied. In this phase of our work the ultimate use (fresh marketing or processing) to which the fruit is to be put will have some influence on which type of treatment is most preferable.

Other factors to be considered in our overall efforts are determination of the fate of the bioregulators that enter the fruit. In this connection C^{14}-labeled bioregulators will be synthesized, and the metabolic and residual fate of these compounds will be examined. We also are concerned with determining the safety of bioregulators and their influence on fruit stability, flavor and other noncolor quality aspects.

Concurrently, we will be continuing our studies on the biosynthesis of carotenoid and related terpenoid compounds in the citrus fruit and on the mode of action of the bioregulators. From our studies it is apparent that the simplistic biosynthetic pathway proposed for carotenoid formation at the microbial level cannot be extrapolated to the citrus fruit; much more complex systems are present in the latter. Bioregulator-membrane interactions need to be examined.

In our continuing studies on structure-activity relations, we are focusing our attention on the development of bioregulators possessing multi-effects.

*Work supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

STUDIES ON PREHARVEST PREVENTION OF REGREENING IN VALENCIA ORANGES*

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The rind color of unripened Valencia oranges [*Citrus sinensis* (L.) Osbeck] is green. As they ripen, the photosynthetic activity decreases, the chlorophyll content decreases and carotenoids accumulate. During this period the fruit usually lose their chlorophyll and develop an orange color while still on the tree. If the mature fruit is not picked and kept on the tree during the summer months, it may revert to green as the chlorophyll is resynthesized, and the carotenoid content decreases. This phenomenon is known as regreening. The degree of regreening is, in part, related to temperature. Coggins and coworkers have found that hot day air temperature is responsible for regreening of Valencia orange fruits; whereas soil temperature has little influence.

Regreening of mature Valencia oranges is a problem in southern California and Florida. Two possible approaches to the problem are being considered. One is to degreen the regreened fruit, that is, to destroy the chlorophyll in the flavedo; the other approach is to prevent the fruit from undergoing the regreening process, that is, to inhibit chlorophyll biosynthesis. Regreened fruit does not respond well to the regular ethylene degreening treatment, although it may be degreened by prolonged exposure to ethylene in a warm and humid atmosphere. However, this procedure hastens rind senescence and thus increases storage problems. 2,4-Dichloro-1-cyanoethanesulphonanilide (C. W. Coggins, Jr. and A. E. Hall, J. Amer. Soc. Hort. Sci. 100:484, 1975) was found to be effective in postharvest degreening of regreened Valencia oranges, however, only when intense light was present. Preharvest treatment with this compound caused leaf abscission.

In view of the impact of regreening on the marketability of fresh fruit, alternative methods need to be developed to overcome the problem. In preliminary tests in our laboratory, a class of bioregulators which showed evidence of causing postharvest degreening of regreened Valencia oranges was discovered. However, the degreening process took about 2 months, which is impractical for commercial purposes. Studies on the mode of action of these bioregulators using kidney bean leaf discs indicated that some bioregulators appear to accelerate the degradation of chlorophyll and others to interfere with biosynthesis of chlorophyll. In the

*Work supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

latter case, up to 40% inhibition was observed. These findings suggested that it might be feasible to inhibit the regreening process itself.

In the past year emphasis was placed on inhibition of regreening. Further studies on the inhibition of chlorophyll biosynthesis were conducted, this time using kidney bean plants rather than leaf discs. Eleven bioregulators that appeared to significantly interfere with chlorophyll formation were then selected for field tests on Valencia oranges at two locations (Indio and Riverside, California). The bioregulators were applied by spraying the 15-20 fruits on a branch. An equal number of fruit on adjacent branches were left untreated and used as controls. Of the 11 compounds examined, 6 appeared to interfere with the regreening process and showed promise as potential inhibitors. In one case the inhibition of regreening also appeared to be accompanied by enhancement of the orange coloration of the flavedo. During the 2 month observation period (May 24 to July 30, 1976), the leaves and new crop small green fruit surrounding the treated fruit appeared to be healthy, and no abscission of either the leaves or the young fruit occurred. These initial field tests provide important and encouraging information about the preharvest prevention of regreening in Valencia oranges. More extensive testing of these bioregulators will be carried out next season. Efforts will also be continued toward the design and synthesis of more effective bioregulators to prevent regreening and at the same time enhance the orange color of Valencia oranges.

CHEMICAL INDUCTION OF PROVITAMIN A CAROTENES IN CITRUS FRUIT*

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Last year we reported that several *para*-substituted diethylaminoethyl benzoates in addition to causing enhanced color due to the large accumulation of the red pigment lycopene, also caused significant increases in the provitamin A cyclic carotenes, β-carotene and to a lesser degree α- and γ-carotene. Because of the advantages of causing color enhancement with large increases in β-carotene instead of lycopene, i.e., a more natural color and an increased nutritional value, further research into the affects of the esters of diethylaminoethanol was undertaken.

The esters of the following aliphatic acids were synthesized and tested on Marsh white seedless grapefruit: phenylacetic, hydrocinnamic, 4-phenylbutyric, 5-phenylvaleric, valeric, hexanoic, heptanoic, octanoic, nonanoic, 5-chlorovaleric, cyclohexanecarboxylic, phenoxyacetic, *p*-chlorophenoxyacetic, 3-phenoxypropionic, cinnamic, and *p*-chlorocinnamic. After 2 weeks the flavedo was analysed and the color enhancement was seen to be due mostly to β-carotene accumulation, which made it the major pigment in several cases, and to a lesser degree to lycopene accumulation. In particular, the hexanoate, the 4-phenylbutyrate and the cinnamate caused up to a 50- to 60-fold increase in the β-carotene content with a lycopene content of only 10-15% of the accumulated β-carotene.

To see whether the β-carotene and lycopene accumulate in the same way with both β-carotene and lycopene inducing compounds, the hexanoate and 2-phenoxytriethylamine, which is a moderately effective lycopene inducer, were applied to grapefruit from the same lot and the flavedo analysed after 1, 2, 3, 4, 7 and 14 days. 2-Phenoxytriethylamine caused a rapid increase in the carotenes, mostly lycopene, the first day and a slow steady increase thereafter. Lycopene comprised 52 and 71% of the total carotenes after 1 and 14 days, respectively, while there was no significant increase in the cyclic carotenes. The response to the hexanoate was much different. The total carotenes increased rapidly during the first day due to the formation of lycopene, not to β-carotene accumulation. Thereafter there was only a slight increase in the total carotenes, but after 4 days the amount of lycopene had decreased to half the initial amount and a similar amount of β-carotene had accumulated. After 7 days β-carotene was the predominate pigment. To see whether the enhanced accumulation of cyclic carotenes caused by the benzoates

*Work supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

occurs in the same way, a lower concentration of *p*-bromobenzoate than was used in the earlier experiments was applied to grapefruit and the flavedo analysed after 2 and 4 weeks. After 2 weeks, lycopene was the predominate pigment with a smaller but significant increase in β-carotene. But after 4 weeks, the lycopene content had decreased although it was still the major pigment and there had been a large increase in β-carotene. Thus the benzoates seem to act in the same way as the aliphatic esters but the conversion of lycopene to β-carotene is greatly delayed. These results can be explained if we assume that the esters are gradually hydrolyzed and thereby lose their biological activity. The rates of hydrolysis of aromatic and aliphatic esters reported for citrus acetylcholinesterases are in general agreement with the relative rates that would hold if our assumption is correct.

The β-carotene inducers therefore seem to work initially in the same way as the lycopene inducers, i.e., derepressing a gene regulating the synthesis of a specific enzyme(s) in the biosynthetic pathway of the carotenoids and inhibiting the cyclase(s) that gives rise to the provitamin A carotenes. However, the β-carotene inducers, the aliphatic and to a certain degree the aromatic esters of diethylaminoethanol, are gradually hydrolyzed so that induced pigment formation ceases. But because the hydrolyzed inducer no longer inhibits the cyclase(s), the accumulated lycopene is converted into β-carotene.

The aliphatic esters, in addition to causing color enhancement, are much more effective at increasing the provitamin A cyclic carotenes than the previously reported lycopene inducers. It should also be possible to achieve better control of the degree of color enhancement of fruit destined for the fresh fruit market because the pigments accumulate only during the first few days after treatment and not indefinitely as is the case with the lycopene inducers.

STUDIES ON PRE- AND POSTHARVEST APPLICATION METHODS OF BIOREGULATORS*

Ernest Hayman, Henry Yokoyama, Stephen M. Poling, Wan-Jean Hsu
and Charles DeBenedict
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In the past most of our efforts centered around the postharvest induction of color in the peel of various citrus fruit under controlled laboratory conditions. In the present work, investigations into the preharvest application of the bioregulators were begun, and the nature of pigment induction in the endocarp was studied. The present work also included pilot scale studies of postharvest peel color induction.

Field-run Valencia oranges were purchased from a packing house and prepared on typical commercial cleaning equipment. Bioregulators were included in the foamer-cleaning solution, three different wax formulations, and a dip treatment. Effectiveness was evaluated by the uniformity of color induction in the peel. A dip treatment appeared to be the most effective with 90% of the fruit showing color induction over 50% or more of the peel area. Inclusion of the inducer in the foamer-cleaner was effective, but this effectiveness was diminished rapidly by the subsequent normal water rinsing step. The inclusion of the inducer in a solvent based wax also showed promise as 68% of the fruit showed color induction on 50% or more of the peel.

Several bioregulators were applied to navel and Valencia oranges in Riverside County by spraying selected branches using a hand held sprayer. Induction of color in the peel was observed. This method resulted in an unacceptable blotchy appearing peel, however, endocarp color was uniform and enhanced. Further experimentation with various surfactants indicated this deficiency may be lessened. At the same time endocarp pigmentation patterns were investigated. A 100% increase in total carotenoids was obtained. Furthermore, there was no accumulation of lycopene as is found in all other systems studied so far with this class of bioregulators. The bright red coloration normally associated with lycopene is not as desirable as the deeper orange color induced by this process in the endocarp. Although there are several possible explanations, the lack of lycopene accumulation here may represent the differences between the carotenoid biosynthetic pathways of endocarp and peel, and therefore may be of considerable biochemical importance.

*Work supported in part by the California Citrus Advisory Board and the Florida Citrus Commission.

In order to study the effects of induction upon the endocarp under more controlled conditions, a laboratory scale pressure infiltration apparatus was developed. It consisted of a thick walled pressure-tight glass container. The fruit to be treated were submerged in a solution of the desired bioregulator, and pressure was applied to the apparatus via a tank of compressed nitrogen. Various operating parameters were tried and the effects of these upon the pigment level of oranges will be discussed.

On a laboratory scale, vacuum infiltration of bioregulator solutions is a very effective means to bring about a uniform color induced action in oranges. The date industry sometimes uses a continuous flow commercial scale vacuum infiltration process. This technology was applied to oranges with the assistance of Carl Vandercook and resulted in uniform external and internal induced coloration.

THE CHARACTERIZATION OF LARGE COLLECTIONS OF FINE PARTICLES BY SIZE MEASUREMENT: A STATUS REPORT

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Pasadena, California

In our early work on lemon juice we fitted particle size distribution data with a cubic equation. The fitted equation readily provided a precise estimate of the effective diameter of the particles making the greatest volume contribution to the cloud-mass. An additional advantage of this method of summarizing particle size data was that it held the subjective aspects of data interpretation to a comparatively low level. Furthermore, when applied to fresh, unpasteurized juices, it facilitated precise evaluation of the widths of the particle size distribution peaks. However, the relative sharpness of the size distribution peaks of certain commercially finished juice specimens made it difficult to use the cubic curve fitting routine. Another difficulty arose when storage tests were carried out on a commercial lemon juice concentrate. In this case it was impossible to obtain a satisfactory fit using the cubic routine.

To overcome the foregoing problems, we adopted some existing numerical procedures that could provide greater flexibility. Curves are now fitted to the particle size data by applying a first-degree, three-point smoothing procedure. Numerical differentiation of the smoothed data is used to specify diameters that correspond to peaks in the particle size distributions. Because of improved compliance with experimental data, the numerical methods make it practical to quantitate the skewness of particle size distributions. Changes in skewness during juice processing evidently correlate with changes in turbidity.

The Model A Coulter Counter initially used by us to measure particle size often malfunctioned because of the heat generated by its large complement of vacuum tubes. Furthermore, being restricted to a single size threshold per instrument setting, it was an inherently slow instrument. Considerations of this kind led us to attempt to build a particle measurement system that would: (a) place minimal reliance on vacuum tubes, and (b) provide several particle size thresholds per instrument setting. The system that we have assembled provides up to 1023 size thresholds per instrument setting,and it appears to be free of the kind of heat-up problems that were previously encountered. Compared with the old Model A, the new particle system is a high-speed, precision instrument. Because of this inherently high speed, its most efficient use will be realized when linked with a computer.

PROGRESS IN THE USE OF MICROORGANISMS TO DETECT ADULTERATIONS OF ORANGE JUICE BEVERAGES

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Last year we reported that the growth of *Lactobacillus plantarum*, under standard assay conditions, was proportional to the amount of orange juice in the assay mixture. A method based on this property was proposed as a means of helping detect adulterated orange juice. Thus, in a standard volume of diluted (or adulterated) juice, the bacterium would grow to a lesser extent than with pure juice. If the level of adulteration were sufficient it could be detected. However, the natural variation of authentic orange juice samples caused a variance in bacterial growth. Even though this variance was about the same or less than many of the single chemical constituents measured in this laboratory, it was large enough that low levels of dilution would be difficult to detect by this single microbiological assay.

It is our opinion that probably no single assay will ever be able to detect adulteration as well as a multiple constituent approach. In this preliminary report we considered the possible use of *L. brevis*, *L. casei*, *L. lactis*, and *Saccharomyces* sp. as supplements to the *L. plantarum* assay. Also, the effects of various possible adulterants were tested on the growth of these organisms. Pure compounds, known mixtures, and compositionally undefined food extracts and ingredients were considered along with ways of detecting their presence.

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