ultraviolet absorption, $\lambda_{max}^{dioxane}$ in mμ ($\epsilon_{max}$): 260 (66,062), 310, sh (13,668), 340 (1594), 357 (1640); infrared absorption (potassium bromide pellet): 3050, 3020, 2987, 2950 (C—H), 1730 cm.$^{-1}$ (C=O); fluorescence in cyclohexane, excitation max. (emission at 390 mμ): 312, 340, 360 mμ; emission max. (excitation at 320 mμ): 365, 385 mμ. Column chromatography of the neutral fraction on Florisil gave the 7,14-quinone and 5,6-quinone, m.p. 252° and 318°, respectively, unchanged upon admixture with authentic materials.

*Anal.* Calcd. for $C_{24}H_{18}O_4$: C, 77.82; H, 4.90. Found: C, 77.54; H, 4.67.

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# Osage Orange Pigments. XIII. Isolation of Three New Pigments from the Root Bark[1,2]

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Three new pigments, characterized as substituted polyhydroxyxanthones, have been isolated from the root bark of the osage orange (*Maclura pomifera* Raf.). These are macluraxanthone (I, bright yellow), $C_{23}H_{22}O_6$; osajaxanthone (II, lemon yellow), $C_{18}H_{14}O_5$; and alvaxananthone (III, yellow), $C_{23}H_{24}O_6$. On standing in air, III decomposed readily but was stable indefinitely in the form of its triacetate or trimethyl ether. I showed insecticidal activity against the tropical termite; both I and III were effective fish poisons and were toxic to mosquito larvae.

Wolfrom and co-workers[4] have described the isolation and complete structure determination of two isoflavone pigments, osajin and pomiferin, obtained from the fruit of the osage orange (*Maclura pomifera* Raf.). Subsequently,[1] the syntheses of dihydroisoosajin and dihydroisopomiferin were reported. The vivid orange color of the root bark of this tree invited further investigation[5] and we report herein the isolation of three new yellow pigments from this source; osajin and pomiferin were not encountered. These three new pigments were found to be polyhydroxyxanthones; they have accordingly been designated macluraxanthone (I), $C_{23}H_{22}O_6$; osajaxanthone (II), $C_{18}H_{14}O_5$; and alvaxanthone (III),[6] $C_{23}H_{24}O_6$. The average yields of the pigments were found to be I, 0.45%; II, 0.02%; and III, 0.15%. However, yields were variable and in some samples of root bark no II or III were encountered; one sample of root bark gave only 0.36% I and 1.42% III. The isolation and separation of I and II were first effected by column chromatography on silicic acid but later work utilized more convenient solution methods and it was on applying such methods that III was found. The beautifully crystalline, yellow pigments were accompanied by large amounts of dark red resins. Although I and II are isolated in admixture and are separable by the chromatographic method, they were separated more conveniently through the 1:1 pyridine complex formed selectively by I. Macluraxanthone (I) and osajaxanthone (II) were stable in the air but alvaxanthone (III) was not; it could, however, be kept indefinitely in the form of its yellow triacetate or yellow trimethyl ether.

All three pigments were methoxyl free and optically inactive. Their acidity was entirely phenolic. All three contained the γ-pyrone ring as indicated by the Perkin test and all gave a deep green ferric chloride–alcohol color test. Macluraxanthone (I) and alvaxanthone (III) formed lead salts, diagnostic of a catechol grouping, while osajaxanthone (II) did not. Macluraxanthone and osajaxanthone showed positive Wilson[7] boric acid tests while alvaxanthone showed a negative reaction. This would indicate the presence in I and II (but not in III) of an auxochrome (presumably a hydroxyl) on the second carbon atom from a carbonyl group. Neither I nor III yielded formic acid on mild alkaline treatment and, therefore, were not isoflavones.

Alvaxanthone (III) formed a bright yellow triacetate under mild or stringent acetylation conditions. Alcoholic ferric chloride gave only a very faint green color with the triacetate; this color deepened on standing during a period of 30 min. Methylation under mild or severe conditions yielded a light yellow trimethyl ether. It was found advisable to convert alvaxanthone to the triacetate or trimethyl ether immediately on isolation to prevent loss due to the decomposition of the pigment.

The similarity of the ultraviolet spectra of macluraxanthone, osajaxanthone, alvaxanthone, and the dimethyl ether and diacetate of alvaxanthone (Table I) showed that all three pigments probably possess the same basic nucleus. A survey of the ultraviolet spectra of the various classes of naturally occurring plant pigments showed that the spectra of our pigments were similar only to xanthones.[8,9] Especially striking is the correspondence between macluraxanthone (I) and jacareubin,[10] and between alvaxanthone and di-

(1) Preceding paper in this series: M. L. Wolfrom and B. S. Wildi, *J. Am. Chem. Soc.*, **73**, 235 (1951).

(2) Preliminary communication, M. L. Wolfrom, J. H. Looker, E. E. Dickey, P. McWain, and A. Thompson, Abstracts, 119th National Meeting of the American Chemical Society, Boston, Mass., April, 1951, p. 16M.

(3) Postdoctoral Fellow of the Graduate School, deceased.

(4) M. L. Wolfrom, W. D. Harris, G. F. Johnson, J. E. Mahan, S. M. Moffet, and B. S. Wildi, *J. Am. Chem. Soc.*, **68**, 406 (1946), and earlier publications cited therein.

(5) First brought to our attention by F. L. Benton.

(6) First isolated by the late A. Thompson.

(7) C. W. Wilson, *J. Am. Chem. Soc.*, **61**, 2303 (1939).

(8) P. Yates and G. H. Stout, *ibid.*, **80**, 1691 (1958).

(9) J. C. Roberts, *Chem. Rev.*, **61**, 591 (1961).

(10) F. E. King, T. J. King, and L. C. Manning, *J. Chem. Soc.*, 3931 (1953).

TABLE I

ULTRAVIOLET SPECTRA OF OSAGE ORANGE ROOT BARK PIGMENTS AND KNOWN HYDROXYXANTHONES[a]

| Compound | $\lambda_{max}^{EtOH}$, mμ (log ε) | | | |
|---|---|---|---|---|
| Macluraxanthone (I) | 242 (4.31) | 283 (4.64) | 338 (4.28) | |
| Osajaxanthone (II) | 240 (3.87) | 285 (4.67) | 339 (3.90) | 382 (3.68) |
| Alvaxanthone (III) | 257 (4.88) | 280 (3.94) | 332 (4.38) | |
| Alvaxanthone trimethyl ether | 249 (4.64) | 276 (4.01)[b] | 323 (4.39) | |
| Alvaxanthone triacetate | 238 (4.62) | 258 (4.56) | 312 (4.20) | 367 (3.72) |
| Jacareubin[c] | 240 (4.09) | 279 (4.61) | 334 (4.26) | |
| Dihydrojacareubin[c] | 251 (4.55) | 284 (4.05) | 330 (4.24) | |

[a] Spectra were taken in absolute ethanol on a Cary Model 14 recording spectrophotometer. [b] Inflection. [c] See ref. 10.

hydrojacareubin.[10] The ultraviolet spectra of most xanthones consist of three intense bands in the region 230–340 mμ, and a fourth less intense band at higher wave lengths which accounts for their yellow color. In the spectra of three of our compounds, I, III, and the trimethyl ether of III, the band in the 340–400-mμ region is apparently not present. This band is also lacking in jacareubin,[10] 1-hydroxy-3,7-dimethoxyxanthone,[8] and in some other xanthones. In our compounds, the band in the 310–340-mμ region extends into the 340–400-mμ region, causing their yellow color. This extension is probably a broad, weak version of the usual xanthone long wave-length band. A xanthone nucleus requires thirteen carbon atoms; inspection of molecular formulas of our pigments shows that a xanthone nucleus leaves residues of ten carbons for I and III and a five-carbon residue for II. Since osajin and pomiferin,[1] isoprenoid-substituted isoflavones, have been found in *Maclura pomifera* Raf., it is considered probable that I, II, and III are indeed xanthones, substituted with two, one, and two isoprenoid groups, respectively.

It is of interest that macluraxanthone was found to be an unusually good antitermite agent.[11] Both I and III were effective fish poisons and were toxic to mosquito larvae. Further work is in progress on the elucidation of the detailed structure of these pigments.

## Experimental[12]

**Isolation of the Pigments.**—The bright orange, paper-thin root bark of *Maclura pomifera* Raf. was reduced to a powder in a Wiley mill. An amount of 400 g. of bark was placed in each of four 1-gal. wide-mouthed jars fitted with screw-top lids. The bark was extracted with ether using a countercurrent principle. The bark in the first jar was covered with ether (2000–2500 ml.) and allowed to stand for 24 hr. On the second day the ether was decanted into the second jar and enough fresh ether was added to the first to cover again the bark. This process was continued until all the jars were full of ether and had soaked at least 1 day. The solvent from the fourth jar was then decanted and filtered. Ether was decanted progressively to the next jar leaving the first jar with spent bark which was discarded. This jar was then filled with 400 g. of fresh bark and placed in the last position. The liquid was again decanted progressively to the next higher numbered jar and fresh ether was placed in the lowest numbered jar. In this way the spent bark was removed on alternate days from the lowest numbered jar which was refilled with fresh bark while the ether solution was removed from the last jar. An alternate method was to extract the root bark in a Soxhlet apparatus using 5 l. of ether/1 kg. of root bark. When 5 l. of extract was obtained, this was concentrated to 1 l. and poured slowly with stirring into 2 l. of commercial *n*-hexane (Skellysolve B). The resulting cloudy solution was decanted from the precipitated red resin (resin fraction 1), concentrated to 500 ml., and decanted from another crop of resin (resin fraction 2). On standing for a week, a gummy, partially crystalline material separated which was removed by filtration, triturated with a small amount of ether, and again filtered.

**Alvaxanthone (III).**—The previous filtrate was evaporated to dryness under reduced pressure and the residue was recrystallized from ether–petroleum ether (b.p. 30–60°) to give pure crystalline **alvaxanthone**, m.p. 155–156°. Yields varied from 0 to several grams; the average was 1.5 g./1 kg. of dry root bark (in one case, over 14 g. of pure III was obtained). X-ray powder diffraction data[13] was 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).

*Anal.* Calcd. for $C_{23}H_{24}O_6$: C, 69.68; H, 6.10; mol. wt., 396. Found: C, 69.31; H, 6.12; mol. wt. (Rast), 386.

**Alvaxanthone Triacetate.**—To a cooled (0°) solution of 0.5 g. of alvaxanthone in 3 ml. of pyridine was added 10 ml. of previously cooled acetic anhydride. The reaction product soon crystallized. The reaction mixture was maintained at 0° overnight and then was poured into water, and the product was filtered, yielding 0.60 g. Recrystallization from benzene–ethanol gave yellow needles, m.p. 209–211°.

The same product was obtained on acetylation of alvaxanthone (0.5 g.) by refluxing for 30 min. with acetic anhydride (10 ml.) and 1 g. of freshly fused sodium acetate. The cooled solution was poured into water and the product was filtered, yielding 0.58 g. Recrystallization, as above, gave yellow needles, m.p. 209–211°, undepressed on admixture with the acetate from the acetic anhydride–pyridine acetylation. The triacetate in ethanol gave a faint green color with alcoholic ferric chloride which changed to a deep green on standing for 30 min.

*Anal.* Calcd. for $C_{23}H_{21}O_6(COCH_3)_3$: C, 66.67; H, 5.79; $CH_3CO$, 24.71; mol. wt., 522.5. Found: C, 66.73; H, 5.76; $CH_3CO$, 22.36; mol. wt. (Rast), 496.

**Alvaxanthone Trimethyl Ether.**—A cooled solution of 1 g. of alvaxanthone in 15 ml. of anhydrous ether was treated with a large excess of diazomethane in ether. A vigorous reaction ensued. The solution was kept at 0° for 2 hr., then overnight at room temperature. The solid obtained on solvent removal was recrystallized from benzene–ethanol, yielding 0.55 g., m.p. 151–152°.

An amount of 500 mg. of alvaxanthone was dissolved in 20 ml. of acetone and 5.0 g. of anhydrous potassium carbonate was added. About 2.5 ml. of dimethyl sulfate was added in three portions over a period of 1 hr. After refluxing overnight the mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was recrystallized from benzene–ethanol, yielding 353 mg., m.p. 150–152°, undepressed on admixture with the product obtained by methylation with diazomethane.

*Anal.* Calcd. for $C_{23}H_{21}O_3(OCH_3)_3$: C, 71.22; H, 6.90; $OCH_3$, 21.21; mol. wt., 438.5. Found: C, 71.23; H, 7.02; $OCH_3$, 21.30; mol. wt. (Rast), 414.

**Separation of Macluraxanthone (I) and Osajaxanthone (II).**—The solid fraction filtered from the ether triturate described before was crystallized from the minimal quantity of benzene. The yellow crystals obtained consisted of a mixture of two yellow pigments. It was found convenient to accumulate several preparations of these crystals before proceeding with the separation of the two pigments. An amount of 10 g. of the crystals was dissolved in 50 ml. of hot pyridine. Hot water was added slowly to the boiling solution until turbidity appeared, about 15 ml. was required. On cooling in the refrigerator overnight, yellow crystals of a pyridine complex of the pigment designated macluraxanthone separated, yielding 10.9 g., m.p. 200–201°.

*Anal.* Calcd. for $C_{23}H_{22}O_6 \cdot C_5H_5N$: C, 71.02; H, 5.75; N, 2.96. Found: C, 71.03; H, 5.76; N, 3.31.

The pyridine complex was dissolved in boiling ethanol and boiled for a few minutes. Addition of hot water to turbidity and cooling yielded **macluraxanthone**. Recrystallization from benzene gave bright yellow crystals which can exist in two dimorphous forms, yielding 8.6 g., m.p. 181–182° and 205–206°. First preparations of this substance yielded the lower melting form, but in later preparations only the higher melting form was encountered. X-ray powder diffraction data[13] (high melting dimorph) was 8.33,

(11) G. N. Wolcott, *J. Agr. Univ. Puerto Rico*, **50**, 136 (1955).

(12) All melting points are corrected and were taken in a Hershberg apparatus using total immersion thermometers [E. B. Hershberg, *Ind. Eng. Chem., Anal. Ed.*, **8**, 312 (1936)].

(13) Interplanar spacing, Å., Cu Kα radiation. Relative intensities were estimated visually and the strongest lines were numbered in order of decreasing intensity.

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.

*Anal.* Calcd. for $C_{23}H_{22}O_6$: C, 70.04; H, 5.62; mol. wt., 394. Found: C, 69.97; H, 5.42; mol. wt. (Rast), 372.

The above pyridine–water mother liquor was heated to boiling and diluted with an equal amount of hot water. On cooling, the substance designated **osajaxanthone** separated. Recrystallization from ethanol yielded tiny yellow needles, m.p. 264–265°. Yields varied from 0 to 0.38 g.; the average yield was 0.2 g./1 kg. of dry root bark. X-ray powder diffraction data[13] was 9.03, 5.84 (1), 4.85, 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.

*Anal.* Calcd. for $C_{18}H_{14}O_5$: C, 69.67; H, 4.54; mol. wt., 310. Found: C, 69.91; H, 4.49; mol. wt. (Rast), 304.

Further amounts (indicated in the above average yields) of pigments were obtained on combining the two resin fractions described previously, and dissolving them in 1 part of ether, pouring into 3 or 4 parts of *n*-hexane, and processing the resultant solution as described above.

The mixture of pigments I and II also could be separated chromatographically on highly active silicic acid–Celite (5:1 w./w.), prepared from Magnesol.[14] A benzene solution of 360 mg. of the crystalline mixture was placed on a 330 × 35 mm. column of the adsorbent and developed with 500 ml. of benzene–*t*-butyl alcohol (500:1 v./v.). Two yellow zones appeared on the column. Extrusion of the column, acetone elution of the leading zone, and evaporation of the eluate gave crude macluraxanthone. Recrystallization from ethanol yielded 270 mg. of bright yellow needles, m.p. 181–182°. Elution of the upper zone with acetone, evaporation, and recrystallization from *n*-butyl ether yielded 16 mg. of light yellow needles of osajaxanthone, m.p. 264–265°.

All three pigments were soluble in acetone, moderately soluble in ethanol and benzene, insoluble in water, and, with the exception of III, insoluble in petroleum ether.

**Qualitative Group Tests. Methoxyl.**—All three pigments were methoxyl free by the Zeisel assay.

**Optical Activity.**—All three substances were optically inactive in 95% ethanol solution (*c* 2) throughout the visible region of the spectrum.

**Phenolic Acidity.**[15]—Each pigment was dissolved in ether and placed in a separatory funnel. The air was displaced with nitrogen and 10% aqueous sodium hydroxide was added with shaking. The ether layer in each case became colorless, and the aqueous layer became dark red. When carbon dioxide was passed through the two layers, the aqueous layer became colorless and the ether layer regained its yellow color. In each case, the pigments were recovered on evaporation of the ether layer.

**Perkin γ-Pyrone Test.**—Each substance was dissolved in glacial acetic acid and a drop of sulfuric acid was added. In each case a deep orange color developed immediately.

**Ferric Chloride–Ethanol.**—Samples of each of the pigments in 95% ethanol gave a deep green color when a drop of a saturated ethanolic (95%) solution of ferric chloride was added.

**Lead Acetate.**—Each pigment was dissolved in 95% ethanol and a saturated solution of methanolic lead acetate was added drop by drop. In the case of osajaxanthone, no precipitate formed. The other two pigments yielded precipitates which could be removed by filtration, dissolved in hot acetic acid, and poured into water to recover the respective pigments.

**Wilson Boric Acid Test.**[7]—Alvaxanthone gave a negative test but the other two pigments gave positive tests.

**Isoflavone Reaction.**—No formic acid was detected on treating either I or III with dilute base.[16]

**Toxicity to Goldfish.**—Colloidal suspensions of I and III were made in water according to Takei, Miyajima, and Ono.[17] The tests were made by placing the fish (weight, each *ca.* 5 g.) in a 200-ml. flask containing 100 ml. of solution and noting the time required "for kill." It was found that a 0.02% solution of III killed the goldfish in 43 min. Concentrations of I ranging from 0.001 to 0.02 were effective in 30 to 60 min. Concentrations of I lower than 0.001% were not lethal.

**Toxicity to Mosquito Larvae.**[18]—The toxicities of I and III to mosquito larvae as compared to rotenone were determined by standard methods. These pigments compared favorably with rotenone (Tables II and III).

TABLE II

TOXICITIES OF ROOT BARK PIGMENTS AND ROTENONE TO THE LARVAE OF THE YELLOW FEVER MOSQUITO (*Aedes aegypti*)

| Concentration (p.p.m.) | | 100 | 10 | 5 | 1 | 0.1 |
|---|---|---|---|---|---|---|
| Macluraxanthone, % kill, | 24 hr. | 100 | 10 | 0 | 0 | 0 |
| | 48 hr. | | 20 | 10 | 0 | 10 |
| Alvaxanthone, % kill, | 24 hr. | 100 | 0 | 10 | 10 | 0 |
| | 48 hr. | | 0 | 40 | 40 | 60 |
| Rotenone, % kill, | 24 hr. | 0 | 0 | 0 | 0 | 0 |
| | 48 hr. | 30 | 0 | 0 | 0 | 0 |

TABLE III

TOXICITIES OF MACLURAXANTHONE AND ROTENONE TO THE LARVAE OF THE MALARIAL MOSQUITO (*Anopholes quadrimaculatus*)

| Concentration (p.p.m.) | | 10 | 5 | 1 | 0.1 |
|---|---|---|---|---|---|
| Macluraxanthone, % kill, | 24 hr. | 0 | 0 | 0 | 0 |
| | 48 hr. | 70 | 38 | 12 | 6 |
| Rotenone, % kill, | 24 hr. | 10 | 0 | 0 | 0 |
| | 48 hr. | 60 | 38 | 10 | 6 |

(14) M. L. Wolfrom, J. H. Frazer, L. P. Kuhn, E. E. Dickey, S. M. Olin, D. O. Hoffman, R. S. Bower, A. Chaney, E. Carpenter, and P. McWain, *J. Am. Chem. Soc.*, **77**, 6579 (1955), footnote 65.

(15) M. L. Wolfrom, F. L. Benton, A. S. Gregory, W. W. Hess, J. E. Mahan, and P. W. Morgan, *ibid.*, **61**, 2832 (1939).

(16) E. Walz, *Ann.*, **489**, 118 (1931); M. L. Wolfrom, J. E. Mahan, P. W. Morgan, and G. F. Johson, *J. Am. Chem. Soc.*, **63**, 1248 (1941).

(17) S. Takei, S. Miyajima, and M. Ono, *Ber.*, **66**, 1826 (1933).

(18) Experimental work was carried out at the Orlando, Fla., Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Entomology Research Division, Insects Affecting Man and Animals Research Branch.