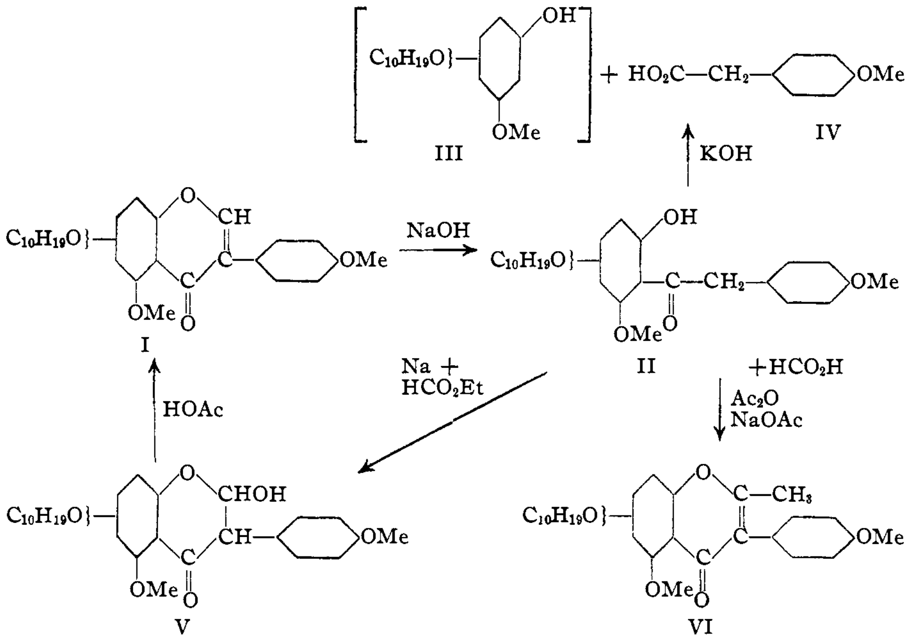
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Osage Orange Pigments. VI.[1] Isoflavone Nature of Osajin[2]

BY M. L. WOLFROM, J. E. MAHAN, P. W. MORGAN AND G. F. JOHNSON

In a previous communication[3] from this Laboratory, a flavone structure for osajin and pomiferin (the pigments of the fruit of the osage orange, *Maclura pomifera* Raf.) was postulated on the basis of color tests, subject to confirmation by degradative experiments. Such experiments have now been effected and an isoflavone structure has been found present. Hexahydro-osajin[3] is therefore an isoflavanone, a general structure not yet found in nature and concerning which very little is known. Anderson and Marrian[4] obtained an isoflavanone by reduction of 7,4'-dimethoxy-isoflavone with hydrogen and platinic oxide in glacial acetic acid solution. Späth and Schläger[5] have reported 7,2',4'-trimethoxy-isoflavanone prepared by oxidation of the corresponding isoflavan and a similar oxidation of an isoflavan derivative to what was believed to be an isoflavanone has been reported by Robertson and co-workers.[6]



It is thus apparent that the reduction color tests cited by Asahina and co-workers[7] as characteristic of flavones and flavanones are also given by isoflavones and isoflavanones and this was found to be true in the case of genistein (5,7,4'-trihydroxy-isoflavone) and pseudobaptigenin (7-hydroxy-3',4'-methylenedioxy-isoflavone).

The isoflavone nucleus was first postulated for the case of the aglucone genistein (prunetol) by Finnemore[8] and established for this substance by Baker and Robinson[9] through synthetic experiments. An isoflavone is characterized definitely by the fact that mild alkali produces quantitatively one mole of formic acid and an hydroxylated benzyl *o*-hydroxyphenyl ketone which on further treatment with strong alkali is cleaved to an hydroxylated phenylacetic acid and a polyhydric phenol.[10]

In the case of tetrahydro-osajin dimethyl ether (I), mild alkaline treatment produced quantitatively one mole of formic acid and tetrahydro-osajetin dimethyl ether (II). This ketone was characterized as an oxime, isolated in two forms. The ketone gave a characteristic dark brown coloration with ferric chloride–alcohol and in

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(1) Preceding publication in this series: M. L. Wolfrom, F. L. Benton, A. S. Gregory, W. W. Hess, J. E. Mahan and P. W. Morgan, THIS JOURNAL, **63**, 422 (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) M. L. Wolfrom, P. W. Morgan and F. L. Benton, THIS JOURNAL, **62**, 1484 (1940).

(4) E. L. Anderson and O. F. Marrian, *J. Biol. Chem.*, **127**, 649 (1939).

(5) E. Späth and J. Schläger, *Ber.*, **73**, 1 (1940).

(6) A. McGookin, A. Robertson and W. B. Whalley, *J. Chem. Soc.*, 787 (1940).

(7) Y. Asahina and M. Inubuse, *Ber.*, **61**, 1646 (1928); **64**, 1256 (1931); Y. Asahina, G. Nakagome and M. Inubuse, *ibid.*, **62**, 3016 (1929).

(8) H. Finnemore, *Pharm. J.*, **85**, 604 (1910), *cf.* H. Molisch and G. Goldschmiedt, *Monatsh.*, **22**, 679 (1901).

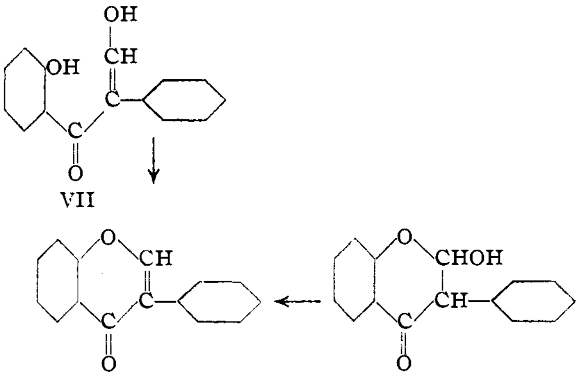
(9) W. Baker and R. Robinson, *J. Chem. Soc.*, 2713 (1926).

(10) H. Hlasiwetz, *J. prakt. Chem.*, **65**, 419 (1855); A. G. Perkin and F. G. Newbury, *J. Chem. Soc.*, **75**, 830 (1899); B. Shibata, *J. Pharm. Soc. Japan*, No. **543**, 380 (1927), *C. A.*, **21**, 3050 (1927); W. Baker, *J. Chem. Soc.*, 1022 (1928); E. Späth and O. Schmidt, *Monatsh.*, **53–54**, 454 (1929); E. Walz, *Ann.*, **489**, 118 (1931).

pyridine solution it vigorously reduced Tollens reagent. On mild acetylation a monoacetate was produced, and on vigorous acetylation a 2-methyl isoflavone derivative (VI) was produced. The latter reaction is well established for these substituted benzyl *o*-hydroxyphenyl ketones.[11]

More vigorous alkaline treatment of tetrahydro-osajetin dimethyl ether yielded homoanisic (*p*-methoxyphenylacetic) acid (IV). The expected fragment III has not been identified but the reaction is under further investigation and crystalline material as yet not characterized, has been found. The alkaline treatment techniques used were adaptations of those employed by Walz[10] in his investigations of daidzein (7,4′-dihydroxyisoflavone) and genistein.

Tetrahydro-osajetin dimethyl ether was converted into the original tetrahydro-osajin dimethyl ether with sodium and ethyl formate followed by treatment with glacial acetic acid. This reaction was first used in chromone synthesis by Perkin and Robinson[12] and applied to isoflavone synthesis by Späth and Lederer.[13] The latter workers synthesized pseudobaptigenin in low yield, employing a temperature of 100° for the condensation. They postulated, but did not isolate, an intermediate of the nuclear structure VII which underwent ring closure on treatment with mineral acids.



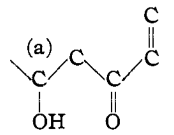
VII

VIII

Venkataraman and co-workers[14] have improved this isoflavone synthesis by employing low temperatures for the condensation with sodium and ethyl formate. They state that the reaction proceeds directly to the isoflavone but they employ glacial acetic acid as a recrystallizing medium. In the work herein reported, we have used the condensation conditions of these workers and have isolated a definite intermediate which differs in composition from tetrahydro-osajin dimethyl ether by one mole of water. Evidence is presented that this is not water of hydration but is constitutionally combined. On treatment with glacial acetic acid the substance yields tetrahydro-osajin dimethyl ether.

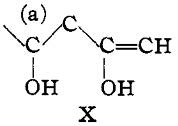
Two formulas (derivatives of VII and VIII) for this intermediate come into question. As evidence for VIII, we present the facts that the substance is much less reducing than tetrahydro-osajetin dimethyl ether, gives no phenol (ferric chloride–alcohol) test and shows a negative Wilson boric acid test.[15] Tetrahydro-osajetin dimethyl ether is strongly reducing, gives a strong phenol test and a positive Wilson boric acid test. It would appear reasonable that a compound of formula VII should be similar to tetrahydro-osajetin dimethyl ether in these respects.

The Wilson boric acid test is indicative of the grouping



IX

Such a grouping is present in VII but not in VIII. The fact that II shows this test is probably due to the closely related enolic system:



X

We have found this test to be very useful. The fully methylated derivatives of osajin[16] and pomiferin[17] give no color reaction with this reagent but the partially methylated ethers[1] do. Accordingly, in these structures at least, methylation of the hydroxyl group in position (a) of formula IX destroys the capacity of the compound to give the color reaction. The free hydroxyl group in posi-

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[11] W. N. Nogai, *Ber.*, **25**, 1284 (1892); St. v. Kostanecki and A. Rózycke, *ibid.*, **34**, 102 (1901); St. v. Kostanecki and L. Lloyd, *ibid.*, **34**, 2942 (1901); F. Wessely and F. Lechner, *Monatsh.*, **57**, 395 (1931); I. M. Heilbron, D. H. Hey and B. Lythgoe, *J. Chem. Soc.*, 295 (1936).

[12] W. H. Perkin, Jr., and R. Robinson, *ibid.*, **93**, 509 (1908).

[13] E. Späth and E. Lederer, *Ber.*, **63**, 743 (1930).

[14] P. C. Joshi and K. Venkataraman, *J. Chem. Soc.*, 513 (1934); H. S. Mahal, H. S. Rai and K. Venkataraman, *ibid.*, 1120, 1769 (1934).

[15] C. W. Wilson, THIS JOURNAL, **61**, 2303 (1939).

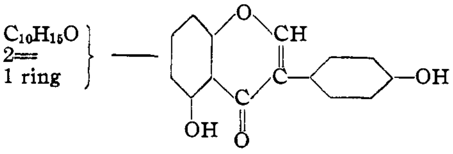
[16] M. L. Wolfrom and A. S. Gregory, *ibid.*, **62**, 651 (1940).

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

tion (a) of formula X is formed in the alkaline treatment (*cf*. II).

The reactions thus established for tetrahydro-osajin dimethyl ether were then extended to osajin dimethyl ether. Analogous results were obtained except that the compound analogous to II was not characterized by derivatives. The intermediate analogous to V was obtained in crystalline condition and yielded osajin dimethyl ether on treatment with glacial acetic acid.

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



XI

To our knowledge, osajin is the first free (non-glycosidic) isoflavone to be isolated from a natural product. The fruit gives a high yield of this pigment and is highly pigmented (yellow). It is probable that the isoflavone is present in the free form in the fruit, since the glycoside would not be expected to be highly colored. This is rendered more probable by the fact that only neutral solvents were employed in its extraction.

Experimental

WITH J. E. MAHAN

Color Tests on Known Isoflavones.—The isoflavones genistein (5,7,4′-trihydroxy-isoflavone) and pseudobaptigenin (7-hydroxy-3′,4′-methylenedioxyisoflavone)[18] were used in these tests. All of these substances gave a yellow coloration on the addition of a few drops of concentrated sulfuric acid to their glacial acetic acid solutions. The reduction tests were performed as described in a previous publication.[3] No coloration was produced on reduction with magnesium and hydrochloric acid but orange-red solutions forming dark red precipitates were obtained on acidification with an excess of concentrated hydrochloric acid of the sodium amalgam reduction mixtures. In the Wilson boric acid test, genistein gave a yellow color but pseudobaptigenin gave a negative test.

Tetrahydro-osajin Dimethyl Ether.—Tetrahydro-osajin[3] (28.5 g.) was dissolved in acetone (500 cc.), the solution heated to boiling under reflux (mechanical stirring) and dimethyl sulfate (15 cc.) added. Then over a period of two and one-half hours dimethyl sulfate (235 cc.) and potassium hydroxide (250 cc. of a 42.5% aqueous solution) were added dropwise at an equal rate after which the solution was refluxed for two hours longer. The cooled solution was poured into water (5 liters) and after standing overnight the precipitate was removed by filtration and washed with water. Pure material was obtained by solution in 95% ethanol (400 cc.) and addition of water to incipient opalescence; yield 27.5 g., m. p. 121–121.5°, unchanged on crystallization from benzene.

The substance crystallized in colorless prisms and exhibited no coloration with ferric chloride–alcohol and gave no reduction with Tollens reagent (pyridine solution). The substance in glacial acetic acid solution gave a deep yellow coloration on the addition of a few drops of concentrated sulfuric acid. The Wilson boric acid test[15] was negative. The compound was very soluble in hot alcohol, benzene and ether; slightly so in petroleum ether; and was practically insoluble in water.

Anal. Calcd. for $C_{25}H_{26}O_3(OCH_3)_2$: C, 74.29; H, 7.39; OCH_3, 14.22. Found: C, 74.35; H, 7.15; OCH_3, 13.9.

This substance also was prepared by the hydrogenation of osajin dimethyl ether.[16] An amount of 2 g. of the latter compound dissolved in 100 cc. of absolute ethanol and ethyl acetate (1:1) was hydrogenated at atmospheric pressure with platinic oxide (0.1 g.) until two moles of hydrogen had been absorbed. The catalyst was removed by filtration and the substance crystallized on the addition of water to the concentrated (75 cc.) filtrate. Pure material was obtained as described above; m. p. 121–121.5°.

(Procedure A) Tetrahydro-osajetin Dimethyl Ether.—Tetrahydro-osajin dimethyl ether (1.000 g.) dissolved in hot absolute ethanol (10 cc.) was added to a hot solution made by dissolving sodium hydroxide (5.5 g., 95%) in absolute ethanol (15 cc.) and water (25 cc.). The solution was held just at the boiling point for fifteen minutes, then cooled and made slightly acid with dilute phosphoric acid. The solution was extracted with petroleum ether (two 150 cc. portions), the petroleum ether removed and the oily residue dissolved in hot 95% ethanol (75 cc.) with water being added to incipient opalescence. The crystals that formed were removed by filtration; yield 0.94 g., m. p. 85°. Pure material was obtained on two further crystallizations from ethanol; yield 0.70 g., m. p. 87°, unchanged on further crystallization from acetic acid–water.

The substance crystallized in colorless elongated prisms. It gave a dark brown ferric chloride–alcohol test and vigorously reduced Tollens reagent (pyridine solution). In glacial acetic acid solution, the substance gave no coloration on the addition of a few drops of concentrated sulfuric acid. The Wilson boric acid test was positive.

Anal. Calcd. for $C_{24}H_{28}O_3(OCH_3)_2$: C, 73.21; H, 8.05; OCH_3, 14.52. Found: C, 73.50; H, 7.89; OCH_3, 14.2.

Oximation of Tetrahydro-osajetin Dimethyl Ether.—Tetrahydro-osajetin dimethyl 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 (0.48 g.) obtained by pouring the cooled reaction mixture into ice and water (400 cc.) was dissolved in ethanol at room temperature and water added to opalescence. A crystalline product separated on seeding; yield 0.32 g., m. p. 79–81°. Then the mother liquor was treated with water to opalescence, seeded and placed in the icebox; yield 0.15 g., m. p. 103–106°.

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[18] We are indebted to Dr. E. D. Walter of Purdue University for a sample of genistin (glycoside of genistein) and to Mr. E. E. Dickey of this Laboratory for the preparation of pseudobaptisin (glycoside of pseudobaptigenin) from the roots of *Baptisia tinctoria*.

The material melting at 79–81° was purified from ethanol–water; yield 0.26 g., m. p. 88.5–89°. This substance crystallized with one mole of water in glistening, elongated, colorless prisms which gave a dark green ferric chloride–alcohol test and were soluble in benzene, pyridine, acetic acid and methanol.

Anal. Calcd. for $C_{26}H_{35}O_5N \cdot H_2O$: C, 67.95; H, 8.12; N, 3.05; H_2O, 3.92. Found: C, 67.96; H, 7.87; N, 2.76; H_2O (loss in weight at 56° under reduced pressure), 3.97.

The material melting at 103–106° was obtained pure from ethanol–water; yield 0.11 g., m. p. 108.5–109.5°. It crystallized in colorless, cottony clusters of needles and gave a light green ferric chloride–alcohol test. Its solubilities were similar to those of the oxime melting at 88.5–89°. A mixture of the two oximes gave a depression in melting point to 79–81°.

Anal. Calcd. for $C_{26}H_{35}O_5N$: C, 70.72; H, 7.99; N, 3.17. Found: C, 70.77; H, 7.80; N, 3.51.

(Procedure B) Formic Acid from Tetrahydro-osajin Dimethyl Ether.—The aqueous solution from which the tetrahydro-osajetin dimethyl ether (obtained from 1.000 g. of tetrahydro-osajin dimethyl ether) was extracted as described in A above, was steam distilled, the volume of the solution being reduced rapidly to 15 cc. and maintained at that point; total volatile acidity (less 0.5 cc. blank), 22.4 cc. of 0.1 *N* sodium hydroxide; calcd. for one mole of formic acid, 22.8 cc. The neutralized distillate was concentrated to a low volume and its formic acid content was determined by oxidation with mercuric chloride[19]; wt. mercurous chloride, 1.052 g.; calcd., 1.079 g.

A qualitative test for formic acid was obtained in the above distillate by reduction of a portion with magnesium and hydrochloric acid followed by application of the formaldehyde milk test.[20] An intense violet color was obtained. The formaldehyde obtained on reduction was identified also by its crystalline dimedon (dimethyl dihydroresorcinol or 3,3-dimethyl-hexadione-1,5) condensation product[21] (m. p 190°, mixed m. p. unchanged).

(Procedure C) Homoanisic Acid from Tetrahydro-osajetin Dimethyl Ether.—Tetrahydro-osajetin dimethyl ether (1.70 g.) was added to a hot solution of potassium hydroxide (22 g., 85%) in absolute ethanol (60 cc.) and the resultant solution was refluxed for forty-eight hours with a stream of hydrogen continually passing through the solution. It was then cooled, diluted with water (100 cc.), made just acid with 6 *N* sulfuric acid and extracted with ether. The ethereal extract (1000 cc.) was concentrated to one-fourth volume and extracted with an aqueous 5% sodium bicarbonate solution (two 200-cc. portions) and then with an aqueous 5% sodium hydroxide solution. The oil (neutral fraction) obtained on solvent removal from the ethereal solution was subjected to steam distillation and the distillate was obtained in crystalline form, m. p. 65–70°. This material is under further investigation.

The above aqueous sodium bicarbonate extract (acid fraction) was made just acid with dilute sulfuric acid and extracted thoroughly with ether. The light brown oil obtained on solvent removal from the dried ethereal extract was treated with boiling water (300 cc.), filtered (decolorizing charcoal) from the residual oil and the filtrate concentrated to 45 cc. and allowed to crystallize at icebox temperature; yield 0.43 g. (calcd. for 1 mole of homoanisic acid, 0.58 g.), m. p. 80–83°. Pure material was obtained on several recrystallizations from water or benzene–petroleum ether; yield 0.15 g., m. p. 86°, unchanged on admixture with an authentic specimen of homoanisic acid (*p*-methoxy-phenylacetic acid) of m. p. 86°.

Anal. Calcd. for $C_7H_6(OCH_3)COOH$ (homoanisic acid): C, 65.2; H, 6.06; OCH_3, 18.65; neutral equivalent, 166.2. Found: C, 65.14; H, 6.10; OCH_3, 18.62; neutral equivalent, 166.7.

When tetrahydro-osajin dimethyl ether was subjected to the above treatment homoanisic acid (m. p. 86°, mixed m. p. unchanged) was obtained; on heating for shorter lengths of time a lower amount of homoanisic acid was formed together with some tetrahydro-osajetin dimethyl ether.

The homoanisic acid isolated above was characterized further by the preparation of its *p*-nitrobenzyl ester according to the general procedure of Shriner and Fuson.[22] An authentic sample of homoanisic acid (0.5 g.) was neutralized with 0.1 *N* sodium hydroxide solution, the total volume reduced to 5 cc., and a few crystals of homoanisic acid added to make the solution slightly acidic. A solution of *p*-nitrobenzyl bromide (0.72 g.) in ethanol (12 cc., 95%) was added and during a period of two hours of refluxing an oil gradually separated. Enough 95% ethanol was added to bring the oil into solution and crystals separated on cooling. Pure material was obtained on recrystallization from ethanol–water; yield 0.3 g., m. p. 73.5–74.5°, unchanged on further crystallization from benzene–petroleum ether.

The ester was soluble in the common organic solvents and crystallized in colorless plates.

Anal. Calcd. for $C_{16}H_{15}O_5N$: C, 63.78; H, 5.02; N, 4.65. Found: C, 63.98; H, 4.98; N, 4.98.

The *p*-nitrobenzyl ester of the homoanisic acid isolated from the alkaline degradation of tetrahydro-osajetin dimethyl ether was prepared as described above; m. p. 73.5–74.5°, mixed m. p. unchanged.

WITH P. W. MORGAN

Tetrahydro-osajetin Dimethyl Ether Monoacetate.—Tetrahydro-osajetin dimethyl ether (0.30 g.) was acetylated overnight at icebox temperature with pyridine (4 cc.) and acetic anhydride (8 cc.). The product obtained on pouring the reaction mixture into an excess of water was purified from ethanol–water; yield 0.25 g., m. p. 79–80°.

The substance crystallized as fine, colorless needles that gave no coloration with ferric chloride–alcohol and showed no reduction toward Tollens reagent (pyridine solution).

Anal. Calcd. for $C_{26}H_{34}O_5(CH_3CO)$: C, 71.78; H, 7.75; CH_3CO, 2.13 cc. 0.1 *N* NaOH per 100 mg. Found: C, 71.91; H, 7.71; CH_3CO, 2.03 cc.

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[19] Scott and Furman, "Standard Methods of Chemical Analysis," D. Van Nostrand Co., New York, N. Y., 5th ed., 1939, Vol. 2, p. 2249.

[20] Klein, "Handbuch der Pflanzenanalyse," J. Springer, Vienna, 1932, Vol. II, p. 376.

[21] D. Vorländer, *Z. anal. Chem.*, **77**, 241 (1929).

[22] Shriner and Fuson, "Identification of Organic Compounds," John Wiley and Sons, New York, N. Y., 2nd ed., 1940, p. 132.

Tetrahydro-osajetin dimethyl ether was recovered from the acetyl analysis (Freudenberg and Harder method[23]) and identified by melting point and mixed melting point.

2-Methyl-tetrahydro-osajin Dimethyl Ether.—Tetrahydro-osajetin dimethyl ether monoacetate (50 mg.) was refluxed for five hours with 2 g. of anhydrous sodium acetate and 15 cc. of acetic anhydride. The gummy solid obtained on pouring the cooled reaction mixture into ice and water was crystallized from ethanol–water and benzene–petroleum ether; yield 30 mg., m. p. 129–130°. If the substance was ground in a mortar before melting or if it was dipped carefully into a bath at 130° and allowed to solidify in the air, it then melted at 146°.

The substance crystallized in colorless plates and gave a negative ferric chloride–alcohol test and showed no reduction with Tollens reagent (pyridine solution). It gave only a dark yellow color with sodium amalgam and subsequent acidification. Wilson's boric acid test was negative.

Anal. Calcd. for $C_{25}H_{25}O_3(CH_3)(OCH_3)_2$: C, 74.64; H, 7.61; OCH_3, 13.8. Found: C, 74.87; H, 7.63; OCH_3, 13.9; CH_3CO, absent.

The above substance also was obtained on treatment of tetrahydro-osajetin dimethyl ether with acetic anhydride and sodium acetate as described above. Identification was made by melting point and mixed melting point.

When 2-methyl-tetrahydro-osajetin dimethyl ether (10 mg.) was refluxed with sodium hydroxide (1 g., 95%) in 10 cc. of 50% ethanol for fifteen minutes, followed by acidification with dilute sulfuric acid and extraction with petroleum ether, tetrahydro-osajetin dimethyl ether (identification by melting point and mixed melting point) was obtained; yield 8 mg.

(Procedure D) 2-Hydroxy-hexahydro-osajin Dimethyl Ether and its Conversion to Tetrahydro-osajin Dimethyl Ether.—A solution of tetrahydro-osajetin dimethyl ether (200 mg.) in 10 cc. of dry, freshly distilled ethyl formate was poured slowly, with cooling (ice-salt) and shaking, on sodium (0.25 g., thinly sliced) under a nitrogen atmosphere. The resultant mixture was kept at icebox temperature for twenty hours and then was poured into an excess of cold water. The insoluble ethyl formate was removed by evaporation with a current of air and the precipitated gum was crystallized from ethanol–water; yield 190 mg., m. p. 160–161° (dec.). Pure material was obtained on further crystallization from 80% ethanol; yield 150 mg., m. p. 173° (dec.), unchanged on recrystallization from benzene–petroleum ether.

The compound crystallized from benzene–petroleum ether in the form of colorless, silvery plates. It was easily soluble in benzene, moderately soluble in 95% ethanol and only slightly soluble in petroleum ether. It gave no coloration with ferric chloride–alcohol and reduced Tollens reagent in pyridine solution very slowly at 100°. This reducing power was much less than that of the tetrahydro-osajetin dimethyl ether. The Wilson boric acid test was negative. The substance showed no loss in weight when dried for three hours at 110° under reduced pressure.

Anal. Calcd. for $C_{25}H_{28}O_4(OCH_3)_2$: C, 71.33; H, 7.54; OCH_3, 13.66. Found: C, 71.49; H, 7.37; OCH_3, 13.53.

When the above product was refluxed for thirty minutes with glacial acetic acid and the solution diluted with water, a good yield of tetrahydro-osajin dimethyl ether was obtained; m. p. 121° (mixed m. p. unchanged).

With George F. Johnson

Alkaline Degradation of Osajin Dimethyl Ether to Formic Acid and Osajetin Dimethyl Ether.—Osajin dimethyl ether[16] (1.000 g.) was treated with alcoholic sodium hydroxide solution as described in procedure A above. The formic acid was determined in the steam distillate according to procedure B above; total volatile acidity 22.9 cc. of 0.1 *N* sodium hydroxide; calcd. for one mole of formic acid, 23.1 cc.; wt. mercurous chloride, 1.061 g.; calcd., 1.088 g.

The yellow oil resulting on solvent removal from the petroleum ether extract obtained from the procedure described in A above, was crystallized from ethanol–water; yield 0.85 g. (two crops), m. p. 62–64°. First nuclei of this substance were obtained with difficulty. Pure material was obtained from ethanol–water; yield 0.77 g., m. p. 65–65.5°, unchanged on further crystallization from glacial acetic acid.

Osajetin dimethyl ether crystallized as fine, lemon-yellow, elongated prisms that gave a brownish-black ferric chloride–alcohol test and rapidly reduced Tollens reagent (pyridine solution). The Wilson boric acid test was positive, although negative for the original dimethyl ether, which also gives no Tollens reduction (pyridine solution). Osajin monomethyl ether[1] gave a positive Wilson boric acid test. The substance gave no coloration with glacial acetic–sulfuric acid. It was soluble in acetone, benzene, pyridine and methanol. It reacted with hydroxylamine and semicarbazide with difficulty and no crystalline reaction product was obtained.

Anal. Calcd. for $C_{24}H_{24}O_3(OCH_3)_2$: C, 73.91; H, 7.16; OCH_3, 14.69. Found: C, 73.69; H, 6.99; OCH_3, 14.78.

Alkaline Degradation of Osajetin Dimethyl Ether to Homoanisic Acid.—Osajetin dimethyl ether (350 mg.) was treated with alcoholic potassium hydroxide as described in procedure C above and the homoanisic acid isolated in the same manner; yield 45 mg. (calcd. for one mole, 138 mg.); m. p. 84–84.5°. Pure material was obtained on further crystallization from hot water; yield 25 mg., m. p. 85° (mixed m. p. unchanged).

When osajin dimethyl ether (2.00 g.) was subjected to the degradation procedure described in C above, homoanisic acid was isolated; yield 0.175 g., m. p. 85–86° (mixed m. p. unchanged).

2-Hydroxy-2,3-dihydro-osajin Dimethyl Ether and its Conversion to Osajin Dimethyl Ether.—Osajetin dimethyl ether (500 mg.) was treated with sodium and ethyl formate as described under procedure D above. The reaction product was isolated in the same manner and was obtained crystalline from ethanol–water; yield 300 mg., m. p. 138–139°. Pure material was obtained from ethanol–water; yield 200 mg., m. p. 139–139.5°, unchanged on further crystallization from acetone–water

The substance crystallized as colorless clusters of small, pointed prisms. It gave no coloration with ferric chloride-alcohol and reduced Tollens reagent (pyridine solution) only slowly on heating. This reduction power was in contrast to the rapid reduction of Tollens reagent shown by

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[23] K. Freudenberg and M. Harder, *Ann.*, **433**, 230 (1923).

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

Anal. Calcd. for $C_{27}H_{30}O_6$: 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).

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[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-

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[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).