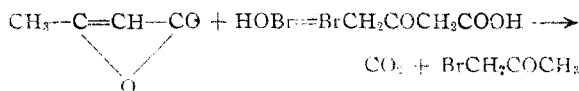


bromouracil (III). The alcohol solution was characterized by its intense and potent lachrymatory odor which was apparently due to the presence of bromoacetone. The latter is probably formed as follows. Dissociation of the 5,5-dibromohydrouracil (II) in the presence of silica gel would give 5-bromouracil (III) and hypobromous acid. The latter acid would be expected to combine immediately with any diketene which might have been formed giving bromoacetoacetic acid which would decompose immediately into bromoacetone as expressed below



In a second experiment treatment of 5,5-dibromoxyhydrouracil (10 g.) with ketene in the presence of silica gel (10 g.) was applied at 60° for four and one-half hours. Extraction of this mixture with ether in a Soxhlet unit was then applied as previously. During this operation crystalline material continued to deposit from the ether extract. This was filtered off and crystallized from water in the form of prisms melting at 200°. It was identified as unaltered dibromoxyhydrouracil (II) and responded immediately to the Wheeler and Johnson⁶ color test for uracil when tested with barium hydroxide.

The ether extract above gave, after removal of the ether, a crystalline product mixed with a small quantity of oil. The solid was easily purified by crystallization from 95% ethanol and 2.6 g. was obtained melting at 175°. It was identified as 3-acetyl-5-bromouracil (VIII). The red oil admixed with this pyrimidine was acidic and possessed a disagreeably lachrymatory odor. It was apparently a mixture of bromoacetic acid mixed with some bromoacetone.

Anal. Calcd. for $\text{C}_6\text{H}_5\text{O}_3\text{N}_2\text{Br}$: N, 12.01. Found: N, 11.96, 11.84.

Action of Ketene on 5,5-Dibromo-4-methoxyhydrouracil.⁹—Ketene addition in the presence of dry silica gel was applied for three hours at 100°, and in a second experiment three and a quarter hours at 80°. In both cases the only definite pyrimidine identified was 4-methyl-5-bromouracil.¹⁰ It melted after crystallization from water between 226–230° with decomposition.

Anal. Calcd. for $\text{C}_8\text{H}_9\text{O}_2\text{N}_2\text{Br}$: N, 13.66. Found: N, 13.57, 13.68.

Summary

1. All the ketene used in this research was prepared by pyrolysis of acetone.
2. Ketene reacts with 5,5-dibromoxyhydrouracil in the presence of silica gel to form 5-bromouracil and 3-acetyl-5-bromouracil. The latter derivative easily undergoes hydrolysis with formation of 5-bromouracil.
3. Ketene failed to interact with uracil, 4-methyluracil, thymine or hydrouracil.
4. 5,5-Dibromo-4-methoxyhydrouracil simply underwent degradation when heated in an atmosphere of ketene giving 4-methyl-5-bromouracil.
5. 5,5-Dibromoxyhydrouracil reacts to form an acetate by treatment with acetic anhydride at ordinary temperature.

(9) Behrend, *Ann.*, **229**, 18 (1885); List, *ibid.*, **236**, 19 (1886).

(10) Behrend, *ibid.*, **229**, 17 (1885); **231**, 249 (1885).

NEW HAVEN, CONNECTICUT RECEIVED OCTOBER 21, 1941

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Osage Orange Pigments. IX.¹ Improved Separation; Establishment of the Isopropylidene Group

BY M. L. WOLFROM AND JOHN MAHAN

In a previous communication from the Laboratory,² the separation of the two pigments of the fruit of the osage orange (*Maclura pomifera* Raf.) was accomplished by a very laborious, fractional crystallization procedure. We now find that the pigment pomiferin, which has the catechol configuration in its molecule, forms an insoluble lead complex while the other pigment, osajin, does not form such a complex. A very simple separation based upon this difference has been devised and is reported herein. One kilogram of the dried fruit yielded 32.5 g. of crude

pigment (m. p. 175–178°) which on separation gave 14.5 g. of pomiferin of maximum purity (m. p. 200.5°) and 9.4 g. of osajin of maximum purity (m. p. 189°), together with lesser amounts of material of lower purity.

We report also that both pomiferin and osajin yield 0.7 mole of acetone on ozonization. This establishes the isopropylidene group as a constituent of both pigments and clarifies the nature of one of the two double bonds present in each of these substances.

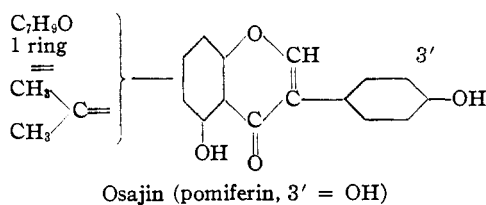
Pomiferin trimethyl ether now has been subjected to fusion at high temperature with potassium hydroxide. From these fusions we have

(1) Previous publication in this series: M. L. Wolfrom and A. S. Gregory, *THIS JOURNAL*, **63**, 3356 (1941).

(2) M. L. Wolfrom and A. S. Gregory, *ibid.*, **62**, 651 (1940).

isolated oxalic acid (from unmethylated pomiferin), catechol and isovaleric acid. The first is of no great significance. The isolation of catechol is in harmony with the isoflavone structure postulated³ for pomiferin. Under the vigorous fusion conditions employed, the substance underwent demethylation and the 3,4-dihydroxyphenylacetic acid, predictable from the isoflavone structure, was very probably further degraded to protocatechuic (3,4-dihydroxybenzoic) acid, since Perkin and Newbury⁴ have demonstrated that *p*-hydroxyphenylacetic acid is converted to *p*-hydroxybenzoic acid under strong conditions of alkali fusion. Since the fusion temperature of 300° was above that of the decarboxylation temperature of protocatechuic acid in an alkali melt,⁵ this fragment then appeared as catechol. The isovaleric acid very probably arose from the same portion of the molecule that produced acetone on ozonization, the double bond merely shifting under the influence of the alkali to produce a saturated carbon chain.

In the light of the above findings, the formulas of osajin and pomiferin may then be extended as shown



Experimental

Lead Acetate Separation of Osajin and Pomiferin.—The dried and coarsely ground meal (3 kg.) of the fruit of the osage orange (*Maclura pomifera* Raf.) was extracted thoroughly with petroleum ether and then with ether in a continuous extractor of the Soxhlet type. The ether extract was concentrated to 250 cc. and allowed to crystallize for two days at ice-box temperature; yield 134 g. This crude material was recrystallized from 450 cc. of xylene (decolorizing charcoal); yield 97.5 g. (2 crops), m. p.⁶ 175–178°. The material (97.5 g.) was dissolved in 1600 cc. of hot 95% ethanol and the warm solution treated with a solution of lead acetate (75 g.) in methanol (130 cc.). A fine, light yellow precipitate formed and the mixture was allowed to stand overnight at room temperature. It was then filtered through a Büchner suction filter pre-coated with filter-cel (Johns-Manville). The precipitate and filter-cel were removed from the filter and shaken with hot acetone (1 liter) and permitted to stand until the lead

complex of pomiferin had settled. It was then filtered through a filter-cel pre-coated filter and washed with hot acetone (100 cc.). The precipitate and filter-cel were removed from the filter and treated with hot glacial acetic acid (1 liter) to dissolve the lead complex of pomiferin. The mixture was filtered to remove the filter-cel and the filtrate was poured slowly, with stirring, into 6 liters of cold water. The precipitated pomiferin was removed by filtration and washed with water; yield 56 g., m. p. 193–195°. Pure material (light yellow crystals) was obtained on two further crystallizations effected by solution in ten parts of hot xylene, filtration through filter-cel, and rapid cooling to room temperature followed by immediate filtration; yield 43.5 g. of m. p. 200.5° (pomiferin of maximum purity) and from the mother liquors, 3.2 g. of m. p. 196–198° and 1.5 g. of m. p. 193–195°.

The above alcohol filtrate and all of the acetone wash liquors were combined, concentrated to 800 cc. and poured slowly, with stirring, into 5 liters of cold water. The precipitated osajin was removed by filtration and washed with water; yield 40 g., m. p. 183–185°. Pure material (light yellow crystals) was obtained on one further crystallization effected by solution in fifteen parts of hot 95% ethanol, filtration through filter-cel, and standing at ice-box temperature overnight; yield 28 g. of m. p. 189° (osajin of maximum purity) and from the mother liquors, 2.8 g. of m. p. 186–188°.

Isovaleric Acid and Catechol from Pomiferin Trimethyl Ether by Alkali Fusion.—Potassium hydroxide (50 g.), to which had been added 8 cc. of water, was melted and heated to 150°. Pomiferin trimethyl ether⁷ (6 g.) was then added, the melt gradually raised to 300 ± 10° over a period of ten minutes and maintained at that temperature for ten minutes longer with constant stirring at all times. The cooled melt was dissolved in 250 cc. of ice and water, acidified with phosphoric acid and steam distilled (distillation A) until the volatile acidity was negligible (3.5 liters of distillate requiring 150 cc. of 0.1 *N* sodium hydroxide for neutralization to phenolphthalein end-point). Ether extraction of the neutralized distillate yielded a small amount (0.1 g.) of organic tar. The neutralized and ether-extracted distillate was concentrated to 40 cc., acidified with 65 cc. of 0.25 *N* sulfuric acid, and the solution refluxed for two hours with a stream of carbon dioxide-free air passing through the solution, to remove any carbon dioxide. The volume was then made to a volume of 150 cc. with water (carbon dioxide-free) and steam distilled (distillation B). The first 100 cc. of distillate (titer 80.5 cc. 0.1 *N* sodium hydroxide) was acidified, after neutralization, with 35 cc. of 0.25 *N* sulfuric acid, made to a volume of 150 cc. with water and again steam distilled (distillation C). The first 200 cc. of distillation C was combined with the second 100 cc. of distillation B (total titer, 86.9 cc. 0.1 *N* sodium hydroxide), all being neutralized, and concentrated to 100 cc. An aqueous solution (15 cc.) of silver nitrate (4.5 g.) was added and a white precipitate formed immediately. This precipitate was removed by filtration, dissolved in 50 cc. of 0.25 *N* sulfuric acid, the volume made to 150 cc. and again steam distilled (distillation D). The first 300 cc. of distillate from distillation D was neutralized with 0.1

(3) M. L. Wolfrom and J. E. Mahan, *THIS JOURNAL*, **63**, 1253 (1941).

(4) A. G. Perkin and F. G. Newbury, *J. Chem. Soc.*, **75**, 830 (1899).

(5) E. Heuser and A. Winsvold, *Ber.*, **56**, 902 (1923).

(6) All melting points are uncorrected and were taken on a 360° thermometer with immersion to the -20° mark.

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

N sodium hydroxide (titer 54.3 cc.), concentrated to 80 cc. and a solution of silver nitrate (3 g.) in 10 cc. of water added. The silver isovalerate was filtered immediately and washed with water; yield 1.01 g.

Anal. Calcd. for C_8H_9COOAg (silver isovalerate): C, 28.7; H, 4.34; Ag, 51.6. Found: C, 28.76; H, 4.26; Ag, 51.5.

That the C_8 acid obtained above was isovaleric acid was determined by transforming the silver salt to the amide through the acid chloride made from the salt with thionyl chloride; m. p. 135° (mixed m. p. unchanged on admixture with an authentic specimen of isovaleramide of m. p. 135°). The melting points of the amides (106°, 111°, 155–156°)⁸ of the other isomeric valeric acids differ significantly from this value.

The distillation residue from distillation A above was neutralized with sodium hydroxide, concentrated to 100 cc. and extracted repeatedly with ether (eight 200-cc. portions). This extract was concentrated to 125 cc. and washed with a saturated sodium bicarbonate solution (two 100-cc. portions). Acidification of the sodium bicarbonate solution and extraction with ether yielded on ether removal only a small amount of tar. The original ether extract, after washing with sodium bicarbonate, was dried and yielded an amorphous dark solid on solvent removal; yield 0.5 g. This solid was extracted with petroleum ether (700 cc.) and yielded a crystalline residue on solvent removal; yield 0.10 g., m. p. 89°. Pure material (colorless crystals) was obtained on sublimation at 150°; yield 75 mg., m. p. 102°. This substance was not depressed in melting point on admixture with an authentic specimen of catechol (m. p. 103°). It exhibited the behavior of catechol with ferric chloride-alcohol, giving a green color changing to red on the addition of sodium carbonate and was precipitated from aqueous solution with lead acetate. The tetrabromide⁹ was prepared (m. p. 191–192° with softening at 185–187°, mixed m. p. unchanged, dark blue color with ferric chloride in 50% ethanol).

Oxalic Acid from Pomiferin by Alkali Fusion.—Pomiferin (2 g.) was fused with ten parts of powdered potassium hydroxide at 260° for seventy-five minutes. The cooled melt was dissolved in water (200 cc.), just neutralized with acetic acid, extracted repeatedly with ether (six 400-cc. portions) and treated with 50 cc. of a solution of lead acetate (4 g. per 100 cc. of water). A light red precipitate formed immediately and was removed by filtration and washed with water; yield 1.2 g. The lead salt was suspended in 10 cc. of water and decomposed with an excess (3 cc.) of sulfuric acid (1:1). The light red precipitate gradually disappeared and a fine white precipitate of lead sulfate settled out. The mixture was extracted repeatedly with ether, and on solvent removal a colorless crystalline solid remained; yield 0.2 g. On recrystallization from water the substance was identified as oxalic acid dihydrate; m. p. 99°, m. p. of di-*p*-toluidide¹⁰ 266–267° (mixed m. p. unchanged).

(8) E. H. Huntress and S. P. Mulliken, "Identification of Pure Organic Compounds, Order I," John Wiley and Sons, Inc., New York, N. Y., 1941, pp. 93, 187, 189, 191.

(9) Ref. 8, p. 227.

(10) Ref. 8, p. 84.

Acetone from Osajin by Ozonization.—Osajin (2 g.), dissolved in acetone-free glacial acetic acid (150 cc.) was ozonized with a stream of 3–4% ozone until the latter was no longer absorbed (70 minutes). A few minutes after the start of the reaction, the color of the solution changed from a yellow to a dark orange, but then gradually reverted to the original yellow color. The ozonide was hydrolyzed by dropping its solution slowly, over a period of twenty minutes, into a boiling solution (reflux) of hydrogen peroxide (10 cc. of 30% hydrogen peroxide in 400 cc. of water). After the addition was completed, the colorless solution was distilled slowly and the distillate was passed through a series of three traps, the first of which contained 100 cc., and the other two contained 50 cc. of a solution of 2,4-dinitrophenylhydrazine prepared by dissolving 4 g. of the hydrazine in concentrated sulfuric acid (28 cc.) and adding to this acetone-free methanol (400 cc.) and water (100 cc.). After each 100 cc. of liquid had distilled, the first trap was replaced with fresh reagent. An amount of 300 cc. of distillate was collected, the last 100 cc. containing no acetone as detected by the absence of hydrazone separation in the first trap. The hydrazone solutions from all of the traps were combined and filtered; yield 0.97 g., m. p. 115–122°. A further amount was obtained from the filtrate by the addition of water, 0.12 g., m. p. 115–121°; 1.09 g. total, m. p. 115–122°. Pure material was obtained on methanol recrystallization; 0.81 g., (calcd. for 1 mole, 1.17 g.), m. p. 124.5–125° unchanged on admixture with an authentic specimen of acetone 2,4-dinitrophenylhydrazone (m. p. 124.5–125°).¹¹

Anal. Calcd. for $C_{10}H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.53. Found: C, 45.37; H, 4.19; N, 23.58.

No substance other than acetone 2,4-dinitrophenylhydrazone could be found in the crude hydrazone preparation.

Acetone from Pomiferin by Ozonization.—Pomiferin (2 g.) was ozonized as described above for osajin; yield of acetone 2,4-dinitrophenylhydrazone, 0.92 g. of crude material melting at 118–120° which on purification from methanol yielded 0.77 g. (calcd. for 1 mole, 1.13 g.) of pure material melting at 124.5–125° (mixed m. p. unchanged).

We acknowledge the assistance of Professor A. L. Henne and Mr. Philip Hill in performing the ozonizations and of Messrs. B. Wildi, E. J. Penka and J. H. Tracht (N. Y. A., Project O. S. U. 181) in preparing the plant material.

Summary

1. An improved separation of osajin and pomiferin is reported.
2. Both osajin and pomiferin yielded 0.7 mole of acetone on ozonization.
3. Oxalic acid, catechol and isovaleric acid have been isolated from alkali fusions of pomiferin.
4. The above facts are in harmony with the isoflavone structure assignment made previously

(11) Shriner and Fuson, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 2nd. ed., 1940, p. 221.

for osajin and pomiferin and in addition establish the presence of the isopropylidene group in each of these pigments.

COLUMBUS, OHIO

RECEIVED OCTOBER 4, 1941

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Osage Orange Pigments. X.¹ Oxidation

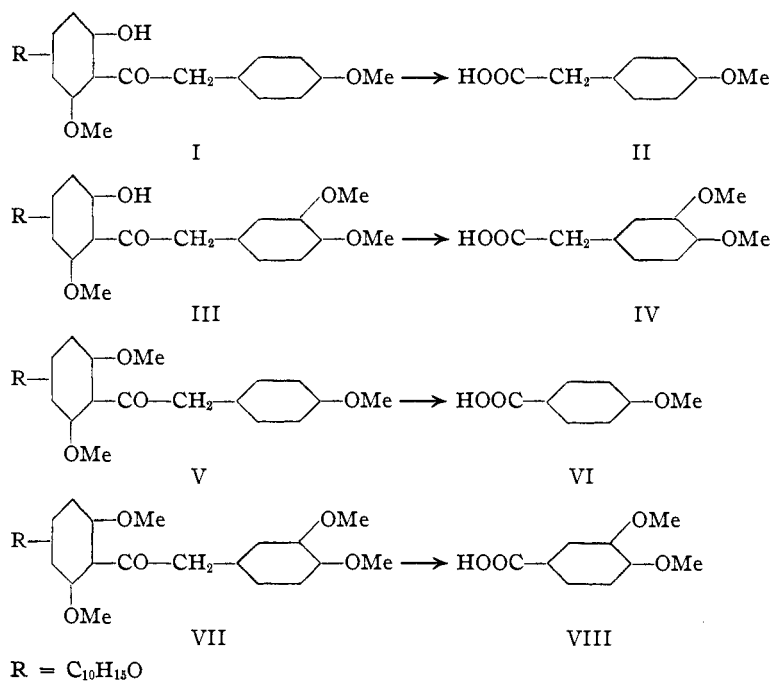
BY M. L. WOLFROM AND SAM M. MOFFETT

In continuation of our studies concerning the structure of osajin and pomiferin, the pigments of the fruit of the osage orange (*Maclura pomifera* Raf.), we have applied to them the $\text{CH}_3\text{-C}$ group analysis of Kuhn and co-workers.² The results, shown in Table I, are consistent with the presence of two $\text{CH}_3\text{-C}$ groups in the molecule of both pigments. Hydrogenation of the double bonds in the compounds (hexahydro-osajin and tetrahydropomiferin) does not decrease the assay value. The Kuhn procedure involves the use of strong acids (sulfuric and chromic) and under these conditions the pigments are very probably initially isomerized,³ even in the case of the fully methylated derivatives (osajin dimethyl ether and pomiferin trimethyl ether). This isomerization involves the disappearance of a double bond. 2-Methyl tetrahydro-osajin dimethyl ether⁴ shows (Table I) one added increment of methyl carbon as is required by its structure.

Oxidation of osajetin dimethyl ether⁴ (I) and of pomiferitin trimethyl ether⁵ (III) by alkaline hydrogen peroxide yielded homoanistic (II) and homoveratric (IV) acids, respectively. Oxidation of the fully methylated ketones, osajetin trimethyl ether (V) and pomiferitin tetramethyl ether (VII), however, yielded anisic (VI) and veratric (VIII) acids, respectively.

Tetrahydro-osajetin⁴ dimethyl ether was com-

pletely methylated to the trimethyl ether (X) and this was oxidized successfully with selenium dioxide⁶ to the diketone (XI), designated tetrahydro-osajetinone trimethyl ether. The analogous



(1) Preceding publication in this series, M. L. Wolfrom and J. Mahan, *THIS JOURNAL*, **64**, 308 (1942).

(2) R. Kuhn and F. L'Orsa, *Z. angew. Chem.*, **44**, 847 (1931); R. Kuhn and H. Roth, *Ber.*, **66**, 1274 (1933).

(3) M. L. Wolfrom, F. L. Benton, A. S. Gregory, W. W. Hess, J. Mahan and P. W. Morgan, *THIS JOURNAL*, **63**, 422 (1941).

(4) M. L. Wolfrom, J. E. Mahan, P. W. Morgan and G. F. Johnson, *ibid.*, **63**, 1248 (1941).

(5) M. L. Wolfrom and J. E. Mahan, *ibid.*, **63**, 1253 (1941).

derivative of pomiferin, designated tetrahydropomiferitinone tetramethyl ether (XIV), was prepared from tetrahydropomiferitin tetramethyl ether (XIII) in a similar manner. XI also was prepared by the deoxygenation of the isonitroso derivative (IX) of X.

The two diketones were then oxidized with alkaline hydrogen peroxide.⁷ Tetrahydro-osajetinone trimethyl ether (XI) yielded anisic acid and an acid (XII), C₁₅H₂₁O(OCH₃)₂COOH, designated tetrahydro-osajylic acid dimethyl ether. This oxidation was effected without carbon loss and the entity XII represents all of the remaining carbon skeleton of the original tetrahydro-osajin,

(6) Cf. H. L. Riley, J. F. Morley and N. A. C. Friend, *J. Chem. Soc.*, 1875 (1932).

(7) Cf. E. Weitz and A. Scheffer, *Ber.*, **54**, 2332 (1921).