STRUCTURAL STUDIES ON ROOT BARK PIGMENTS
OF THE OSAGE ORANGE

DISSERTATION

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University

By

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INTRODUCTION

A. Occurrence of the Osage Orange Tree.

The osage orange tree (Maclura pomifera Raf.) belongs to the Mulberry family. It is native to the south central United States from Kansas and Missouri to the eastern part of Texas. However, this tree is cultivated in many other sections of the United States for hedges or for ornamental purposes. Large quantities of greenish-yellow fruit are produced annually by the female trees of bearing size. The wood, sometimes called bow-wood (or bois d'arc) is very hard, strong and flexible. It is utilized in railway ties and fence posts. It is the wood of choice for archery bows. It was formerly much used in wagon axles since its dimensional changes with temperature and moisture are the lowest of any wood. The wood is known to be completely termite-proof. It finishes to a beautiful orange-colored surface. The tree is small and does not readily yield saw timber. Water extracts (solids) of the leaves, twigs and branches are available on the market. These are used in tanning and are sold as "Osage Orange Crystals" although the material is amorphous.

B. Pigments in the Fruit of the Osage Orange.

Nearly all of the quantitative studies of the chemical constitution of the pigments present in the fruit, wood
and bark of the osage orange have been conducted within the past twenty years. The shortness of this period of chemical investigation is indeed interesting in view of the fact that the osage orange has long been available in abundant supply and its many uses have long been appreciated.

In 1934 Dr. E. D. Walter, Department of Agricultural Chemistry, The Ohio State University, isolated a yellow crystalline substance (1) from the fruit of the osage orange.


Subsequent research on this crystalline material was directed by Dr. M. L. Wolfrom of The Department of Chemistry, The Ohio State University.

Wolfrom and co-workers found that the fruit contained two yellow pigments (2) which were present in high and approximately equal amounts (3). The names osajin and


(approximately equal amounts (3). The names osajin and

pomiferin were assigned to these two pigments. An intensive investigation by Wolfrom and his research group led to the elucidation of the complete structures (4) of osajin and pomiferin.


The only structural difference between osajin and pomiferin is that pomiferin has an additional phenolic group in the
3' position. The substances are to be considered as hydroxy-isoflavones upon which are substituted two isoprene residues.

C. Pigments in the Root Bark of the Osage Orange.

(a) The Isolation of Substance I and Substance II:

The coloring matter in the wood and bark of the osage orange first received attention in 1941, when Wolfrom and Dickey (5) attempted to isolate the pigment in crystalline form. The initial attempts were unsuccessful and it was 1945 before Dickey isolated a yellow crystalline substance, m.p. 177-180° from the root bark. Chromatography of a chloroform extract of the root bark provided the key to this isolation. The adsorbent used was Magnesol-Celite (5:1) and the developer was benzene-ethanol (100:1).

An improved chromatographic procedure for the isolation of the yellow pigment was developed by Mosby (6) who

W. L. Mosby, "Chemistry 701 Report", The Ohio State University (1946).

found that a sharper yellow zone on the chromatographic column could be obtained by pre-washing the Magnesol-Celite with acetic acid. Mosby also showed by melting point studies that the yellow crystalline material, m.p. 181-181.7°, from this zone was different from the two pigments osajin (1) and pomiferin (2) which had been isolated from the fruit of the osage orange.

Further improvements in the isolation of the yellow pigment, m.p. 181-181.7°, were devised by McWain (7). The essential feature of McWain's method involved a "pre-washing" of the Magnesol-Celite with hydrochloric acid to give an essentially silicic acid-Celite mixture. McWain chromatographed the crude crystalline rootbark pigment (itself isolated via chromatography) on the acid-washed Magnesol-Celite (5:1) and developed the column with benzene-tertiary-butyl alcohol (500:1). By this procedure he isolated a new yellow
pigment, m.p. 264-265.5°, which he termed Substance II. The previously isolated pigment, m.p. 181-181.7°, was designated Substance I.

The over-all yields (based on the weight of dry flakes of osage orange root bark) of Substance I and Substance II were 0.7% and 0.04% respectively.

In an attempt to characterize the two root bark pigments, McWain employed many of the techniques which had previously been of great value in the determination of the structures of osajin and pomiferin (4). He presented the following data. Analysis of Substance I: C, 69.81, 69.97; H, 5.42, 5.32; absence of S, N, P, halogen and OCH3; molecular weight (Rast), 372. Analysis of Substance II: C, 69.83, 69.50; H, 5.18, 5.09; absence of S, N, P, halogen and OCH3; molecular weight (Rast), 305. On the basis of these data the two substances were considered to be isomeric and to possess the molecular formula of C22H2O06. Substance I gave a deep green color with alcoholic ferric chloride, a decolorization of bromine, an orange-yellow color with sulfuric acid in acetic acid, a positive Wilson boric acid test, a negative Molisch test, positive reductive color tests with both magnesium-hydrochloric acid and sodium amalgam. Attempted alkaline degradation of I did not yield any formic acid. A partially crystalline lead salt was formed upon the treatment of Substance I with methanolic lead acetate. Substance II
gave a deep green color with alcoholic ferric chloride, a deep yellow color with sulfuric acid in acetic acid, a positive Wilson boric acid test, a negative Molisch test, positive reductive color tests with both magnesium-hydrochloric acid and sodium amalgam. Substance II did not form a lead salt upon treatment with methanolic lead acetate. Neither Substance I nor Substance II displayed optical activity. The ultraviolet absorption spectra of I indicated a strong maximum at 2825 Å and a smaller peak at 3375 Å. Substance II showed maxima at 2850 Å, 3400 Å and 3800 Å.

On the basis of the above tests, McWain concluded

*The interpretation of each of these tests, in terms of molecular structure, is discussed in a summary of the work of J. H. Looker. See page 10.

that Substance I possessed a phenol group, a γ-pyrone ring and the structural unit

```
  C   C   C
  |   |   |
  C   C   O
  |   |
  OH  O
```

It seemed that Substance I might belong to the flavone class of compounds with the probability of a catechol group in the
side ring. From the tests upon Substance II, McWain con­
cluded that Substance II also contained a phenol group, a
\[ \begin{array}{c}
\text{i} \\
\text{OH}
\end{array} \]
\[ \begin{array}{c}
\text{C} \\
\text{C}
\end{array} \]
\[ \begin{array}{c}
\text{C} \\
\text{C}
\end{array} \]
\[ \begin{array}{c}
\text{O}
\end{array} \]
evidence pointed to a flavone structure.

In July, 1947, an intensive investigation of Sub­
stances I and II was started by J. H. Looker. The objective
was to develop further the structures of the two root bark
pigments. The entire project was under the supervision of
Dr. M. L. Wolfrom. Looker's approach to the problem was
essentially the same as that used in the proof of structure
of osajin and pomiferin (4). The following discussion is a
summary of his work (8). Looker rechecked McWain's

(8) J. H. Looker, "Root Bark Pigments of the Osage
Orange (Maclura pomifera Raf.) and Their Structure," Ph.D.
Dissertation, The Ohio State University (1949).

analytical data (7) for Substance I and showed that the
molecular formula \( C_{23}H_{22}O_6 \) was more in agreement with the
observed carbon and hydrogen values than was the formula
\( C_{22}H_{20}O_6 \) as reported by McWain. It is of considerable inter­
est to note that this revised molecular formula for Substance
I is isomeric with rotenone. For this reason, the name
neo-rotenone was proposed for Substance I. This name is no longer considered desirable for this compound.

Looker (8) improved the methods previously used for the isolation of the crude root bark pigment and developed a pyridine-water crystallization technique for obtaining pure Substance I from this crude product. Dry flakes of osage orange root bark were extracted with diethyl ether for five days at room temperature. The solution was then filtered and the residue discarded. The filtrate was precipitated by pouring it into boiling Skellysolve B (b.p. 65-69°). The orange-red precipitate which formed was removed by filtration and discarded. The filtrate was evaporated under an air jet until a red amorphous product had formed on the container walls. The supernatant liquid was decanted and the process of "evaporation-decantation" was repeated fifteen times, following which the solid that separated was crystalline. The crystalline material was added to the final residue obtained by complete solvent removal. Crystallization of the combined solids from benzene gave a yellow pigment, m.p. 165-175°. This product was dissolved in boiling pyridine; water was then added and the solution was kept at room temperature for eighteen hours. The yellow crystalline solvate, m.p. 194-197°, which appeared was collected by filtration and then treated with 95% ethanol. The ethanolic solution was filtered
and the filtrate was boiled. Storage of this filtrate for
two days at room temperature produced yellow plates of
Substance I, m.p. 201-203.5° (see page 24).

A crystalline product was obtained from the pyridine-
water mother liquor. This product was chromatographed on
acid-washed Magnesol-Celite (5:1) using benzene-tertiary-
butyl alcohol (500:1) as developer. Two compounds were
isolated: Substance I, m.p. 195-197° and Substance II, m.p.
263-265° (see page 22 ).

Analysis of the Substance I pyridine solvate revealed
a 1:1 complex, \( \text{C}_{23}\text{H}_{22}\text{O}_6 \cdot \text{C}_5\text{H}_5\text{N} \). Substance I was shown to exist
in dimorphous forms. When the lower melting form, m.p. 181-
182°, was ground in a mortar, it was converted to the higher
melting dimorph. Recrystallization of the higher melting
dimorph from benzene produced the lower melting form.

(b) The Partial Characterization of Substance I:

Before describing the derivatives of Substance I, it
seems pertinent to review the main color tests which have
been employed in the structural studies of the osage orange
pigments.

The Ferric Chloride Test:

The formation of a green color, upon the addition of
a few drops of ferric chloride to an alcoholic solution of
a compound, reveals the presence of a phenol or enol group.
If the color from the ferric chloride test changes to reddish-violet upon the addition of dilute ammonium hydroxide, an ortho dihydric phenol is indicated (1).

The Perkin Test:

If an intense yellow color is produced when sulfuric acid is added to an acetic acid solution of a compound, then the compound contains a γ-pyrone ring (7, 8).

The Wilson Boric Acid Test (9, 10):


A compound is dissolved in dry acetone and the solution is divided into two equal parts. To one part is added 2 ml. of "boric acid-citric acid-acetone" reagent. To the other part is added 2 ml. of "citric acid-acetone" reagent. Any slight darkening in the color of the solution containing the boric acid-citric acid-acetone is interpreted as a positive test. According to Wilson (9), the structural requirement for a positive test is the unit $C - R'$.
in which "a" is an auxochromic group. This test has been successfully used to distinguish between certain types of flavonoids (10). The generic term "flavonoid" includes the following structures:

Flavones (derivatives of 2-phenylchromone).

Isoflavones (derivatives of 3-phenylchromone).

Flavonols (derivatives of 3-hydroxyflavone).

Flavanones (derivatives of 2-phenylchromanone).

Isoflavanones (derivatives of 3-phenylchromanone).

The flavones, isoflavones and flavonols give a positive Wilson boric acid test, whereas the flavanones and isoflavanones give a negative Wilson boric acid test.

The reductive color tests (7, 8, 10):

The two most widely used tests in this group utilize magnesium-hydrochloric acid for acid reduction and sodium amalgam for alkaline reduction. The reduction products exist
in or separate from hydrochloric acid solutions as red substances of a flavylium salt nature. Some research workers (11) have claimed that these reductive color tests offer a fully reliable means of differentiating between flavones, flavonols and flavanones. However, more recent work (12) has shown that the reductive color tests do not always differentiate between the flavonoids and their main value is that they indicate the presence of the phenylchromone structure.

Acetylation (8) of Substance I at 0° C with acetic anhydride and pyridine produced a yellow diacetate, C_{23}H_{20}O_{4}(OCOCH_{3})_{2}, m.p. 195°, which gave a positive Perkin test and a positive Wilson boric acid test. Acetylation of Substance I under vigorous conditions, using acetic anhydride and sodium acetate at 140°, produced a white triacetate, C_{23}H_{19}O_{3}(OCOCH_{3})_{3}, m.p. 132°. This triacetate gave a positive
Perkin test and a negative ferric chloride test.

Methylation (8) of Substance I with an excess of diazomethane in diethyl ether produced Substance I dimethyl ether, C$_{23}$H$_{20}$O$_4$(OCH$_3$)$_2$, m.p. 219° (dec.). This yellow crystalline product gave a positive Perkin test and a positive Wilson boric acid test. A green color formed slowly with alcoholic ferric chloride. When Substance I was methylated under vigorous conditions, using dimethyl sulfate and 50% aqueous potassium hydroxide in acetone, a colorless Substance I trimethyl ether, C$_{23}$H$_{19}$O$_3$(OCH$_3$)$_3$, m.p. 98.5°, was formed. This product gave a positive Perkin test, a negative Wilson boric acid test and a negative ferric chloride test.

Substance I gave positive reductive colors with both magnesium-hydrochloric acid and sodium amalgam (7).

The following conclusions (8) were based on the above acetylation and methylation experiments. Substance I contains three hydroxyl groups, either phenolic or enolic. The color tests show that the free hydroxyl group of Substance I dimethyl ether is phenolic or enolic and is probably located at position five of a flavone or isoflavone derivative. The positive Perkin test given by Substance I, its acetates and ethers indicates the presence of a γ-pyrone ring. Since both Substance I diacetate and Substance I dimethyl ether give a positive Wilson boric acid test, indicative of the unit
then it seems that the hydroxyl group at position five is the one which cannot be acetylated by acetic anhydride-pyridine at 0° or methylated by diazomethane. Since vigorous conditions are required to acetylate and methylate the five hydroxyl, then this phenolic group must be di-ortho substituted (4). The positive reductive tests indicate a phenylchromone structure with the phenyl group at either position two or three. The fact that formic acid was not formed upon the treatment of Substance I with mild alkali (7) offers strong evidence that the phenyl group is on position two rather than on position three. Thus the partial structural formula for Substance I can be written as

\[
\begin{align*}
(C_8H_{13}O) & \\
\text{(C\textsubscript{6}H\textsubscript{13}O)} & \text{ortho (OH)}_2
\end{align*}
\]

The nature of five of the six oxygen atoms is established. It was pointed out by Looker (8) that only part of the \( C_8H_{13}O \) group need be located at position six.

Substance I in absolute ethanol was hydrogenated (8)
stepwise (at room temperature and atmospheric pressure) over platinum catalyst to give dihydro-Substance I, $C_{23}H_{24}O_6$, m.p. 181-182° and tetrahydro-Substance I, $C_{23}H_{26}O_6$, m.p. 203° (dec.).

Dihydro-Substance I gave a positive ferric chloride test, a positive Perkin test and positive Wilson boric acid test. Mild acetylation of dihydro-Substance I produced dihydro-Substance I diacetate, $C_{23}H_{22}O_4(OCOCH_3)_2$, m.p. 179-180.5°. This diacetate gave a positive ferric chloride test, a positive Perkin test and a positive Wilson boric acid test. Vigorous acetylation of dihydro-Substance I produced dihydro-Substance I triacetate, $C_{23}H_{21}O_3(OCOCH_3)_3$, m.p. 170-171°. This product gave a negative ferric chloride test, positive Perkin test and a negative Wilson boric acid test.

Tetrahydro-Substance I gave a positive ferric chloride test, a positive Perkin test and a positive Wilson boric acid test. Acetylation of tetrahydro-Substance I with acetic anhydride and sodium acetate at 140° produced colorless needles of tetrahydro-Substance I triacetate, $C_{23}H_{23}O_3(OCOCH_3)_3$, m.p. 179.5-180.5°. This triacetate gave a negative ferric chloride test, a positive Perkin test and a negative Wilson boric acid test. Vigorous methylation of tetrahydro-Substance I produced colorless plates of tetrahydro-Substance I trimethyl ether, $C_{23}H_{23}O_3(OCH_3)_3$, m.p. 111-112°. This product was also prepared (8) by the catalytic hydrogenation of
Substance I trimethyl ether.

The hydrogenation studies (8, 10) enabled Looker to draw the following conclusions: Substance I contains two double bonds which are not aromatic. Neither of the double bonds reduced is the one of the γ-pyrone ring since tetrahydro-Substance I gives a positive Perkin test and a positive Wilson boric acid test. Thus the partial structural formula of tetrahydro-Substance I may be written as

\[
\begin{align*}
\text{(C}_6\text{H}_{17}0) & \quad \{ \text{ ortho (OH)}_2 \\
\text{OH} & \quad \text{C} \\
\text{O} & \quad \text{Y-pyrene ring} \\
\end{align*}
\]

The formation of a triacetate upon the vigorous acetylation of tetrahydro-Substance I indicates that no oxide is opened (at least not to give an acetylatable hydroxyl) during the stepwise hydrogenation of Substance I.

No maleic anhydride adduct was formed upon treatment of Substance I with refluxing xylene, thus the double bonds in the C6H13O-part of Substance I are not conjugated to each other.

Terminal methyl-group analysis (8) gave the following values: Substance I, 1.19; Dihydro-Substance I, 1.02; Tetrahydro-Substance I, 1.16. These data indicate that no simple allyl group, CH2=CH-CH2-, is present in Substance I.
Propoxyl and amoxyl values, obtained upon analysis of tetrahydro-Substance I, eliminated the possibility that the C₈H₁₇O-fragment contained either a propyl group or an amyl group.

More data concerning the position of the two double bonds in the C₈H₁₃O-group were obtained from ultraviolet absorption spectra (8). Application was made of the "styrene-ethyl benzene" effect—in which the reduction of a double bond conjugated to an aromatic system causes a shift in the ultraviolet absorption bands toward shorter wave lengths, with an attendant lowering in the height of the bands. The reduction of Substance I to the dihydro or tetrahydro derivatives did not cause a shift in the ultraviolet absorption bands toward the region of shorter wave lengths with a concomitant lowering in the height of the bands. Looker (8) thereby concluded that neither of the readily reducible double bonds of Substance I is conjugated to any part of the phenylchromone nucleus. Ultraviolet spectra data (8) were obtained for the triacetate and trimethyl ether of Substance I. Vigorous acetylation of Substance I caused the expected shift of the bands toward shorter wave lengths. Vigorous methylation of Substance I caused an unexpected shift of the bands toward shorter wave lengths. The work of Skarzynski (8, 13) has shown that etherification of the hydroxyl groups

(13) B. Skarzynski, Biochem. Z., 301, 150-169 (1939).
in positions 5, 6, 7, 2', and 3' does not alter the absorption spectra of the hydroxylated flavone; that etherification of the hydroxyl group at position 3 causes a radical shift to shorter wave length; that etherification of the hydroxyl group at the 4' position also causes a shift to shorter wave length. In the light of Skarzynski's work, it was pointed out by Looker (8) that a flavonol structure for Substance I could be neither accepted nor rejected on the basis of the ultraviolet absorption spectra.

Numerous color tests were applied (8) to Substance I in an attempt to determine the relative positions of two of the hydroxyl groups. (Evidence previously discussed has established the third hydroxyl group at position five.) Substance I formed a lead salt upon treatment with methanolic lead acetate and hence the two hydroxyl groups would seem to be ortho to each other somewhere on the phenyl chromone system. Only three pairs of positions are possible: 7 and 8; 2' and 3'; 3' and 4'. Looker (8) observed that these possibilities do not include a flavonol structure and thus Substance I does not appear to be a flavonol. The addition of one drop of neutral aqueous silver nitrate to an ethanolic solution of Substance I produced a red color in thirty seconds and when the test solution was permitted to stand overnight, a silver mirror was formed. This reaction (8, 14)

offered further evidence in favor of an ortho-dihydric phenol structure for Substance I. A deep green color was formed upon treatment of Substance I with alcoholic ferric chloride. This deep green color changed to olive green, then gradually to yellow, upon the addition of dilute ammonium hydroxide. Since the ammonium hydroxide did not produce a red-violet color, which is characteristic of most catechol groups, it was inferred by Looker (8) that Substance I may not possess an ortho-dihydric phenol unit. The reaction of Substance I with Millon's reagent (a solution of mercurous nitrate in nitric acid) did not give the colored precipitates known to be characteristic of compounds containing at least two adjacent phenolic hydroxyl groups.

Looker (8) attempted to isomerize Substance I with concentrated sulfuric acid, a technique which had been employed in the osajin and pomiferin studies (15). Although


no crystalline products were isolated from the reaction, Substance I seemed to be acid-sensitive. Looker reported that Substance I trimethyl ether formed "an apparent
isomerization product, $C_{28}H_{28}O_{8}$, when treated under mild alkaline conditions". Thus a phenolic or enolic group is formed during the isomerization. All attempts to break the $\gamma$-pyrone ring of Substance I trimethyl ether by alkaline hydrolysis were unsuccessful. These data definitely eliminate the isoflavone unit as a possible classification for Substance I.

Upon the basis of the reactions described, Looker (8) considered the following formulas as the main possibilities for the structure of Substance I.
It is of interest to note that Substance I displays an insecticidal activity (8) that is quite similar to that of rotenone.

(c) The Partial Characterization of Substance II:

Looker (8) rechecked McWain's analytical data (7) for Substance II and on the basis of acetylation studies established the molecular formula for Substance II as C₁₆H₁₄O₅. The techniques used for the partial characterization of Substance I were applied to Substance II. Upon the basis of color tests (see page 8), McWain (7) concluded that Substance II contained the structural unit \(\text{OH} \quad \text{O} \)

and that a flavone structure was strongly indicated.

Acetylation (8) of Substance II with acetic anhydride and sodium acetate at 140° produced white needles of Substance II diacetate, C₁₆H₁₂O₃(OCOCH₃)₂, m.p. 203-204°. This diacetate gave a negative ferric chloride test and a positive Perkin test.

Methylation (8) of Substance II with either a tremendous excess of methyl iodide and a large amount of anhydrous potassium carbonate in acetone or with dimethyl sulfate and potassium hydroxide in acetone produced colorless crystals of Substance II dimethyl ether, C₁₆H₁₂O₃(OCH₃)₂, m.p. 167-168°.
This dimethyl ether gave a negative ferric chloride test.

The acetylation and methylation data show that Substance II contains two phenolic or enolic hydroxyl groups. Color tests offer evidence that one of these hydroxyl groups seems to be at position five of a phenyl chromone structure.

Looker (8) presented the following partial structural formula for Substance II as the one which is in best agreement with the experimental data. He also suggested that a synthetic sequence of reactions might provide the shortest route to the complete elucidation of the Substance II structure.

(d) An Improved Method of Isolation for Substance I: the Isolation and Partial Characterization of Substance III.

Structural studies of Substance I and Substance II were greatly hampered because of the scarcity of the two pigments.

Dr. Wolfrom assigned the problem of increasing the supply of these pigments to Dr. A. Thompson, a Research Associate in the Department of Chemistry, The Ohio State University.
Thompson (16) developed an improved method for the isolation of Substance I. Substance II was not isolated but a third pigment, designated Substance III, was discovered (16). The following discussion is a summary of Thompson's work.

The root bark, ground to pass a 5/8 inch screen, was extracted with ether using the countercurrent principle. The ether solution was concentrated to one-fifth its volume and poured into Skellysolve B. The precipitated resin was removed by filtration and eventually discarded. The Skellysolve solution was concentrated by distillation and decanted from any resinous material. The concentrated Skellysolve solution was cooled under an air jet and decanted from any resin which formed. When the solution was permitted to stand overnight, a crystalline product formed. Trituration of this product with ether produced two fractions; an ether-soluble fraction and an ether-insoluble fraction.

The ether-soluble fraction gave crystalline material upon evaporation of the solvent. This product was passed through a layer of acid-washed Magnesol and recrystallized from ether-petroleum ether. When this recrystallized mater-
ial was chromatographed on Magnesol-Celite (3:1) using benzene-tertiary-butyl alcohol (500:1) as developer, a yellow zone appeared. Elution of this zone with acetone, followed by evaporation and recrystallization from ether-petroleum ether, gave yellow crystals, m.p. 155-156°. The designation Substance III was assigned to this new pigment (16).

The ether-insoluble fraction from the trituration consisted mainly of crude Substance I. Recrystallization of this crude product from hot acetone gave orange crystals of Substance I, m.p. 192-198°. Thompson (16) further purified a sample of this Substance I by the pyridine-complex method described by Looker (8).

From 21 Kg. of root bark, Thompson obtained 2150 g. of resin, 95 g. of Substance I and 30 g. of crude Substance III.

The following data were presented by Thompson (16) in an attempt to partially characterize Substance III.

Analysis: C, 69.31, 69.21; H, 6.12, 6.40; molecular weight (Rast), 384, 386; absence of sulfur and nitrogen. The molecular formula \( \text{C}_{23}\text{H}_{24}\text{O}_{6} \) was thereby assigned to Substance III.

Substance III is insoluble in water; slightly soluble in petroleum ether and very soluble in diethyl ether, acetone and benzene. The pigment is somewhat unstable and becomes highly colored upon standing. It gives a dark green
precipitate with ferric chloride. There is no loss in weight upon heating Substance III at 80° for six hours over phosphorus pentoxide. Thus Substance III is not a hydrate.

Acetylation (16) of Substance III under mild conditions (acetic anhydride and pyridine at 0°), vigorous conditions (acetic anhydride and sodium acetate at 135°) or reductive conditions (acetic anhydride, sodium acetate and zinc dust at 130°) gave the same yellow crystalline product. Analysis showed this product to be Substance III triacetate, $C_{23}H_{21}O_3(OCOCH_3)_3$, m.p. 209-211°. The triacetate is optically inactive throughout the visible spectrum.

Methylation (16) of Substance III with diazomethane in diethyl ether produced pale yellow crystals of Substance III trimethyl ether, $C_{23}H_{21}O_3(OCH_3)_3$, m.p. 150-151°.

Preliminary but inconclusive tests (16) indicate that Substance III is toxic to goldfish and mosquito larvae.

No attempt was made to assign a partial structural formula to Substance III.
STATEMENT OF PROBLEM

The object of the present investigation is to elucidate further the structures of Substances I and III.

A definitive proof of structure must involve a systematic degradation of each pigment, followed by an identification of the fragments. Substance II is the logical starting point for degradative studies. However, the total available quantity of this pigment is less than 10 mg. and hence such studies are almost impossible.

Evidence as outlined in the Introduction favors a substituted flavone structure for Substance I. Attention is to be focused upon the possibility of oxidizing Substance I with alkaline permanganate. An isolable degradation product from this reaction would contribute much to a structural interpretation of the group(s) attached to the phenylchromone nucleus. Alkaline hydrogen peroxide is to be employed in an attempt to split the \( \gamma \)-pyrone ring of tetrahydro-Substance I trimethyl ether. A successful cleavage of this ring would reveal the nature of the side phenyl group.
It is well known that flavones are quite resistant to degradation. A cleavage between atoms 1 and 2 is extremely difficult, even under the most drastic treatment with alkali. However, the flavanones are readily hydrolyzed with dilute aqueous or alcoholic potassium hydroxide. Thus if Substance I is a flavone, then hydrogenation of the 2-3 double bond is very desirable. Conditions more vigorous than those described by Looker are to be used for this proposed catalytic hydrogenation. There are numerous examples in the literature where the use of glacial acetic acid as an hydrogenation solvent has enhanced the reducing power of the platinic oxide catalyst.

Hydrogenation of Substance III (or its derivatives) would detect and measure any olefinic unsaturation in the molecule. These data might also provide the key to any structural relationship between Substances I and III. The available quantities of Substance III derivatives are so small that an investigation of these compounds is greatly limited.
X-ray diffraction techniques, "specific" color tests and spectrographic analyses are to be employed for comparative purposes.
EXPERIMENTAL

X-ray Powder Diffraction Patterns of Root Bark Pigments (Substances I, II and III) of the Osage Orange (Maclura pomifera, Raf.).

All data were obtained with CuK$_{\alpha}$ radiation, $\lambda = 1.5418$ Å, exposure time as indicated. The first figure is the d spacing in Å. The second figure is the relative intensity as estimated visually: if parenthetically expressed, it denotes the most intense lines in decreasing order of intensity. Substance I (high melting dimorph), C$_{23}$H$_{22}$O$_6$; exposure time 1.25 hours. 8.33, 7.38, 5.81(3), 5.04, 4.52(2), 3.93(5), 3.65(1), 3.26(4), 2.99, 2.75, 2.53, 2.28, 2.18, 2.04, 1.81, 1.38, 1.23, 1.18.

Substance II, C$_{18}$H$_{14}$O$_6$; exposure time 1 hour. 9.03, 5.84(1), 4.35, 4.26, 3.88, 3.45(2), 3.17(3), 2.92, 2.33, 2.09(4), 1.96, 1.81(5), 1.28, 1.09.

Substance III, C$_{23}$H$_{24}$O$_6$; exposure time 1.25 hours. 9.39(4), 8.33(5), 5.93, 5.31(3), 4.75(2), 3.72, 3.38(1), 2.71, 2.17, 2.07, 1.72(6).
The Hydrogenation Apparatus.

A micro-hydrogenator (8, 17, 18) was constructed as shown in Figure 1. The apparatus was thoroughly checked for leaks and then tested with a trial run. Cinnamic acid, dissolved in absolute ethanol, was hydrogenated at room temperature and pressure using platinic oxide as catalyst. 1.06 moles of hydrogen were absorbed per mole of cinnamic acid.

Hydrogenation of Substance I with Platinic Oxide in Glacial Acetic Acid.

Preliminary solubility tests showed that the addition of a small amount of absolute ethanol to a warm glacial acetic acid solution of Substance I would keep Substance I in solution for at least ten hours at room temperature.
FIGURE 1
MICRO - HYDROGENATOR
Substance I (205.5 mg.) was dissolved in 16 ml. of hot glacial acetic acid. The solution was cooled to about 50° and 5 ml. of absolute ethanol was added. This solution was allowed to cool to room temperature. The platinic oxide (19) catalyst (52.7 mg.) was introduced via an aluminum boat into the hydrogenation flask (type b, Figure 1) and covered with 5 ml. of glacial acetic acid. The gas burette was flushed four times with hydrogen, then the remainder of the apparatus was flushed with a slow stream of hydrogen for ten minutes. The gas pressure was brought to that of the atmosphere and the apparatus was allowed to stand for five minutes to bring the system to equilibrium. The shaker was started and the hydrogenation was continued until the catalyst was fully activated. The shaker was then stopped and the solution of Substance I was introduced into the hydrogenation flask. An additional 10 ml. of glacial acetic acid was used as washing. The apparatus was flushed very slightly with hydrogen and was then brought to equilibrium. The shaker was started and the hydrogenation was continued for thirty-six minutes, after which time
there was no further uptake of hydrogen. The activation of the catalyst required 9.9 ml. of hydrogen at 27° and 750 mm. Substance I absorbed 36.6 ml. of hydrogen at 27° and 750 mm. The catalyst was removed by suction filtration and the filtrate was evaporated to dryness by distillation in vacuo. The crude yellow residue was dried overnight in a vacuum desiccator. This material, m.p. 203-204° (dec.), was recrystallized several times from glacial acetic acid according to the following scheme:

reaction product (labelled AI)

A crop of fluffy light yellow crystals was obtained from the 4₁ fraction. This crop weighed 20.7 mg. immediately following filtration and 17.2 mg. after being dried in an Abderhalden chamber for forty hours at 100° C. and 4 mm.; a weight loss of 16.8%. An additional 51 mg. of hydrogenated product was obtained from the 5₂ fraction (this material was shown to be identical with the 4₁ fraction).
A glacial acetic acid solution of the hydrogenated product (AI₄₁) gave a bright yellow color immediately upon the addition of one drop of concentrated sulfuric acid. The product gave a positive Wilson boric acid test and a positive ferric chloride test.

**Anal.** Calcd. for C₂₃H₂₆O₆: C, 68.98; H, 7.05.
Calcd. for C₂₃H₂₆O₆: C, 69.33; H, 6.58. Found (Huffman; C and H corrected for residue): C, 69.30; H, 6.64; residue 0.24.

**Comparative Studies of AI₄₁ (Hydrogenated Substance I) and Looker's Tetrahydro-Substance I.**

**The Melting Points:** These data were inconclusive because of decomposition effects. The melting points were determined on a Fisher hot stage and the values differed greatly, depending on the rate of heating. Upon fairly rapid heating, 4₁ started to decompose at 201.5°, melted at 206-207°. Looker's tetrahydro-Substance I started to decompose at 195° and melted at 204-205°.

**X-ray Powder Diffraction Data:** All data were obtained with CuKα radiation, λ = 1.5418 Å. The first figure is the d spacing in Å. The second figure is the relative intensity as estimated visually: if parenthetically expressed, it denotes the most intense lines in decreasing order of
intensity. Fraction Al4X (exposure time 1 hour): 8.03(3),
6.04(2), 4.85, 4.47(4), 4.01, 3.30(1), 3.15, 2.92, 2.66,
2.44, 2.32, 2.22, 2.14(5), 2.04, 1.91, 1.63, 1.68. Looker's
tetrahydro-Substance I (exposure time 1 hour): 8.50,
6.62(2), 5.48, 4.77, 4.28(1), 3.88(3), 3.50, 3.31(4),
2.96(4), 2.67(4), 2.34, 2.22, 2.13, 1.93, 1.67.

Reductive Color Tests: Positive reductive colors
(see page 10) were given by Al4X and Looker's tetrahydro-
Substance I with both magnesium-hydrochloric acid and
sodium amalgam. Known flavones, flavonols and flavanones
were run as controls. For the acid reduction (7) the
sample (2 mg.) was dissolved in 1.5 ml. of 95% ethanol and
then one drop of mercury plus five drops of concentrated
hydrochloric acid were added. Powdered magnesium was added
and the reaction mixture was kept at a constant (40°)
temperature. More magnesium and more concentrated hydro­
chloric acid were introduced from time to time. A summary of
these data is presented in Table 1.

Hydrogenation of Substance I with Platinic Oxide in
Absolute Ethanol (8): In order to determine whether or not
Al4X has the same molecular structure as Looker's tetrahydro-
Substance I, it was decided to hydrogenate Substance I by
Looker's procedure, then crystallize one half of the reaction
product from benzene (used by Looker) and the other half from
**TABLE 1.**

Magnesium-Hydrochloric Acid Reduction Tests

<table>
<thead>
<tr>
<th>Sample</th>
<th>Wt.</th>
<th>Vol. 95% C₂H₅OH</th>
<th>Classification of Sample</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 min.</td>
</tr>
<tr>
<td>Substance I</td>
<td>2 mg.</td>
<td>1.5 ml.</td>
<td>light brown</td>
<td>deep red</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>brown-orange change</td>
<td>orange</td>
</tr>
<tr>
<td>Looker's Tetrahydro Substance I</td>
<td>2 mg.</td>
<td>1.5 ml.</td>
<td>deep red</td>
<td>deep red</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>change</td>
<td>orange</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>change</td>
<td>orange</td>
</tr>
<tr>
<td>Fraction AI₄₁</td>
<td>2 mg.</td>
<td>1.5 ml.</td>
<td>light brown-red</td>
<td>deep red</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>brown-red</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>deep red</td>
<td>orange</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>brown</td>
<td>deep red</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>change</td>
<td>orange</td>
</tr>
<tr>
<td>Quercitrin</td>
<td>2 mg.</td>
<td>1.5 ml.</td>
<td>light purple-red</td>
<td>deep red</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>purple-then cherry red</td>
<td>change</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cherry red</td>
<td>change</td>
</tr>
<tr>
<td>Hesperidin (glycoside)</td>
<td>2 mg.</td>
<td>1.5 ml.</td>
<td>very faint purple</td>
<td>light medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>purple</td>
<td>medium purple</td>
</tr>
<tr>
<td>Rutin</td>
<td>2 mg.</td>
<td>1.5 ml.</td>
<td>light purple-medium</td>
<td>medium purple</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>purple</td>
<td>pink</td>
</tr>
<tr>
<td>Luteolin Tetraacetate</td>
<td>0.2 mg.</td>
<td>0.15 ml.</td>
<td>light purple-medium</td>
<td>medium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>orange</td>
<td>change</td>
</tr>
<tr>
<td>Blank</td>
<td></td>
<td></td>
<td>colorless</td>
<td></td>
</tr>
</tbody>
</table>

*insufficient sample available*
glacial acetic acid. Hydrogenation of Substance I in absolute ethanol gave a value of 1.64 moles of hydrogen per mole of Substance I. Upon further hydrogenation with a fresh batch of catalyst, this value was increased to 2.19 moles of hydrogen per mole of Substance I. The crude hydrogenation product was divided into two parts; part (a) was crystallized from benzene and part (b) from glacial acetic acid. X-ray diffraction patterns were taken at each stage in the procedure. A comparison of these data is given in Table 2.

**Ultraviolet Absorption Spectra:** The absorption spectrum of product Alβ₂ (obtained by the hydrogenation of Substance I in glacial acetic acid; product dried at 100°/4 mm./44 hours) is shown in Figure 2. The measurements were made by Mr. Albert Antoine with a Beckman spectrophotometer, model DU, cell length 1 cm. Looker (8) had reported three maxima, at wave lengths 2560 Å., 2900 Å., 3330 Å., for his tetrahydro-Substance I.

Infrared spectra for Substance I and its hydrogenated product (Alβ₂) are shown in Figure 3 and Figure 4.

The hydrogenation of Substance I in glacial acetic acid was repeated and a hydrogen uptake of 2.70 moles of hydrogen per mole of Substance I was obtained. This product was used in the following methylation reaction.
### TABLE 2.

**X-ray Diffraction Data for Products Obtained from the Hydrogenation of Substance I.**

(All films designated by a check (✓) have the same X-ray pattern; all films designated by a dot (*) have the same pattern.)

<table>
<thead>
<tr>
<th>Description</th>
<th>Hydrogenation of Substance I in absolute ethanol (as a repetition of Looker's work). The product was divided into two parts, (a) and (b).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Looker's tetrahydro-Substance I, prepared by hydrogenation of Substance I in absolute ethanol and crystallization of the product from benzene. film no. 17 (✓)</td>
<td>Hydrogenation of Substance I in glacial acetic acid and crystallization of the product from glacial acetic acid. This product (Al₄₁) was dried at 100°/4 mm./40 hours. Weight loss 16.8% film no. 16 (*)</td>
</tr>
<tr>
<td>Hydrogenation of Substance I in absolute ethanol (as a repetition of Looker's work). The product was divided into two parts, (a) and (b).</td>
<td>Part (a) was crystallized from benzene and dried only at the water pump. Weight loss 19% film no. 22 (✓)</td>
</tr>
<tr>
<td>Part (b) was crystallized from glacial acetic acid and dried at 100°/4 mm./44 hours. Weight loss 19% film no. 21 (*)</td>
<td>A sample of (a) was recrystallized from glacial acetic acid and the product dried at 100°/5 mm./24 hours. Weight loss 12.3% film no. 25 (*)</td>
</tr>
<tr>
<td>A sample of (a) was recrystallized from glacial acetic acid and the product dried at 100°/5 mm./24 hours. Weight loss 12.3% film no. 25 (*)</td>
<td>Film no. 24 (*)</td>
</tr>
</tbody>
</table>
FIGURE 2
Absorption Spectrum of Product Al₅₂
Solvent, 95% ethanol
Concentration, 0.00054 g/100 ml.
Cell length, 1 cm.
FIGURE 3
Substance I
Baird I.R. Spectrophotometer
NaCl prism

FIGURE 4
Product Al\(\text{I}_2\)
Baird I.R. Spectrophotometer
NaCl prism
Methylation of Hydrogenated Substance I.

The hydrogenated material used in this experiment was obtained by the above preparation. The methylation procedure is the same as that described by Looker (8). Hydrogenated Substance I (58 mg.) was dissolved in 6.0 ml. of C.P. acetone and the solution was heated to boiling under reflux. Dimethyl sulfate (0.8 ml.) followed by 50% aqueous potassium hydroxide (0.5 ml.) were added through the condenser to the boiling solution. Then six alternative portions of 0.2 ml. of dimethyl sulfate and 0.25 ml. of 50% aqueous potassium hydroxide were added. The reaction mixture was refluxed for one-half hour, cooled for one-half hour, then poured into 40 ml. of an ice-water mixture and allowed to stand at ice-box temperature for five hours. The product which oiled out upon filtering was washed with water. The oil and any residue was dissolved in hot methanol; a few drops of water were added and the solution was placed in the ice-box overnight. Since no crystals appeared, the methanolic solution was concentrated slightly and stored again at 0° for one day. Upon removal from the ice box, the material was allowed to stand at room temperature for two hours. Beautiful almost-colorless crystalline plates formed. This product (labelled DI) was recrystallized from methanol; yield 27 mg.
Product DI was compared with Looker's tetrahydro-Substance I trimethyl ether. The melting points of these two compounds were taken simultaneously on a Fisher-Johns hot stage. Looker's compound melted at 114-115° (Looker recorded a m.p. of 111-113°) and DI melted at 122.5-123°. A mixture of the two samples melted at 116-117°.

Attempted Formation of a Carbonyl Derivative of Substance I.

The "Procedure Number" or "Experiment Number" for each of the following reactions refers to the procedure described by Shriner and Fuson (20). Attempts to form an


oxime (Procedure 42B), a phenylhydrazone (Experiment 23) and a 2,4-dinitrophenylhydrazone (Procedure 15) of Substance I were unsuccessful. Approximately one-half of the starting material was recovered in each case.

Attempted Oxidation of Tetrahydro-Substance I Trimethyl Ether with Alkaline Peroxide.

A large quantity (1.7 g.) of tetrahydro-Substance I
was prepared by essentially the same method as that employed by Looker (8), that is, by the hydrogenation of Substance I in absolute ethanol using platinic oxide (19) as catalyst. The only difference in procedure is that a Parr hydrogenator (21) was used instead of a micro-hydrogenator as used by Looker. Methylation (see page 42) of the tetrahydro-Substance I produced the trimethyl ether derivative in 75% yield, m.p. 128-130° (Fisher hot stage).

The procedure used in the attempted oxidation of tetrahydro-Substance I trimethyl ether is that of Wolfrom and Gregory (3). Tetrahydro-Substance I trimethyl ether (415 mg.), dissolved in C.P. acetone (35 ml.) was mechanically stirred in a conical flask and then treated with 0.4 ml. of 50% aqueous potassium hydroxide, followed by the dropwise addition of 5 ml. of 30% aqueous hydrogen peroxide. Almost immediately a white flock formed in the bottom of the flask. (A blank produced a white cloudiness which disappeared when the solution was allowed to stand overnight.) The oxidation was continued under stirring. After
forty-eight hours the solution tested oxidative to potassium iodide starch paper; a color developed on the paper after thirty seconds. The solution was permitted to stand overnight without stirring. Saturated aqueous sodium bisulfite solution was added dropwise until the solution was neutral to potassium iodide starch paper. At this point the solution was acid to litmus. The addition of the sodium bisulfite produced a white crystalline solid. This material was removed by filtration and was shown to be inorganic. The acetone in the filtrate was removed under reduced pressure and then 30 ml. of 1% hydrochloric acid was added. This acidified solution was extracted several times with ether. The water layer, upon evaporation in vacuo, gave only inorganic residue. The ether extract was concentrated to about 25 ml. and then extracted several times with a 5% ammonium carbonate solution. The ether solution, remaining after the ammonium carbonate extraction, was washed with water, dried with anhydrous sodium sulfate and then concentrated to a volume of about 12 ml. Pale yellow crystals formed, which upon two recrystallizations from hot methanol gave a product (300 mg.) of m.p. 128-129°. No depression in this melting point was observed when the product was mixed with a sample of starting material, tetrahydro-Substance I trimethyl ether.

The ammonium carbonate extract was reduced to a
volume of 15 ml. and filtered. The filtrate was acidified with 1% hydrochloric acid. No precipitate formed at this stage. The acidified filtrate was reduced to dryness in vacuo. The residue was redissolved in 8 ml. of water and the aqueous solution was then extracted several times with ether. No crystalline organic material was isolated from this ether extract.

**Attempted Oxidation of Substance I with Alkaline Permanganate.**

The procedure used for this reaction is that described by Harris (4, 22). Substance I (420 mg.) was dissolved in 15 ml. of a 5% potassium hydroxide solution. The cooled solution was diluted to 75 ml. and a 3% aqueous solution of potassium permanganate was added slowly, with shaking, until a persistent color of permanganate was noted. The total volume of permanganate used was 100 ml.: the first 50 ml. was added over a three hour period; the second 50 ml. over a six hour period. The temperature was kept at

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20° during the addition of this reagent. After standing overnight the excess permanganate was destroyed with hydrazine hydrate. The reaction mixture was heated on a water bath at 100° for one and one-half hours, then filtered. The filtrate, colored by some colloidal manganese dioxide was cleared by passing in a stream of sulfur dioxide. This solution was again filtered and labelled "filtrate 1". The manganese dioxide was suspended in about 200 ml. of water and decomposed by a stream of sulfur dioxide. The cleared solution was filtered and this filtrate was united with "filtrate 1". To the combined filtrate was added 10 ml. of concentrated hydrochloric acid, then the solution was saturated with sodium chloride. The solution was ether extracted in three batches, each batch being extracted with five 80 ml. portions of ether. The combined ether extract was reduced to about 300 ml. and this was extracted with three 50 ml. portions of 1 N. ammonium hydroxide. In order to remove any oxalic acid, about 40 ml. of 10% calcium chloride was added to the combined ammonium hydroxide extract and the solution was filtered. The filtrate was acidified with 35 ml. of 6 N. hydrochloric acid and extracted with eight 100 ml. portions of ether. The combined ether extract was dried with anhydrous sodium sulfate, then reduced nearly to dryness in vacuo. A brown syrup formed. All attempts to obtain a crystalline product from this syrup
Hydrogenation of Substance III Triacetate.

The choice of a suitable solvent for the hydrogenation of Substance III triacetate presented a problem. The triacetate is not completely soluble in either glacial acetic acid or ethanol. An attempted hydrogenation of the triacetate in a benzene-glacial acetic acid solution provided a very interesting experiment. It was found that the benzene was hydrogenated at room temperature and pressure using platinic oxide as catalyst. Indeed, in a separate experiment C.P. benzene was hydrogenated (procedure as described on page 31) in glacial acetic acid and the hydrogen uptake was 3.05 moles of hydrogen per mole of benzene. When C.P. benzene was hydrogenated in absolute ethanol, the hydrogen uptake was 2.08 moles of hydrogen per mole of benzene.

Further search for a solvent revealed ethyl acetate to be satisfactory. Substance III triacetate (100 mg. of the material prepared by Thompson—see page 25) was dissolved in 10 ml. of C.P. ethyl acetate and hydrogen via platinic oxide (25.5 mg.) according to the procedure already outlined for Substance I (page 31). Activation of the catalyst required 6.0 ml. of hydrogen at 30° and 730 mm. The sample of Substance III triacetate absorbed 34 ml. of
hydrogen at 30° and 730 mm. Time required for this hydrogen uptake was thirty-five minutes. The catalyst was removed by filtration and the ethyl acetate was removed by distillation in vacuo. The m.p. of the crude hydrogenation product was sharp; 162-162.5°. This product was re-crystallized from benzene-ethanol according to the following scheme:

reaction product (labelled CII)

Beautiful "wool-like" pale yellow crystals were obtained from this recrystallization procedure; total combined yield 74.6 mg. The 3₁ fraction was dried in an Abderhalden chamber at 78° and 5 mm. for forty-six hours; m.p. of dried material, 170-171°.

Anal. of 3₁. Calcd. for C₂₉H₃₄O₉: C, 66.14; H, 6.51. Found (Huffman; C and H corrected for residue): C, 66.05; H, 6.64; residue 1.77.

The appearance of a residue in the analytical sample was probably due to colloidal platinum, the removal of
which is very difficult. In an attempt to obtain a platinum-free product, a sample of crude hydrogenated material was dissolved in ethyl acetate and filtered through a one inch layer of acid-washed Magnesol. More ethyl acetate was poured over the filter mat, then the filtrate was reduced to dryness in vacuo. The product obtained upon solvent removal was recrystallized five times from benzene-ethanol.

**Anal.** of this recrystallized product. Calcd. for \( \text{C}_{29}\text{H}_{34}\text{O}_9 \): C, 66.14; H, 6.51. Found (Warfel; C and H corrected for residue): C, 66.44; H, 6.68; residue 0.51.

**Attempted Acetylation of Hydrogenated Substance III Triacetate.**

Hydrogenated Substance III triacetate (32 mg., from the above preparation), acetic anhydride (3.5 ml.) and twice fused sodium acetate (200 mg.) were heated under reflux for two hours, then kept at about 100° for another two hours. The reaction mixture was poured into 10 ml. of an ice-water mixture and the suspension was stored in the ice box for two days. The crude crystalline product was collected by filtration. This material was dissolved in ether and washed with water. The ether solution was dried by anhydrous sodium sulfate, then the solvent was removed by distillation. The yellow residue was recrystallized from
95% ethanol to which was added a few drops of benzene. Very pale yellow crystals were formed; yield 16 mg., m.p. 169-170°. This melting point was unchanged on admixture with a sample of starting material (hydrogenated Substance III triacetate).

**Color Tests on Hydrogenated Substance III Triacetate**

(Product CII\textsubscript{3}I, See page 49).

**The Perkin test:** A sample of the CII\textsubscript{3}I product was dissolved in warm glacial acetic acid and the pale yellow solution was divided into two parts. The addition of two drops of concentrated sulfuric acid to one of the parts produced a deeper yellow color, the intensity of which became very pronounced after one minute. Substance III triacetate also gave a yellow color with the Perkin test. It should be noted however, that neither Substance III triacetate nor its hydrogenated product gave a yellow color as intense as that obtained from the Perkin test upon tetrahydro-Substance I (page 35).

**The ferric chloride test:** Treatment of Substance III triacetate and its hydrogenation product with an alcoholic solution of ferric chloride, produced in each case a very faint green color. Upon standing for one-half hour, these two solutions turned to a much deeper green.
A blank showed no change over the same period.

The reductive color tests: Substance III triacetate and hydrogenated Substance III triacetate were subjected to both acid and alkaline reduction by the methods described on pages 12 and 36. With the magnesium-hydrochloric acid test, both Substance III triacetate and hydrogenated Substance III triacetate maintained their initial pale yellow colors over a four hour period. After eight hours, the solution containing the hydrogenated Substance III triacetate turned to a light purple; the color of the Substance III triacetate solution did not change. With the sodium-mercury amalgam test, both Substance III triacetate and hydrogenated Substance III triacetate gave greenish-yellow solutions after a two hour period. Acidification of these solutions with concentrated hydrochloric acid produced a pale yellow solution in each case. Aqueous dilution of each acidified solution produced a murky yellow color; no distinct precipitate was formed.

The Wilson boric acid test (see page 11): This test was negative for both Substance III triacetate and its hydrogenation product.

The antimony pentachloride test for chalcones (12): A 5-10 mg. sample of each of the following compounds was
dissolved in 5 ml. of anhydrous carbon tetrachloride and 1 ml. of a 2% anhydrous carbon tetrachloride solution of antimony pentachloride was then added. (A positive test is indicated by an intense red or violet-red precipitate.) When this test was applied to Substance III triacetate, yellow flocks formed immediately and after one minute these changed to dark brown flocks. Hydrogenated Substance III triacetate reacted in exactly the same way. Substance III trimethyl ether gave yellow flocks immediately and this color did not change over a five minute period.

Ultraviolet and Infrared Spectra of Substance III Triacetate and Hydrogenated Substance III Triacetate (Product CII3). The ultraviolet absorption spectra of Substance III triacetate and its hydrogenation product are shown in Figures 5 and 6. The measurements were made by Mr. Albert Antoine with a Beckman spectrophotometer, model DU, cell length 1 cm.

The infrared spectra for Substance III triacetate and its hydrogenated product are shown in Figures 7 and 8. The measurements were made by Mr. Robert Lieberman.
**FIGURE 5**
Absorption Spectrum of Substance III Triacetate

Solvent, 95% ethanol
Concentration, as indicated
Cell length, 1 cm

Extinction Coefficient, $\log \left( \frac{I_0}{I} \right)$

Concentration
0.0027 g/100 ml.

Concentration
0.0036 g/100 ml.

$\lambda$, mμ

220 240 260 280 300 320 340 360
FIGURE 6
Absorption Spectrum of Product C II 31
Solvent, 95% ethanol
Concentration, 0.0032g/100 ml.
Cell length, 1 cm.
Attempted Deacetylation of Hydrogenated Substance III Triacetate.

All attempts to deacetylate this hydrogenated product were unsuccessful. Conditions for acid hydrolysis and those for basic hydrolysis produced in each case a dark brown syrup, from which no crystalline material was obtained.

Attempted Hydrogenation of Substance III Triacetate to the Dihydro State.

A survey of the data for the product CII3 (page 48) indicated a tetrahydro-derivative of Substance III triacetate. The object of this experiment was to obtain the intermediate dihydro-product.

Substance III triacetate (40.3 mg.) was dissolved in 10 ml. of C.P. ethyl acetate and hydrogenated via platinic oxide (25 mg.) until 1.90 ml. of hydrogen was absorbed. This controlled hydrogen uptake represents the theoretical volume of hydrogen at 24° and 755 mm. which is required to convert the sample of Substance III triacetate to its dihydro derivative. The procedure for the hydrogenation was the same as that described for Substance I (page 31). The catalyst was removed by filtration and the filtrate was reduced to dryness in vacuo. The yellow product melted at 186-188° (Fisher hot stage); upon admixture with tetrahydro-Substance
III triacetate, m.p. 175-176°.

Attempted Formation of a Carbonyl Derivative of Substance III.

The light brown colored material used in these experiments was obtained and labelled by Thompson (16) as "crude Substance III". Attempts to form an oxime and a phenylhydrazone (see page 43) from samples (200 mg.) of this material were unsuccessful. In each case a dark brown solution resulted, from which no crystalline material was isolated.

Some Comparative Studies of Substance III Derivatives and Anthraquinone.

Although there is as yet no structural correlation between Substances I and III, it is interesting to note that the triacetate of Substance III is highly colored, whereas the triacetate of Substance I is white. Substance III is quite sensitive to air and it undergoes at least partial decomposition upon standing.

The possibility that Substance III contains a quinonoid system seemed worthy of investigation. A survey (23, 24) of quinone chemistry revealed that anthraquinones

(23) L. Fieser and M. Fieser, "Organic Chemistry,"
are extremely resistant to acetylation on the quinonoid grouping. However, one reference (25) stated that anthraquinone could be acetylated by means of acetic anhydride, sodium acetate and zinc chloride. Before attempting to use this procedure on derivatives of Substance III, it was decided to check the validity of the reaction on a known pure sample of anthraquinone.

A mixture of anthraquinone (1.0 g.), acetic anhydride (25 ml.), twice fused sodium acetate (2.0 g.) and twice fused zinc chloride (2.0 g.) was refluxed for two hours. The solution was allowed to cool, then it was poured into a mixture of ice and water and stored in the ice box for two days. The product, pale yellow needles, was collected by filtration and recrystallized from ethyl acetate. This recrystallized material, obtained in high yield, melted at 286° with partial sublimation at 170°; upon admixture with anthraquinone, m.p. 286° with partial
sublimation at 170°.

It is well established that nearly all anthraquinones give characteristic red vat dyes (23) when treated with aqueous alkali and sodium hydrosulfite. Indeed, when a mixture of anthraquinone, aqueous sodium hydroxide and sodium hydrosulfite was allowed to stand for three minutes, a cherry-red "vat" was formed. When this test was applied to crude Substance III and to Substance III triacetate, a light yellow color was produced in each case.

The infrared spectrum for anthraquinone is shown in Figure 9 and that for Substance III triacetate is shown in Figure 7 (see page 56).
FIGURE 9
Anthraquinone
Baird I. R. Spectrophotometer
NaCl prism
DISCUSSION OF RESULTS

The catalytic hydrogenation of Substance I has been further studied on a micro scale. A survey of the literature reveals that there are many variations in both design and operation of the micro-hydrogenation apparatus. Usually, each compound to be hydrogenated presents its own unique problems and hence modifications to the so-called "standard procedure" must be made accordingly. In the present investigation, more consistent results were obtained by flushing (18) the micro-hydrogenator with a slow stream of hydrogen, rather than by water pump evacuation (8) of the system. However, special precautions must be taken in preparation for activation of the catalyst. It is essential that all the catalyst has settled to the bottom of the hydrogenation flask before the apparatus is flushed with hydrogen; otherwise the measured volume of hydrogen for activation of the catalyst will be far below the theoretical uptake.

The hydrogenation of 205.5 mg. of Substance I with platinic oxide in glacial acetic acid required 36.6 ml. of hydrogen at 27° and 750 mm. These data are equivalent to 2.81 moles of hydrogen per mole of Substance I. Thus, at this stage of the investigation, it appeared that an hexa-hydro derivative of Substance I had been produced. Looker
(8) had prepared tetrahydro-Substance I by the catalytic hydrogenation of Substance I in absolute alcohol. The fact is well established that glacial acetic acid enhances the reducing power of the platinic oxide catalyst (26)


and thereby promotes the hydrogenation of double bonds which would not be affected in other solvents. In spite of the absorption of almost three moles of hydrogen during the hydrogenation of Substance I in glacial acetic acid (product designated $AI_{41}$), the analytical data indicates a tetrahydro derivative. Product $AI_{41}$ gave a positive Perkin test (7, 8), indicative of a $\gamma$-pyrone; a positive ferric chloride test, indicative of a phenol; and a positive Wilson boric acid test (9, 10), indicative of the unit

\[
\begin{align*}
R & \quad C - R' \\
\text{C} & \quad \text{C} \\
\text{II} & \quad \text{O} \\
\text{a} & \quad \text{C} \\
\end{align*}
\]

in which "a" is an auxochromic group. Thus the hydrogenation of Substance I in glacial acetic acid did not affect the 2-3
double bond of the postulated flavone structure. The following conclusions may be drawn from the comparative studies of product AI4_1 and Looker's tetrahydro-Substance I.

The melting point data for the two compounds are of little value because of decomposition effects.

Product AI4_1 and Looker's tetrahydro-Substance I gave identical positive reductive colors (see page 12) upon treatment with magnesium and hydrochloric acid; both gave identical positive colors upon reduction with sodium amalgam. Thus both compounds contain a phenylchromone system (12).

The ultraviolet absorption spectrum of hydrogenated Substance I reveals three maxima, at wave lengths 2560 Å., 2900 Å., 3330 Å. These values coincide exactly with those reported by Looker (8) for his tetrahydro-Substance I.

The X-ray powder diffraction patterns for AI4_1 and Looker's tetrahydro-Substance I are definitely different. Crystallization of a sample of tetrahydro-Substance I (prepared by Looker's method) from glacial acetic acid gave a product, which when dried at 100°/5 mm./24 hours had the same X-ray powder diffraction pattern as that for AI4_1. This same pattern was obtained from a sample of tetrahydro-Substance I which had been dried at 100°/5 mm./27 hours.
It is apparent that prolonged vacuum drying of the hydrogenation products gives rise to the same X-ray diffraction pattern. This evidence indicates that tetrahydro-Substance I is dimorphous.

Hydrogenated Substance I (produced by the absorption of nearly three moles of hydrogen per mole of Substance I) was dissolved in acetone and methylated by Looker's procedure (8) with dimethyl sulfate and 50% aqueous potassium hydroxide. The product of almost-colorless crystalline plates had a melting point (122.5-123°) about ten degrees above that of Looker's tetrahydro-Substance I trimethyl ether (m.p. 111-113°). A mixture of the two samples melted at 116-117°. The total available quantity of Looker's tetrahydro-Substance I trimethyl ether was too small to permit a study of the X-ray powder diffraction patterns of the two products.

The failure to form an oxime, a phenylhydrazone or a 2,4-dinitrophenylhydrazone of Substance I may actually reinforce the earlier postulation (8) of the existence of a chromone unit in Substance I. The carbonyl group of chromones has been shown to be very inactive (27) whereas

(27) P. W. Morgan, "Studies in the Determinative
the carbonyl group of flavanones and chalcones is quite reactive.

The fact that tetrahydro-Substance I trimethyl ether was recovered in 75% yield from an attempted oxidation of the material with alkaline peroxide indicates that the postulated γ-pyrone ring remained intact during the reaction. This high stability to alkaline peroxide is characteristic of flavones but it is not characteristic of flavanones and isoflavones. Indeed, under the above conditions, Wolfrom and Gregory (3) obtained anisic acid from osajin dimethyl ether (an isoflavone) and veratric acid from pomiferin trimethyl ether (an isoflavone).

The oxidation of Substance I with alkaline permanganate produced a brown syrup. All attempts to obtain a crystalline product from this syrup were unsuccessful. It was hoped that this experiment would reveal the nature of the unsaturation of the C₆H₁₂O-residue (no chromene ring) which is attached to a phenylchromone nucleus in Substance I. Wolfrom and Harris (4, 22) obtained α-hydroxy-isobutyric acid from the oxidation of pomiferin with alkaline permanganate and thereby established the presence of a
2,2-dimethyl-chromene structure in that pigment. It is to be noted that only 10 mg. of α-hydroxy-isobutyric acid was obtained from 2 g. of pomiferin.

In the hydrogenation of Substance III triacetate (100 mg.) with platinic oxide in ethyl acetate, 34 ml. of hydrogen at 30° and 730 mm. was absorbed. This hydrogen uptake represents an absorption of 6.8 moles of hydrogen per mole of Substance III triacetate. The recrystallized hydrogenation product (labelled CII3) melted at 170-171°.

In spite of the uptake of 6.8 moles of hydrogen during the hydrogenation of Substance III triacetate, the analytical data for the hydrogenated product indicates a tetrahydro-derivative.

In a separate experiment, C. P. benzene was hydrogenated at room temperature and pressure using Adam's catalyst, and glacial acetic acid as the solvent. The hydrogen uptake was 3.05 moles of hydrogen per mole of benzene. Whether this hydrogen absorption by benzene bears any significance to the hydrogen uptake by Substance III triacetate, is not yet known. One would expect the phenyl group in the phenylchromone system to be more resistant to hydrogenation than benzene itself.

The acetylation of hydrogenated Substance III triacetate with acetic anhydride and sodium acetate,
returned the starting material, unchanged. This experiment indicates that no new hydroxyl group was formed in the hydrogenation of Substance III triacetate to the tetrahydro-derivative.

Substance III triacetate and its tetrahydro-derivative each gave a positive Perkin test (7, 8), indicative of a γ-pyrone unit. Thus hydrogenation of Substance III triacetate did not produce a flavanone. The two compounds gave only a very faint green color with an alcoholic solution of ferric chloride. Upon standing for one-half hour, the two solutions turned to a much deeper green, whereas the blank did not change color. These observations may indicate the presence of a hindered phenol or enol group in the two compounds.

Results from the reductive color tests upon Substance III triacetate are inconclusive. This is at least partially due to the fact that acetylated compounds had to be employed in place of the (unstable) free hydroxylated pigments as specified in the tests (7, 8, 12).

The negative Wilson boric acid test given by Substance III triacetate and its tetrahydro-derivative indicates either the absence of the unit

\[ \text{R} - \overset{\alpha}{\text{C}} - \text{C} - \overset{\omega}{\text{C}} - \text{C} - \text{C} - \text{C} - \text{C} - \text{R} \]

or that the auxochromic group "\( \alpha \)" has been blocked during
acetylation.

A negative antimony pentachloride test (12) for Substance III triacetate, tetrahydro-Substance III triacetate, and Substance III trimethyl ether indicates the absence of a chalcone structure. All three compounds gave yellow flocks when treated with the test reagent, a result indicative of a flavonoid structure (12).

The ultraviolet absorption spectrum for Substance III triacetate reveals three maxima, at wave lengths 2390 Å, 2580 Å, 3110 Å. The ultraviolet absorption spectrum of tetrahydro-Substance III triacetate reveals well defined maxima at 2390 Å, 2580 Å, 3350 Å, and a slight dip at 3200 Å. Application of the "styrene-ethyl benzene effect" (see page 18) to these data would apparently indicate that neither of the double bonds reduced (in the formation of tetrahydro-Substance III triacetate from Substance III triacetate) is conjugated to any part of an aromatic system.

It is possible that we have here a case where the elimination of this side chain conjugation to a large poly-ring conjugated system does not significantly affect the ultraviolet absorption. Thus in osajin and pomiferin derivatives (4), this effect while present, was slight in the derivatives that had not been degraded to the benzyl phenyl ketone structure. Ultraviolet absorption data for Substance I and derivatives (8)
and for Substance III derivatives are summarized in Table 3.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Max. III</th>
<th>Max. II</th>
<th>Max. I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Å</td>
<td>Å</td>
<td>Å</td>
</tr>
<tr>
<td>Substance I</td>
<td>2280</td>
<td>2325</td>
<td>3375</td>
</tr>
<tr>
<td>Dihydro-Substance I</td>
<td>2430</td>
<td>2360</td>
<td>3380</td>
</tr>
<tr>
<td>Tetrahydro-Substance I</td>
<td>2560</td>
<td>2900</td>
<td>3330</td>
</tr>
<tr>
<td>Substance I triacetate</td>
<td>2400</td>
<td>2710</td>
<td>3220</td>
</tr>
<tr>
<td>Substance I trimethyl ether</td>
<td>2440(?)</td>
<td>2735</td>
<td>3200</td>
</tr>
<tr>
<td>Substance III triacetate</td>
<td>2390</td>
<td>2580</td>
<td>3110</td>
</tr>
<tr>
<td>Tetrahydro-Substance III triacetate</td>
<td>2390</td>
<td>2580</td>
<td>3350 slight dip at 3200</td>
</tr>
</tbody>
</table>

It may be concluded from these data that Substance I and Substance III seem to be closely related. Indeed, the data indicate that for these two Substances (or derivatives) there is the same system of bands in the regions 230-240 m\(\mu\); 260-280 m\(\mu\); and 300-350 m\(\mu\).

It seems appropriate at this stage in the discussion to comment on the fact that Substance III triacetate, an apparently fully acetylated compound, is colored (yellow).
Tetrahydro-Substance III triacetate possesses a pale yellow color. The slight yellow color of a substance is sometimes due to the tailing-off of a band which is far out in the ultraviolet region, into the visible part (400-700 μm) of the spectrum. Blocking the hydroxyl groups (by acetylation or methylation) in the substance, ordinarily eliminates that portion of the band which is extended into the visible region. However, this does not occur in the case of Substance III triacetate and tetrahydro-Substance III triacetate.

All attempts to deacetylate tetrahydro-Substance III triacetate produced a dark brown syrup, from which no crystalline material was isolated. This experiment indicates the presence of a group within the molecule which is highly sensitive to both acid and base. The fact that a dark brown solution resulted from the attempts to form carbonyl derivatives of Substance III is also indicative of a highly sensitive group within the parent molecule.

The results from the attempt to prepare dihydro-Substance III triacetate are indefinite. Reliable data for this experiment will be obtained only when a relatively large amount of Substance III triacetate becomes available for hydrogenation. The negative vat dye test (23) given by Substance III
triacetate sheds strong doubt on the possibility that this compound contains an anthraquinone system. If an anthraquinone nucleus is present, then that nucleus remains intact during acetylation; that is, the formation of a triacetate is not preceded by a reduction of the anthraquinone.
SPECULATION ON THE COMPLETE
STRUCTURES OF SUBSTANCES I, II, III

The isoprene unit has long been upheld as the fundamental building block in numerous natural products (23) where the number of carbon atoms is an even multiple of the \( C_5H_8 \) unit. Since isoprene itself has never been found in plants, there is considerable doubt that it is the actual precursor of these natural products. Nevertheless, use is made of the above postulation in the structural studies of terpenes and other alicyclic compounds. If several structures are possible for a given terpene, the one which shows the largest number of isoprene units as the building blocks is considered the most probable. This rule, called the "Isoprene Rule" (28), has however, been known to have exceptions.


On the basis of the experimental evidence which has been presented in the preceding pages, Substances I and II have been assigned the following partial structural formulas.
Since Substance II contains the smaller \(\text{C}_3\text{H}_5\text{O}^-\) group of undetermined structure, it seems logical that this substance should be the starting point for speculation on a complete molecular structure. The fragment \(\text{C}_3\text{H}_5\text{O}^-\) has been derived on the basis of one point of attachment of this group to the phenylchromone system. The following isomeric groups seem worthy of consideration.

\[
\begin{align*}
\text{CH}_2=\text{CH}-\text{CH}_2-\text{O}^- & \quad \text{CH}_3-\text{CH}=\text{CH}-\text{O}^- \\
\text{CH}_3-\text{C}-\text{O}^- & \quad \text{CH}_2-\text{C}-\text{O}^- \\
\text{CH}_2 & \quad \text{CH}_2
\end{align*}
\]
It is unfortunate that the available data for Substance II is too incomplete to state definitely whether or not a single point of attachment (of the C\textsubscript{3}H\textsubscript{5}O\textsuperscript{-} group) must involve position six of the phenylchromone system. Experimental evidence eliminates any postulated optically active compounds.

If two points of attachment are considered, then the residue C\textsubscript{3}H\textsubscript{5}O\textsuperscript{-} becomes C\textsubscript{3}H\textsubscript{6}O\textsuperscript{-}, and this gives rise to the following structures:

CH\textsubscript{2}=CH\textsuperscript{-} plus a CH\textsubscript{3}O\textsuperscript{-} attached to another position in the aromatic ring. This arrangement is eliminated on the basis of methoxyl analyses.

![Chemical structure diagram]

There are several possible structures of the above type in which "A" may be either a five or a six membered ring.

The difference between the residues on Substances I and II, is C\textsubscript{5}H\textsubscript{8}, an isoprene unit. This residue upon attachment to a ring, becomes C\textsubscript{5}H\textsubscript{9} and its possible structure might be
In a comparison of Substances I and II, it seems possible that the same \( \text{C}_3\text{H}_5\text{O}^- \) group might be common to both compounds. Available data indicate that the hydroxyl group at position five of Substance I is definitely di-ortho substituted. Thus the following structures might be written as purely speculative formulations for Substances I and II.

Indeed, it is realized that these structures may not satisfy all the known data; for example, terminal methyl group analyses. If the \( \text{C}_6\text{H}_{13}\text{O}^- \) fragment is composed of two separate groups (for example \( \text{C}_3\text{H}_5\text{O}^- \) and \( \text{C}_5\text{H}_9^- \)), then the known data seems to favor a double bond in each of the two groups. An isomerization product could be formed by interaction of the \( \text{C}_5\text{H}_9^- \) group on position six with an hydroxyl group at position five. Any one of the other non-cyclic structures for the \( \text{C}_3\text{H}_5\text{O}^- \) group seems possible.
The fact that Substance III can be readily acetylated under mild, vigorous or reductive conditions, to give a triacetate, suggests the possibility that all three hydroxyl groups are on the side phenyl group of the postulated phenylchromone nucleus. The following formula may be considered as a possibility for the complete structure of Substance III.

\[
\begin{array}{c}
\text{C}_2\text{H}_2\text{C} = \text{C} \text{H}_2 \\
\text{O} \\
\text{C}_2\text{H}_2 = \text{C} - \text{C}_2\text{H}_3 \\
\text{O} \\
\text{C}_2\text{H}_2 \text{C} = \text{C} \text{H}_3 \\
\end{array}
\]

It is to be noted however, that the molecular formula for the above compound is \( \text{C}_2\text{H}_2\text{O}_6 \), whereas the reported molecular formula for Substance III is \( \text{C}_2\text{H}_4\text{O}_6 \). A survey of the carbon and hydrogen analyses for Substance III reveals that the data is not sufficiently reliable to be used in the derivation of a molecular formula. Indeed, a statistical treatment of these data indicates that \( \text{C}_2\text{H}_2\text{O}_6 \) is almost as probable as \( \text{C}_2\text{H}_4\text{O}_6 \) for the molecular formula of Substance III.
SUMMARY

1. The X-ray powder diffraction patterns of root bark pigments (Substance I, high melting dimorph; Substance II; Substance III) of the Osage Orange (Maclura pomifera, Raf.) have been measured.

2. Hydrogenation of Substance I with platinic oxide in glacial acetic acid produced tetrahydro-Substance I.

3. The tetrahydro-Substance I obtained by the above procedure, was shown to be dimorphous with the tetrahydro-Substance I obtained by Looker (8). This fact was established by the use of X-ray powder diffraction measurements, color tests, and spectrographic analyses. These data are herein recorded.

4. Tetrahydro-Substance I was methylated with dimethyl sulfate and 50% aqueous potassium hydroxide to yield a product which melted at 122.5-123°, about ten degrees higher than that reported by Looker for tetrahydro-Substance I trimethyl ether. An insufficient quantity of the Looker material prevented a comparative study of the two products.

5. All attempts to form an oxime, a phenylhydrazone, or a 2,4-dinitrophenylhydrazone of Substance I, were unsuccessful. The starting material was recovered in approximately 50% yield. These data indicate the inactive character
of the carbonyl group in Substance I and hence offer further evidence for the existence of a chromone unit in this pigment.

6. Tetrahydro-Substance I trimethyl ether was recovered in 75% yield from an attempted oxidation of the material with alkaline peroxide. This result indicated that the postulated Y-pyrone ring in tetrahydro-Substance I trimethyl ether remained intact during the reaction. This high stability to alkaline peroxide is characteristic of flavones.

7. Oxidation of Substance I with alkaline permanganate produced a brown syrup. All attempts to obtain a crystalline product from this syrup were unsuccessful.

8. Hydrogenation of Substance III triacetate with platinic oxide in ethyl acetate, produced tetrahydro-Substance III triacetate, C_{23}H_{25}O_{2}(OCOCH_{3})_{3}, m.p. 170-171°.

9. Hydrogenation of C.P. benzene with platinic oxide in glacial acetic acid involved a hydrogen uptake of 3.05 moles of hydrogen per mole of benzene. This experiment reveals the enhancing effect of glacial acetic acid, when used as a solvent for catalytic reductions with platinic oxide. These data also indicate that the phenyl group(s) in a flavonoid structure may also be (although less likely) subject to hydrogenation.

10. Attempted acetylation of tetrahydro-Substance III
triacetate with acetic anhydride and sodium acetate at 140° returned the starting material, unchanged. This experiment indicates that no new hydroxyl group was formed during the hydrogenation of Substance III triacetate.

11. Substance III triacetate and its tetrahydro derivative each gave a positive (although not immediately strong) Perkin test, indicative of a γ-pyrone unit.

12. Substance III triacetate, a distinctly yellow substance, and tetrahydro-Substance III triacetate, pale yellow in color, gave only a very faint green color upon the addition of an alcoholic solution of ferric chloride. This result may be indicative of a hindered phenolic or enolic group within the two compounds.

13. Substance III triacetate and tetrahydro-Substance III triacetate gave a negative Wilson boric acid test.

14. Substance III triacetate, tetrahydro-Substance III triacetate, and Substance III trimethyl ether, each gave a negative antimony pentachloride test. These results indicate the absence of a chalcone structure.

15. The ultraviolet absorption spectra of Substance III triacetate and tetrahydro-Substance III triacetate
have been measured. A comparison of these data with the ultraviolet absorption data for Substance I derivatives (Looker) indicates that Substance I and Substance III (or derivatives) are closely related.

16. Attempts to obtain crystalline material by the deacetylation of tetrahydro-Substance III triacetate were unsuccessful.

17. Inconclusive results were obtained from the attempt to stop the hydrogenation of Substance III triacetate at the dihydro stage.

18. All attempts to form a carbonyl derivative of Substance III were unsuccessful.

19. Comparative studies between Substance III derivatives and anthraquinone point to the absence of the anthraquinone nucleus in the Substance III derivatives.

20. Infrared spectra are recorded for Substance I, tetrahydro-Substance I, Substance III triacetate, tetrahydro-Substance III triacetate, anthraquinone.

21. Speculation of the complete structures of Substances I, II and III is discussed.
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I, Percy Meldrum Mundell, was born in Vancouver, Canada, December 14, 1921. I received my secondary school education in the public schools of the city of Vancouver. My undergraduate and early graduate training was obtained at The University of British Columbia, from which I received the degree of Bachelor of Arts in 1943 and the degree of Master of Arts in 1945. For the year 1945-46 I was an instructor at The University of British Columbia. In October, 1946 I enrolled in the graduate school of The Ohio State University. During the period 1946-1952 I received graduate assistantships and research fellowships. Since September, 1952 I have been a member of the Department of Chemistry at Miami University, Oxford, Ohio.