osajetin dimethyl ether. The Wilson boric acid test was negative. The substance was unchanged on heating at 100° under reduced pressure.

Anal. Calcd. for $C_{27}H_{30}O_8$: C, 71.98; H, 6.71. Found: C, 71.68; H, 6.52.

2-Hydroxy-2,3-dihydro-osajin dimethyl ether (50 mg.) was refluxed for thirty minutes with glacial acetic acid (10 cc.). Water was added (incipient opalescence) to the cooled solution and the material crystallized on standing overnight at icebox temperature; yield 40 mg., m. p. 110–111°. Pure material was obtained on two crystallizations from ethanol-water and this was identified as osajin dimethyl ether by melting point (115°) and mixed melting point (115–116°) with an authentic specimen of osajin dimethyl ether (m. p. 116–116.5°).

We are indebted to Mr. Gail Clark and to Messrs. Bernard S. Wildi and Joseph Tracht (N. Y. A. Projects O. S. U.-166 and O. S. U.-169) for assistance in preparing the plant material.

Summary

1. Osajin dimethyl ether (and tetrahydro-osajin dimethyl ether) was degraded by mild alkali to one mole of formic acid and osajetin dimethyl ether (and tetrahydro-osajetin dimethyl ether).

2. Osajetin dimethyl ether (and tetrahydro-osajetin dimethyl ether) reacted with sodium and ethyl formate to produce an intermediate which on mild acid treatment (glacial acetic acid) produced osajin dimethyl ether (and tetrahydro-osajin dimethyl ether).

3. Evidence is presented that the above intermediate is 2-hydroxy-2,3-dihydro-osajin dimethyl ether (and V, 2-hydroxy-hexahydro-osajin dimethyl ether).

4. Osajetin dimethyl ether (and tetrahydro-osajetin dimethyl ether) was degraded by strong alkali to homoanisic (*p*-methoxy-phenylacetic) acid.

5. Tetrahydro-osajetin dimethyl ether has been characterized as an oxime, isolated in two forms.

6. Mild acetylation of tetrahydro-osajetin dimethyl ether produced a monoacetate and vigorous acetylation yielded 2-methyl-tetrahydro-osajin dimethyl ether.

7. The above evidence establishes the isoflavone nature of osajin (XI).

COLUMBUS, OHIO RECEIVED FEBRUARY 17, 1941

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

Osage Orange Pigments. VII.[1] Isoflavone Nature of Pomiferin[2]

BY M. L. WOLFROM AND J. E. MAHAN

In the preceding communication,[1] we have established the isoflavone structure in the pigment osajin. We wish to report now the extension of the same type of degradation experiments to the related pigment pomiferin. Tetrahydropomiferin trimethyl ether (I) was degraded by mild alkaline treatment to formic acid (one mole, quantitatively) and the ketone tetrahydropomiferitin trimethyl ether (II), characterized as its crystalline oxime. Further and more vigorous alkaline treatment of II yielded homoveratric (3,4-dimethoxyphenylacetic) acid (IV), characterized as its crystalline phenacyl ester. The alkaline degradation techniques used were adaptations of those described by Walz[3] in his work on other members of the isoflavone group. The expected fragment III was not identified but a crystalline fraction has been obtained which is under further investigation.

The ketone II was treated with sodium and ethyl formate according to the general procedure of Venkataraman and co-workers.[4] Contrary to the results of these workers, an intermediate (V) was isolated from which tetrahydropomiferin trimethyl ether (I) was obtained on treatment with glacial acetic acid. Evidence was presented in the preceding communication[1] for the structure of the derivative analogous to compound V, the latter being designated 2-hydroxy-hexahydropomiferin trimethyl ether. The properties of substance V were similar to those exhibited by the analogous intermediates described for osajin derivatives in the preceding communication.

The above-described reactions were then extended to pomiferin trimethyl ether[5] and analogous results were obtained except that the com-

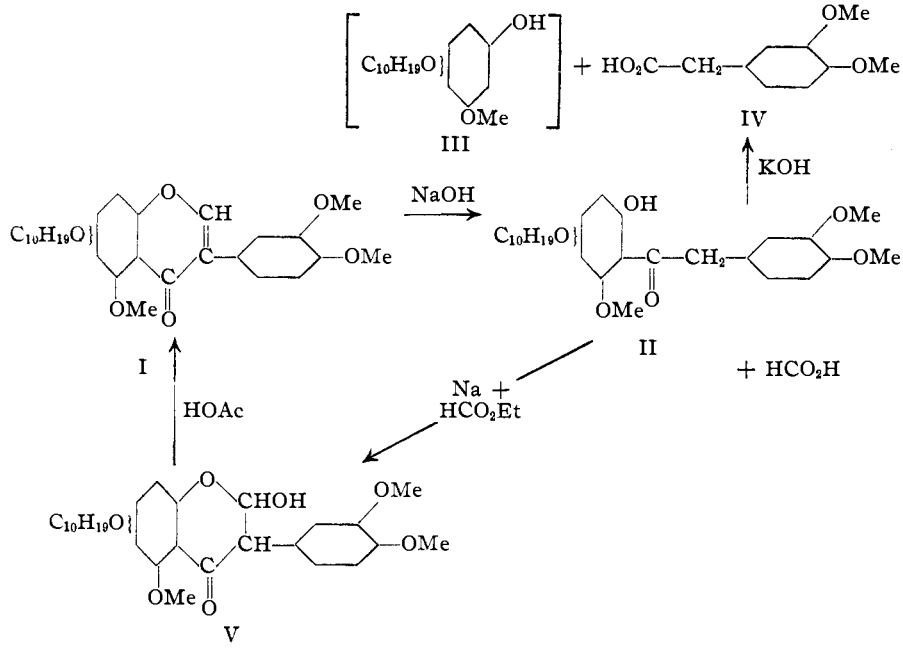
(1) Preceding publication in this series: M. L. Wolfrom, J. E. Mahan, P. W. Morgan and G. F. Johnson, THIS JOURNAL, **63**, 2481 (1941).

(2) Presented before the Division of Organic Chemistry at the 101st Meeting of the American Chemical Society, Saint Louis, Missouri, April 8, 1941.

(3) E. Walz, *Ann.*, **489**, 118 (1931).

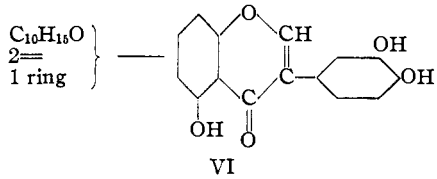
(4) H. S. Mahal, H. S. Rai and K. Venkataraman, *J. Chem. Soc.*, 1769 (1934).

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



pound analogous to II was not characterized by a derivative. The intermediate analogous to V and designated 2-hydroxy-2,3-dihydropomiferin trimethyl ether, was obtained in crystalline condition and yielded pomiferin trimethyl ether on treatment with glacial acetic acid.

These reactions definitely establish the isoflavone nucleus in pomiferin. The formula of pomiferin, which is methoxyl-free, may then be developed as follows



The above formula is analogous to that assigned to osajin[1] and thus a second free isoflavone has been added to the small and recently established group of isoflavones and isoflavone glycosides occurring in the plant world.

Experimental

Tetrahydropomiferin Trimethyl Ether.[6]—Tetrahydropomiferin[7] (12 g.) was methylated as described in the preceding communication[1] for the preparation of tetrahydro-osajin dimethyl ether and the reaction product was isolated in the same manner. Pure material was obtained on two recrystallizations from 95% ethanol; yield 9.7 g., m. p. 127–128°, unchanged on further crystallization from benzene–petroleum ether.

The substance crystallized in colorless needles and was very soluble in benzene; moderately so in ethanol; and was practically insoluble in petroleum ether and water. It gave no coloration with ferric chloride–alcohol but gave a yellow coloration in glacial acetic acid solution on the addition of a few drops of concentrated sulfuric acid. It did not reduce Tollens reagent (pyridine solution) and the Wilson boric acid test[8] was negative.

Anal. Calcd. for $C_{25}H_{25}O_3(OCH_3)_3$: C, 72.07; H, 7.35; OCH_3, 19.95. Found: C, 72.30; H, 7.28; OCH_3, 19.72.

This substance also was prepared by the hydrogenation of pomiferin trimethyl ether[5] (2 g.) according to the procedure described in the preceding communication for the preparation of tetrahydro-osajin dimethyl ether from osajin dimethyl ether; yield 1.3 g., m. p. 127–128°.

Alkaline Degradation of Tetrahydropomiferin Trimethyl Ether to Formic Acid and Tetrahydropomiferitin Trimethyl Ether.—Tetrahydropomiferin trimethyl ether (1.000 g.) was treated with alcoholic sodium hydroxide solution as described in procedure A of the preceding communication. The formic acid was determined in the steam distillate according to procedure B of the preceding communication; total volatile acidity, 21.3 cc. of 0.1 *N* sodium hydroxide; calcd. for 1 mole of formic acid, 21.4 cc.; wt. mercurous chloride, 1.005 g.; calcd., 1.010 g. A positive test for formic acid was obtained by reduction of a portion of the distillate with magnesium and hydrochloric acid followed by application of the formaldehyde milk test. An intense violet color was obtained.

The yellow oil resulting on solvent removal from the petroleum ether extract obtained by following procedure A of the preceding communication, was crystallized from ethanol–water; yield 0.88 g., m. p. 77–78°. Pure material was obtained on further crystallization from ethanol–water; yield 0.84 g., m. p. 78.5–79.5°, unchanged on recrystallization from glacial acetic acid.

The substance formed cube-like crystals of a faint yellow color. It gave a dark brown ferric chloride–alcohol test and vigorously reduced Tollens reagent (pyridine solution). The Wilson boric acid test[8] was positive. In glacial acetic acid solution, the substance gave no yellow color on the addition of a few drops of concentrated sulfuric acid.

Anal. Calcd. for $C_{24}H_{27}O_3(OCH_3)_3$: C, 71.02; H, 7.95; OCH_3, 20.39. Found: C, 70.89; H, 7.87; OCH_3, 20.0.

(6) Experimental work by Mr. A. S. Gregory.

(7) M. L. Wolfrom, P. W. Morgan and F. L. Benton, THIS JOURNAL, **62**, 1484 (1940).

(8) C. W. Wilson, *ibid.*, **61**, 2303 (1939).

Tetrahydropomiferitin Trimethyl Ether Oxime.—Tetrahydropomiferitin trimethyl ether (0.50 g.) was dissolved in methanol (25 cc.) and refluxed for six hours with hydroxylamine hydrochloride (2.5 g.) and anhydrous sodium acetate (3 g.). The crude material obtained on pouring the cooled reaction mixture into ice and water (400 cc.) was obtained crystalline by dissolving in ethanol at room temperature and adding water to incipient opalescence; yield 0.3 g., m. p. 125°. Pure material was obtained on further crystallization from ethanol-water; yield 0.1 g., m. p. 133–133.5° unchanged on further crystallization from acetone-water.

The substance crystallized as colorless, flat, short prisms and gave a dark green coloration with ferric chloride-alcohol. It was very soluble in ethanol, methanol, benzene and pyridine.

Anal. Calcd. for $C_{27}H_{37}O_6N$: C, 68.77; H, 7.91; N, 2.97. Found: C, 69.07; H, 7.75; N, 3.13.

Alkaline Degradation of Tetrahydropomiferitin Trimethyl Ether to Homoveratric Acid.—Tetrahydropomiferitin trimethyl ether (1.30 g.) was treated with alcoholic potassium hydroxide as described in procedure C of the preceding communication. Steam distillation of the neutral fraction yielded crystalline material (m. p. 65–70°) which is under further investigation. In the acid fraction, the brown oil obtained on solvent removal from the final, dried ethereal extract was treated with hot benzene (40 cc.), filtered (decolorizing charcoal) from the residual oil and the filtrate concentrated to 25 cc. and allowed to crystallize at icebox temperature. The material was recrystallized once from benzene-petroleum ether; yield 0.32 g., m. p. 85–86°. Pure material was obtained on one recrystallization from hot water followed by one recrystallization from benzene-petroleum ether; yield 0.15 g., m. p. 97.5–98°, unchanged on admixture with an authentic specimen of anhydrous homoveratric (3,4-dimethoxy-phenylacetic) acid[9] of melting point 97.5–98°.[10]

Anal. Calcd. for $C_7H_5(OCH_3)_2COOH$ (homoveratric acid): C, 61.21; H, 6.17; OCH_3, 31.6; neutral equivalent, 196.2. Found: C, 61.3; H, 6.18; OCH_3, 31.3; neutral equivalent, 196.9.

The homoveratric acid isolated above was characterized further by the preparation of its phenacyl ester according to the general procedure of Shriner and Fuson.[11] An authentic sample of homoveratric acid (1.30 g.) was neutralized with 0.1 *N* sodium hydroxide solution, the total volume reduced to 5 cc., and a few crystals of homoveratric acid added to make the solution slightly acidic. A solution of phenacyl bromide (1.3 g.) in ethanol (10 cc., abs.) was added and the mixture refluxed for ninety minutes. Colorless, fine, elongated prisms separated on cooling; yield 1.50 g., m. p. 66–67°. Further recrystallization from absolute ethanol or benzene-petroleum ether slightly sharpened this melting point to 66.5–67°. The ester was soluble in acetone, methanol, pyridine and chloroform.

Anal. Calcd. for $C_{18}H_{18}O_5$: C, 68.78; H, 5.77. Found: C, 68.81; H, 5.66.

The phenacyl ester of the homoveratric acid isolated from the alkaline degradation of tetrahydropomiferitin trimethyl ether was prepared as described above; m. p. 66.5–67°, mixed m. p. unchanged.

2-Hydroxy-hexahydropomiferin Trimethyl Ether and its Conversion to Tetrahydropomiferin Trimethyl Ether.—Tetrahydropomiferitin trimethyl ether (400 mg.) was treated with sodium and ethyl formate as described in procedure D of the preceding communication. The reaction product was isolated in the same manner and was obtained crystalline from ethanol-water; yield 310 mg. Pure material was obtained on several crystallizations from benzene-petroleum ether; yield 150 mg., m. p. 129–130°.

The substance was colorless. It gave no coloration with ferric chloride-alcohol and reduced Tollens reagent (pyridine solution) only slowly on heating. This reduction was in contrast to the rapid reduction of Tollens reagent shown by tetrahydropomiferitin trimethyl ether. The Wilson boric acid test was negative. The substance was unchanged on heating at 100° under reduced pressure.

Anal. Calcd. for $C_{28}H_{36}O_7$: C, 69.40; H, 7.49. Found: C, 69.26; H, 7.69.

The above substance (70 mg.) was refluxed for thirty minutes with glacial acetic acid (20 cc.) and on adding water to the cooled solution to the point of incipient opalescence and cooling to icebox temperature, crystallization ensued; yield 60 mg., m. p. 125–126°. Pure material was obtained on further crystallization from ethanol-water and identified by melting point (127–128°) and mixed melting point (unchanged) as tetrahydropomiferin trimethyl ether.

Alkaline Degradation of Pomiferin Trimethyl Ether to Formic Acid and Pomiferitin Trimethyl Ether.—Pomiferin trimethyl ether[5] (1.000 g.) was treated with alcoholic sodium hydroxide solution as described in procedure A of the preceding communication. The formic acid was determined in the steam distillate according to procedure B of the preceding communication; total volatile acidity, 21.3 cc. of 0.1 *N* sodium hydroxide; calcd. for 1 mole of formic acid, 21.6 cc.; wt. mercurous chloride; 1.004 g.; calcd., 1.020 g.

The yellow oil resulting on solvent removal from the petroleum ether extract obtained by following procedure A of the preceding communication, was crystallized from ethanol-water; yield 0.85 g., m. p. 63.5–64°. Pure material was obtained on crystallization from ethanol-water; yield 0.81 g., m. p. 64.5–65°, unchanged on recrystallization from acetic acid-water.

Pomiferitin trimethyl ether crystallized in thick, yellow prisms. It gave a dark brown coloration with ferric chloride-alcohol and vigorously reduced Tollens reagent (pyridine solution). The substance reacted with hydroxylamine and semicarbazide with difficulty and no crystalline reaction product was obtained. The substance gave a positive Wilson boric acid test. Pomiferin trimethyl ether[5] gave a negative and pomiferin dimethyl ether[5] a positive Wilson boric acid test. Pomiferitin trimethyl ether, in glacial acetic acid solution, gave no deepening of the slight yellow initial color on the addition of a few drops of concentrated sulfuric acid.

(9) R. D. Haworth, W. H. Perkin, Jr., and J. Rankin, *J. Chem. Soc.*, **125**, 1693 (1924); *cf.* "Organic Syntheses," John Wiley and Sons, New York, N. Y., Vol. XV, 1935, p. 31.

(10) We are indebted to Mr. E. E. Dickey of this Laboratory for the preparation of a sample of homoveratric acid.

(11) Shriner and Fuson, "Identification of Organic Compounds," John Wiley and Sons, New York, N. Y., 2nd ed., 1940, p. 132.

Anal. Calcd. for $C_{21}H_{23}O_3(OCH_3)_3$: C, 71.67; H, 7.13; OCH_3, 20.6. Found: C, 71.89; H, 7.03; OCH_3, 20.2.

Alkaline Degradation of Pomiferitin Trimethyl Ether to Homoveratric Acid.—Pomiferitin trimethyl ether (6 g.) was treated with alcoholic potassium hydroxide as described in procedure C of the preceding communication and the homoveratric acid isolated in the same manner; yield 1.8 g., m. p. 93–94°. Pure material was obtained on further crystallization from hot water (decolorizing charcoal) and from benzene–petroleum ether; yield 0.5 g., m. p. 97–98° (mixed m. p. unchanged). The substance was further characterized by preparation of its phenacyl ester as described above; m. p. 66.5–67° (mixed m. p. unchanged).

2-Hydroxy-2,3-dihydropomiferin Trimethyl Ether and its Conversion to Pomiferin Trimethyl Ether.—Pomiferitin trimethyl ether (400 mg.) was treated with sodium and ethyl formate as described in procedure D of the preceding communication. The reaction product was isolated in the same manner and was obtained crystalline from ethanol–water; yield 270 mg., m. p. 98–105°. Pure material was obtained on further crystallization (decolorizing charcoal) from benzene–petroleum ether; yield 170 mg., m. p. 143–144°.

The substance crystallized as fine, colorless prisms. It gave no coloration with ferric chloride–alcohol and reduced Tollens reagent (pyridine solution) only slowly on heating. This reduction was in contrast to the rapid reduction of Tollens reagent shown by pomiferitin trimethyl ether. The Wilson boric acid test was negative. The substance was unchanged on heating at 100° under reduced pressure.

Anal. Calcd. for $C_{28}H_{32}O_7$: C, 69.98; H, 6.73. Found: C, 69.78; H, 6.68.

The above substance (50 mg.) was refluxed for thirty minutes with glacial acetic acid (15 cc.). Water was added (incipient opalescence) to the cooled solution and the material crystallized on standing overnight at icebox temperature; yield 45 mg., m. p. 137–138°. Pure material was obtained on one further crystallization from ethanol–water and this was identified as pomiferin trimethyl ether by melting point (139–139.5°) and mixed melting point (139–139.5°) with an authentic specimen of pomiferin trimethyl ether (m. p. 139.5°).

We are indebted to Mr. Gail Clark and to Messrs. Bernard S. Wildi and Joseph Tracht (N. Y. A. Projects O. S. U.-166 and O. S. U.-169) for assistance in preparing the plant material.

Summary

1. Pomiferin trimethyl ether (and tetrahydropomiferin trimethyl ether) was degraded by mild alkali to one mole of formic acid and pomiferitin trimethyl ether (and tetrahydropomiferitin trimethyl ether).

2. Pomiferitin trimethyl ether (and tetrahydropomiferitin trimethyl ether) reacted with sodium and ethyl formate to produce an intermediate which on mild acid treatment (glacial acetic acid) produced pomiferin trimethyl ether (and tetrahydropomiferin trimethyl ether).

3. Evidence is presented that the above intermediate is 2-hydroxy-2,3-dihydropomiferin trimethyl ether (and V, 2-hydroxyhexahydropomiferin trimethyl ether).

4. Tetrahydropomiferitin trimethyl ether was characterized as an oxime.

5. Pomiferitin trimethyl ether (and tetrahydropomiferitin trimethyl ether) was degraded by strong alkali to homoveratric (3,4-dimethoxyphenylacetic) acid.

6. The above evidence establishes the isoflavone nature of pomiferin (VI).

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

3,7-Dihydroxy-1,2,5,6-dibenzanthraquinone

BY JAMES CASON[1] AND LOUIS F. FIESER

In an earlier paper on the subject of carcinogen metabolism,[2] we described the synthesis of 4′,8′-dihydroxy-1,2,5,6-dibenzanthracene and reported that the substance very probably is identical with the product of the metabolism of dibenzanthracene by rats and mice.[3] There remains for identification the rabbit metabolite[3,4] isolated by Levi and Boyland[4] and characterized as a dihydroxydibenzanthracene having the hydroxyl groups at some positions other than 4, 8, 9 and 10. Although we are led by certain speculative considerations to believe that the hydroxyl groups are most likely to be found at the 3′ and 7′ positions[2] and are attempting (with D. M. Bowen) to synthesize this isomer, we have in the meantime investigated one other possibility.

It seemed likely that the symmetrically substituted 3,7-isomer might be synthesized in the form of the quinone by the intermolecular con-

(1) Research Fellow on a grant from the National Cancer Institute (1938–1940); present address, Department of Chemistry, DePauw University, Greencastle, Indiana.

(2) Cason and Fieser, THIS JOURNAL, **62**, 2681 (1940).

(3) Dobriner, Rhoads and Lavin, *Proc. Soc. Exptl. Biol. Med.*, **41**, 67 (1939).

(4) Levi and Boyland, *Chemistry and Industry*, **15**, 446 (1937).