[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL CHEMISTRY AND THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

A Yellow Pigment from the Osage Orange (*Maclura pomifera* Raf.)[1]

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Large quantities of osage oranges are produced annually in the United States on female osage orange (*Maclura pomifera* Raf.) trees that have grown to bearing size. The range of natural distribution of this tree is throughout southeastern Oklahoma and eastern Texas, but it is widely planted elsewhere for hedges or for ornament. It seems strange that this large, greenish-yellow fruit has received so little attention from the investigator. McHargue[2] in 1915 determined the inorganic constituents of the ash, the protein content of the dry pulp, and constants of the oil from the seeds. His analyses showed that the pulp and seeds contain valuable feed, fertilizer, oil and resin constituents. In 1912 Fox[3] reported an investigation of the fruit of the osage orange as a source of rubber, which showed that this rubber was of little commercial value.

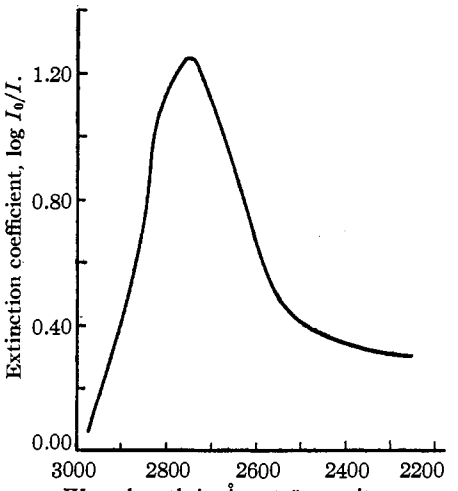

Fig. 1.—Absorption spectra of osajin: solvent, absolute ethyl alcohol; concentration, 0.0007 g./100 cc.; cell, 1.0 cm.

In the fall of 1934 some osage oranges were gathered and extracted with various solvents. A yellow crystalline substance was found in rich amounts in some of the extracts and a procedure was devised whereby this material could be obtained in a yield as high as 6% of the dried fruit. When purified the compound was of a light lemon-yellow color and melted at 189° (uncorr.) or 193° (corr.). It was insoluble in water but was soluble in the usual organic solvents except petroleum ether. It was optically inactive. The absorption spectrum (Fig. 1) showed one band with a maximum at 2750 Å.

A preliminary chemical investigation of this substance has been carried out and several of its derivatives have been prepared. The analyses and the equivalent and molecular weights obtained indicate a provisional formula of $C_{25}H_{24}O_5$. The name osajin is suggested for the pigment. It reduces Fehling's solution and gives a silver mirror when heated with Tollens' reagent in pyridine solution. On the addition of a few drops of ferric chloride to an alcoholic solution of the compound, a green coloration is obtained which changes to a reddish-violet upon the addition of ammonia. This is indicative of an *o*-dihydric phenol. When a few drops of sulfuric or hydrochloric acid are added to a solution of the substance in glacial acetic acid a deep orange coloration is obtained. Osajin was recovered unchanged when an attempt was made to determine whether it would undergo oxime or semicarbazone formation. When osajin was oxidized with nitric acid, oxalic acid was the only product isolated.

The substance consumes one equivalent of alkali when treated with an excess of base and may be recovered unchanged on acidification. Direct titration of osajin in dilute acetone solution yields an alkali consumption that is approximately 70% of that obtained in the presence of an excess of alkali. This would seem to be indicative of a lactone.

On acetylation of osajin with pyridine and acetic anhydride a monoacetate (m. p. 159°, cream-yellow) is formed, from which the osajin may be regenerated on saponification followed by neutralization. The monoacetate exhibits a green coloration with ferric chloride. When either this monoacetate or the osajin is acetylated with sodium acetate and acetic anhydride a colorless di-

(1) Presented in essentially its present form before the Division of Organic Chemistry at the 93rd meeting of the American Chemical Society, Chapel Hill, North Carolina, April 13, 1937.

(2) J. S. McHargue, *J. Ind. Eng. Chem.*, **7**, 612 (1915).

(3) C. P. Fox, *Orig. Com. 8th Intern. Congr. Appl. Chem.* (Appendix), **25**, 593 (1912).

acetate (m. p. 152°) is produced which no longer shows the ferric chloride coloration. One of the acetate groups in the diacetate is saponified with difficulty.

The osajin also yields a di-*p*-toluenesulfonate (m. p. 148°, slight yellow color) which no longer shows Fehling's reduction but gives a green coloration with ferric chloride. The information at present available on this derivative would indicate that it is best characterized as a di-*p*-toluenesulfonate of osajin in which the lactone ring has opened with the production of a new hydroxyl group.

The data so far obtained would thus indicate a provisional formula of $C_{24}H_{22}O(COO)(OH)_2$. Further work is in progress in this Laboratory on the elucidation of the structure of this substance.

Experimental[4]

Isolation and Properties of Osajin.—The fruit of the osage orange tree (*Maclura pomifera* Raf.) was chopped with a hatchet into pieces weighing 10–25 g. and oven dried at 80–90° until the pieces became brittle. The brittle material was ground in a hammer mill, and the ground meal was extracted in 1.6-kg. quantities with 4 l. of low-boiling petroleum ether in an extractor of the Soxhlet type. Extraction was continued until the extract was colorless, approximately thirty-six hours being required. The meal was then extracted with 4 l. of commercial grade ether until the extract was colorless, twelve to sixteen hours being required. The clear golden-colored ethereal extract was treated with decolorizing charcoal and filtered through a layer of fuller's earth and charcoal. Yellow crystals separated on concentration of the filtrate to 500 cc.; yield 50 g., m. p. 182–186°. Concentration of the mother liquor yielded further material in two crops; 23 g. of m. p. 173–180° and 20 g. of m. p. 170–176°; total yield, 93 g. or 5.8%.

Purification of the crude crystalline material was effected by several recrystallizations from xylene followed by recrystallization from 95% ethanol. The melting point was then 189° (uncorr.) or 193° (corr.), unchanged on further recrystallization. The color of the crystals was a light lemon-yellow. The purified substance was completely adsorbed from benzene solution by activated alumina in a Tswett column. Elutriation was effected with difficulty but finally was accomplished by extraction with glacial acetic acid, the elutriated crystals being unchanged in color and melting point.

The substance was optically inactive, numerous settings throughout the visible spectrum being made by means of a polarimeter with a spectroscope monochromator attachment. The absorption spectrum (Fig. 1) of the substance was taken and showed one band with a maximum at 2750 Å., extending into the visible in sufficiently concentrated solutions.[5]

Osajin reduced Fehling's solution and in pyridine solution it reduced Tollens' reagent. The Legal test was negative. When a few drops of aqueous ferric chloride solution were added to an alcoholic solution of the substance a dark green coloration was obtained which changed to a reddish-violet on the addition of a few drops of dilute aqueous ammonium hydroxide. A few drops of concentrated sulfuric or hydrochloric acid added to the yellow solution of the compound in glacial acetic acid produced a deep orange color. A chloroform solution of osajin readily decolorized bromine. The substance was insoluble in sirupy phosphoric acid at room temperature but dissolved in concentrated sulfuric acid to form a reddish-brown solution. The substance was insoluble in boiling, concentrated potassium carbonate solution and also in boiling concentrated ammonium hydroxide, but dissolved in boiling 5% potassium hydroxide to form a dark yellow solution from which a flocculent, brown precipitate could be obtained on acidification. Osajin was recovered unchanged when treated with an alcoholic solution of either hydroxylamine acetate or semicarbazide acetate (refluxed for ten minutes and allowed to stand overnight).

That the substance contained no water of hydration was indicated by its water-insolubility. It was unchanged in weight when heated successively under reduced pressure at 111° (three hours); at 135–140° (two hours); at 160° (two hours). The pure substance (m. p. 189°) will resolidify after melting and then melts at 188–189°. The substance showed a negative Zeisel and negative tests were also obtained for nitrogen, sulfur, halogen and phosphate.

Osajin was very soluble in chloroform, ether, acetone and pyridine; moderately so in benzene, alcohol and warm carbon tetrachloride; and was practically insoluble in water and petroleum ether.

Anal.[6] Calcd. for $C_{25}H_{24}O_8$: C, 74.3; H, 5.98; mol. wt., 404.4; saponification value (one equivalent), 2.47 cc. 0.1 *N* NaOH per 100 mg. Found: C, 74.3; H, 6.06; equivalent weight by saponification, 415 (2.41 cc.), 405 (2.47 cc.), 408 (2.45 cc.).

The saponification values were obtained by dissolving 100 mg. of osajin in 50 cc. of acetone, cooling to −4°, adding 15 cc. of 0.1 *N* sodium hydroxide, allowing to stand for three hours and back-titrating with standard acid using the glass electrode to obtain the end-point. A blank was run on the acetone used. This procedure yielded the value 415 (2.41 cc.) and is essentially the Kunz[7] procedure for the analysis of acetates of alkali-sensitive sugars. The saponification value was also determined by using the sodium ethylate saponification procedure described below under osajin diacetate but operating at room temperature for one hour 405 (2.47 cc.) and for two hours 420 (2.38 cc.).

Osajin (100 mg.) was dissolved in 100 cc. of 90% ace-

(4) The isolation of the substance was first effected by Dr. E. D. Walter and subsequently improved by Mr. F. L. Benton of this Laboratory. The remainder of the experimental work was performed by Mr. W. W. Hess.

(5) We are indebted to Professor W. R. Brode of this Laboratory for the absorption spectra measurements.

(6) All combustion analyses herein recorded were made by Dr. Ing. A. Schoeller, Berlin.

(7) A. Kunz and C. S. Hudson, THIS JOURNAL, **48**, 1982 (1926).

tone and titrated directly with 0.1 *N* sodium hydroxide, using phenolphthalein indicator (5 cc. of a 50% ethanol solution containing 50 mg. of phenolphthalein per 100 cc.). The end-point was determined by withdrawing 0.05-cc. portion of the solution at intervals and diluting with five volumes of water in order to show the color of the phenolphthalein. A blank determination was run under the same conditions. The direct titer was 1.65 cc. of 0.1 *N* sodium hydroxide. When 10 cc. of 0.1 *N* sodium hydroxide was added to the osajin solution and a back-titration performed, the titer was 2.45 cc. of 0.1 *N* sodium hydroxide per 100 mg., corresponding to an equivalent weight of 408. The ratio 1.65:2.45 is 0.67.

Osajin (1.0 g.) was dissolved in 100 cc. of absolute ethanol, 10 cc. of 0.5 *N* sodium methylate added and the whole allowed to stand for two days at room temperature. The solution was then made just acid with hydrochloric acid and concentrated to the crystallization point; yield 0.86 g. in two crops (0.25 g., m. p. 184–186°; 0.61 g., m. p. 187–188°). Recrystallization from 90% ethanol gave light yellow crystals of melting point 188–189° which were identified as osajin (mixed m. p. unchanged). Application of the same procedure to osajin that had been saponified at 90° according to the method described below under osajin diacetate, yielded material identified as osajin by melting point and mixed melting point.

Osajin Monoacetate.—Osajin (1 g.) was added to a solution of pyridine (6 cc.) and acetic anhydride (12 cc.) with preliminary cooling and allowed to stand overnight. Occasionally some of the acetate separated in the reaction mixture. The solution was poured into 50 cc. of water, ice added and the crystalline product removed by filtration and washed with water. Pure material was obtained on recrystallization from absolute ethanol; yield 0.7 g.; m. p. 159°, unchanged on further recrystallization. The substance was a cream-yellow crystalline material and showed the same green ferric chloride test as the original compound. It reduced Fehling's solution and showed the orange coloration with acetic acid–sulfuric acid, the color appearing more slowly than with osajin. It was water-insoluble, but was soluble in the usual organic solvents, being very slightly soluble in boiling petroleum ether.

Anal. Calcd. for $C_{25}H_{33}O_4(OCOCH_3)$: C, 72.6; H, 5.87; saponification equivalent weight, 223.2 (4.48 cc. 0.1 *N* NaOH per 100 mg.). Found: C, 72.5; H, 5.84; equivalent weight by saponification, 221 (4.52 cc.), 220 (4.54 cc.).

The first saponification value cited above was determined by the Kunz procedure and the second by the sodium ethylate procedure (ninety minutes at room temperature) as described under osajin diacetate. The neutralized solution from the Kunz procedure was allowed to stand for several days and a yellow, crystalline substance separated which was recrystallized from ethanol and identified as osajin by melting point and mixed melting point.

Osajin Diacetate.—Osajin (100 mg.) was refluxed for three hours with 10 cc. of acetic anhydride and 4 g. of fused sodium acetate. The mixture was poured into water and kept overnight at ice-box temperature. The crystalline material was removed by filtration; yield 0.12 g.; m. p. 158–160°. Pure material was obtained by recrystallization from ethanol by the addition of water; m. p. 162°, unchanged on further recrystallization. Repetition of this procedure at a later date always resulted in a product of melting point 152° and it was then found that the sample originally melting at 162° exhibited a melting point of 152°. It would appear that we were dealing with a case of crystalline pseudomorphism.

The solubilities of the substance were similar to those of the monoacetate. The compound was colorless and its alcoholic solution showed no coloration with ferric chloride. It reduced Fehling's solution and showed the orange coloration in acetic acid on the addition of sulfuric acid, the color appearing slowly. The same substance was obtained on subjecting the monoacetate to the same acetylating conditions.

Anal. Calcd. for $C_{25}H_{22}O_3(OCOCH_3)_2$: C, 71.3; H, 5.78; saponification equivalent weight, 162.8 (6.14 cc. 0.1 *N* NaOH per 100 mg.). Found: C, 71.1; H, 5.68; equivalent weight by saponification, 163 (6.12 cc.), 161 (6.19 cc.).

Osajin diacetate showed varying degrees of incomplete saponification under the mild conditions which were sufficient to saponify osajin and its monoacetate. The following procedure gave consistent and complete saponification. The substance (200 mg.) was dissolved in 100 cc. of absolute ethanol in a strong-walled flask and a rapid stream of oxygen-free nitrogen was bubbled through the solution for ten minutes. Ten cc. of 0.5 *N* sodium ethylate was added, the flask was stoppered tightly and heated at 90° for ninety minutes. The solution was cooled and the excess sodium ethylate was titrated with 0.1 *N* hydrochloric acid, using phenolphthalein indicator (5 cc. of a 50% ethanol solution containing 50 mg. of phenolphthalein per 100 cc.). The solution titrated was dark green in color and it was necessary to remove 0.05 cc. portion of the solution at intervals and to dilute these with five volumes of water in order to show the color of the phenolphthalein. A blank determination was run under the same conditions. When analyzed according to the above procedure, osajin gave an equivalent weight by saponification of 417 or 2.40 cc. (calcd., 404 or 2.47 cc.) and osajin monoacetate yielded the value 221 or 4.52 cc. (calcd., 223 or 4.48 cc.).

Osajic Acid Di-*p*-toluenesulfonate.—Osajin (1 g.) was dissolved in 10 cc. of dry pyridine and treated at room temperature with a solution of *p*-toluenesulfonyl chloride (4 g.) in 15 cc. of dry pyridine. The solution stood at room temperature for eighteen hours and was then poured with stirring into 150 cc. of ice and water and allowed to stand overnight at ice-box temperature. The creamy yellow, gummy solid that separated was removed by filtration and washed with water. The material was dissolved in the minimum amount of toluene at room temperature and petroleum ether was added to incipient opalescence. Crystalline material separated; yield 0.9 g.; m. p. 120–128°. Pure material was obtained on recrystallization from glacial acetic acid followed by recrystallization from 95% ethanol; yield 0.6 g.; m. p. 148°, unchanged on further recrystallization. The color of this compound was a faint yellow but repeated recrystallizations and treatments with decolorizing charcoal failed to remove this color. The solubilities of the com-

pound were similar to those of the monacetate. An alcoholic solution of the substance gave a dark green coloration with a few drops of ferric chloride solution but showed no violet coloration on the subsequent addition of ammonia. The substance in glacial acetic acid solution gave a deep orange coloration on the addition of a few drops of concentrated sulfuric acid. The compound showed a negative Fehling's reduction and in pyridine solution it did not reduce Tollens' reagent. On acetylation of the *p*-toluenesulfonate derivative with sodium acetate and acetic anhydride, a product was obtained which no longer exhibited the green coloration with ferric chloride, but as the product could not be obtained in crystalline form it was not further investigated.

Anal. Calcd. for $C_{25}H_{22}O_3(OSO_2C_6H_4CH_3)_2$: C, 65.70; H, 5.09; S, 9.00; mol. wt., 712.8. Calcd. for $C_{25}H_{24}O_4(OSO_2C_6H_4CH_3)_2$: C, 64.1; H, 5.24; S, 8.77; mol. wt., 730.8. Found: C, 64.4; H, 5.08; S, 8.75; mol. wt. (Rast), 680, 650.

An amount of 200 mg. of the *p*-toluenesulfonate derivative was dissolved in 150 cc. of acetone and titrated directly with 0.1 *N* sodium hydroxide solution, using phenolphthalein indicator and the procedure described under the direct titration of osajin. The direct titer found was 2.65 cc. or 1.33 cc. of 0.1 *N* sodium hydroxide per 100 mg.; calcd. for one equivalent on $C_{25}H_{24}O_4(OSO_2C_6H_4CH_3)_2$: 1.37 cc.

Oxidation of Osajin.—Osajin (0.5 g.) was treated with 10 cc. of concentrated nitric acid. A vigorous reaction ensued, nitrogen dioxide was evolved and the color changed from yellow through brown to black, and then back to reddish-yellow. On concentration to near dryness, 0.16 g. of crystals was obtained which were identified as oxalic acid dihydrate (m. p. 98–99°; mixed m. p. unchanged; equivalent weight by titration, 70; calcd., 63; m. p. of oxal *p*-toluidide derivative, 266°; mixed m. p. unchanged).

Summary

1. A crystalline yellow substance has been isolated from the fruit of the osage orange (*Maclura pomifera* Raf.).

2. Two acetates and a *p*-toluenesulfonate of the above compound have been prepared in pure crystalline condition.

3. It is shown that the data on all the above compounds agree best with the formula $C_{25}H_{24}O_5$ as extended to $C_{24}H_{22}O(COO)(OH)_2$, for the naturally occurring substance. This formula is put forth provisionally and the name osajin is suggested for the compound.

COLUMBUS, OHIO RECEIVED JANUARY 15, 1938

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UNIVERSAL OIL PRODUCTS COMPANY]

Alkylation of Benzene with Cycloparaffins in the Presence of Sulfuric Acid

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It is well known that the olefins alkylate benzene in the presence of sulfuric acid,[1] and since the lower cycloparaffins may be considered to contain a potential double bond, it was interesting to investigate whether they also could alkylate benzene under similar conditions.

Cyclopropane was found to alkylate benzene in the presence of cold sulfuric acid to yield *n*-propylbenzene, part of the cyclopropane being found in the catalyst acid in the form of *n*-propyl sulfate. Evidently, the cyclopropane did not first isomerize to propene,[2] in which case the alkylated product would have been isopropylbenzene. The reaction may be represented as follows.

$$\begin{array}{l} CH_2{-}CH_2 \\ \quad\diagdown\diagup \\ \quad CH_2 \end{array} + HOSO_2OH \longrightarrow CH_3CH_2CH_2OSO_2OH$$

$$CH_3CH_2CH_2OSO_2OH + C_6H_6 \longrightarrow CH_3CH_2CH_2C_6H_5 + HOSO_2OH$$

The cyclobutane ring also alkylates benzene in the presence of cold sulfuric acid, *t*-amylbenzene being obtained from the interaction between methylcyclobutane and benzene. The formation of *t*-amylbenzene involves a rearrangement which can be visualized as follows.

(1)
$$CH_3{-}CH(CH_2{-}CH_2) \longrightarrow CH_3{-}CH(CH_3){-}CH_2OSO_2OH \longrightarrow$$

$$CH_3{-}C(CH_3)=CH_2 \longrightarrow CH_3{-}CH(CH_3){-}CHOSO_2OH \longrightarrow$$

$$CH_3{-}C(CH_3)(OSO_2OH){-}CH_2$$

(2)
$$CH_3{-}C(CH_3)(OSO_2OH){-}CH_2 + C_6H_6 \longrightarrow CH_3{-}C(CH_3)(C_6H_5){-}CH_2 + HOSO_2OH$$

(1) Ipatieff, Corson and Pines, THIS JOURNAL, **58**, 919 (1936).
(2) Ipatieff and Huhn, *Ber.*, **36**, 2014 (1903).