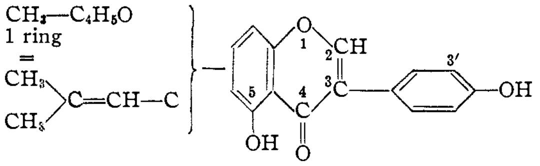
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE OHIO STATE UNIVERSITY]

Osage Orange Pigments. XI. Complete Structures of Osajin and Pomiferin[1]

BY M. L. WOLFROM, WALTER D. HARRIS, GEORGE F. JOHNSON, J. E. MAHAN, SAM M. MOFFETT AND BERNARD WILDI

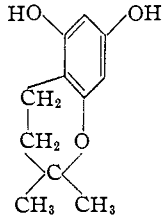
In previous publications in this series, the partial structures shown have been established for osajin and pomiferin, the yellow pigments of the fruit of the osage orange, *Maclura pomifera* Raf.



Osajin (pomiferin, 3' = OH)

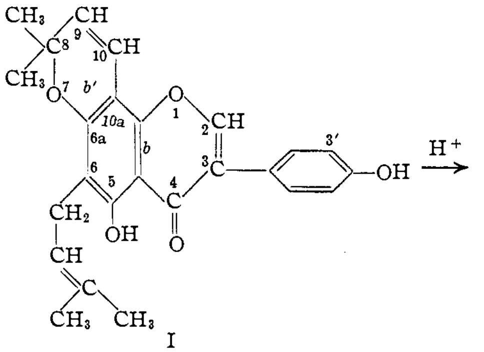
The compounds are optically inactive. It was shown[2] that both pigments possess the same carbon nucleus. The identity in the position of the second double bond was not demonstrated for the two substances. Both compounds were shown to isomerize in the presence of mineral acids to yield high-melting, colorless products, designated iso-osajin and isopomiferin, in which the phenolic group in position 5, *peri* to the carbonyl of the γ-pyrone ring, had added to one of the ethylenic bonds.

In the work herein reported, iso-osajin and isopomiferin were subjected to treatment ("fusion") with alcoholic alkali under vigorous conditions and 2,2-dimethyl-5,7-chromandiol was isolated from the reaction mixture. This crystalline substance was identical with an authentic synthetic specimen prepared from phloroglucinol according to the directions of Robertson and co-workers.[3] The diacetate of the isolated material was made and was likewise found identical with an authentic synthetic[3] sample. Robertson and collaborators[3,4] have recorded a sound proof of structure for 2,2-dimethyl-5,7-chromandiol.



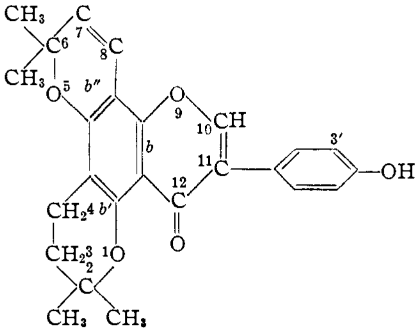
2,2-Dimethyl-5,7-chromandiol

The isolation of the above compound establishes the phloroglucinol nucleus in the central benzene ring of the isoflavone structure and thereby accounts for all of the oxygen in both pigments. The nature of the isomerization is also clearly established, including the six-membered size of the new ring formed in the isomerization reaction. The formulas of osajin and pomiferin (I) and their iso-compounds (II) may then be developed as shown. The nature of the C_5H_8 residue and its attachment at positions 6a and 10a of I will be demonstrated later. The resistance toward substitution of the phenolic group in position 5 is clarified by its formulation not only as *peri* to the carbonyl group of the γ-pyrone ring but also as di-orthosubstituted. The side chain in position 6 accounts for the formation of isovaleric acid on prolonged alkaline fusion of pomiferin trimethyl ether.[5]



I

Osajin (pomiferin, 3' = OH)



II

Iso-osajin (isopomiferin, 3' = OH)

In establishing the isoflavone nature of osajin[6] and pomiferin,[7] it was demonstrated that on mild alkaline fusion, the methyl ethers of each of these

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[1] Preceding publication in this series, M. L. Wolfrom and S. M. Moffett, THIS JOURNAL, **64**, 311 (1942). A brief preliminary notice of the work described herein appeared in THIS JOURNAL, **65**, 1434 (1943), and was read before the Division of Organic Chemistry at the 106th meeting of the American Chemical Society, Pittsburgh, Pennsylvania, September 6, 1943.

[2] Preceding communication, *cf.* note 1.

[3] W. Bridge, R. G. Heyes and Alexander Robertson, *J. Chem. Soc.*, 279 (1937).

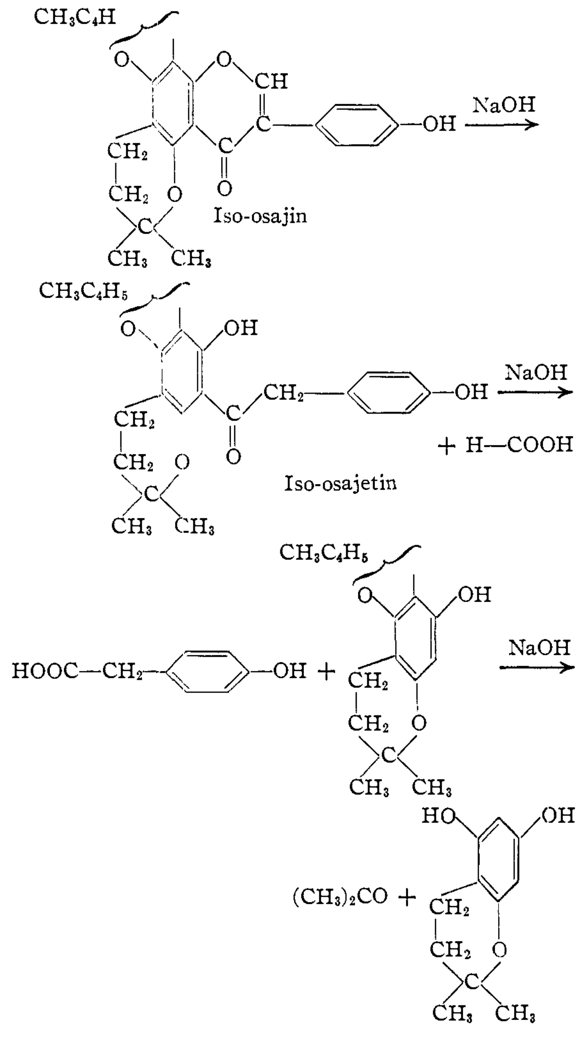
[4] W. Bridge, A. J. Crocker, T. Cubin and Alexander Robertson, *ibid.*, 1530 (1937).

[5] M. L. Wolfrom and J. Mahan, THIS JOURNAL, **64**, 308 (1942).

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

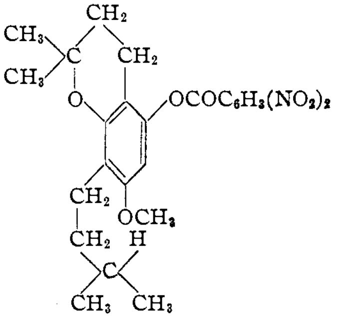
[7] M. L. Wolfrom and J. E. Mahan, *ibid.*, 1253 (1941).

pigments (and their tetrahydro derivatives) yielded formic acid and a substituted phenyl benzyl ketone. This mild alkaline fusion has now been applied to iso-osajin and the ketone, designated iso-osajetin, has been isolated in crystalline form. The concomitant formation of formic acid was demonstrated. The successive steps in the alkaline fusion of iso-osajin may then be delineated.



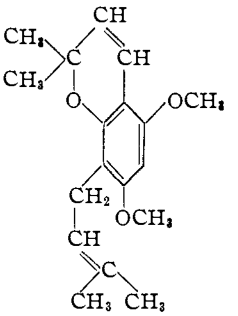
It is probable that the fusion of isopomiferin follows the same general course. Acetone was detected in the fusion mixtures from both substances and *p*-hydroxyphenylacetic acid was isolated in the case of iso-osajin. When the hydroxyls in the 3′ and 4′ positions were methylated, as in the previously described work,[6,7] the formation of homoveratric and homoanisic acids from the pomiferin and osajin non-isomerized structures, respectively, could readily be demonstrated.

In previous publications[6,7] from this Laboratory, a crystalline fraction of low melting point had been isolated from the alkaline fusion of both tetrahydro-osajin dimethyl ether and tetrahydropomiferin trimethyl ether. Further investigation of this fraction has now shown that the material is a complex mixture and is not always obtained in crystalline form. In one such experiment, employing tetrahydro-osajin dimethyl ether, a pure component of this mixture was isolated as its crystalline 3,5-dinitrobenzoate. An insufficient amount was obtained for further degradative work but from its analysis and from our present established knowledge of the structure of osajin, it is highly probable that it is tetrahydro-osajinol-1-(3′,5′-dinitrobenzoate) 3-methyl ether of the structure shown.



Tetrahydro-osajinol-1-(3′,5′-dinitrobenzoate)-3-methyl ether

In similar experiments employing pomiferin trimethyl ether, the crude mixture of fusion products was methylated and from this a substance was isolated in pure crystalline form which decolorized alkaline permanganate solution and readily absorbed hydrogen (platinum catalyst) This compound likewise was obtained in an amount insufficient for further degradative characterization, but from its analysis and from our present knowledge of the structure of pomiferin, it is highly probable that it is the unsaturated analog of the above-described dinitrobenzoate and may be designated osajinol dimethyl ether. Both of these fragments would appear to have the C_5H_8O ($C_5H_{10}O$ for the tetrahydro derivative) ring intact.

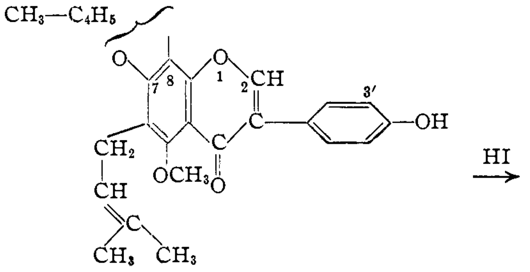


Osajinol dimethyl ether

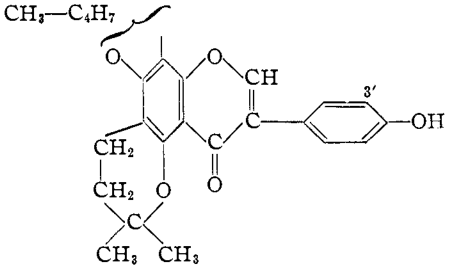
To complete the structural formulas of osajin and pomiferin, there remains to be elucidated only the nature of the C_5H_8 or isoprene (presumably) residue attached to the oxygen in position 7 of the isoflavone nucleus. Herein it may be stated:

that the formation of acetone in the alkaline fusion may be pertinent; and that some evidence of a negative character for the absence of a furan nucleus was obtained by the failure to isolate furan-carboxylic acids as oxidation (hydrogen peroxide and alkali) products of osajin on the application of a procedure employed by Späth and co-workers[8] in demonstrating the presence of the condensed furan ring system in furanocoumarones.

The phenolic groups of osajin and pomiferin have been completely acetylated[9,10] and methylated.[10,11] In the case of the acetates, the substituent groups have been removed with the regeneration of the parent substances[9,12] or of their iso compounds.[10,13] In order to remove the substituent groups of the fully methylated ethers of osajin and pomiferin, a mild treatment with hydriodic acid was employed. Osajin dimethyl ether yielded dihydro-iso-osajin under these conditions and pomiferin trimethyl ether yielded dihydro-isopomiferin. The same products were obtained when unmethylated osajin and pomiferin were subjected to identical treatment. In some of the experiments recorded, identification of the dihydro iso compounds was made through their acetates. Dihydro-iso-osajin monoacetate was found to exhibit dimorphism. It is thus demonstrated that hydriodic acid demethylates but at the same time isomerizes one olefinic linkage in these pigments and reduces the other olefinic linkage.



Osajin dimethyl ether
(pomiferin trimethyl ether, 3′ = OCH₃)



Dihydro-iso-osajin
(dihydro-isopomiferin, 3′ = OH)

The above reaction allows some deductions to be made regarding the nature of the C_5H_8O residue in osajin and pomiferin. An acyclic ether (C_5H_7) structure is eliminated since this should have been cleaved by the hydriodic acid with the formation of a phenolic group in position 7 of the isoflavone nucleus. Therefore the residual grouping is attached to the benzene ring at two points and these points must be positions 7 and 8 of the isoflavone nucleus. From the known degree of unsaturation[12] of these pigments, the C_5H_8O group must contain a ring bearing the ethylenic linkage which is the first reduced and is the one not involved in the isomerization.[13]

It is well established[14] that the reduction of a double bond conjugated to an aromatic system causes a shift in the ultraviolet absorption bands toward shorter wave lengths with a concomitant drop in the heights of the bands. The absorption spectra of styrene and ethylbenzene show this effect and represent its simplest illustration. If a double bond is not conjugated to the aromatic system, reduction of such a bond changes the absorption bands only slightly or not at all. The three ethylenic bonds of osajin and pomiferin can be hydrogenated in a step-wise fashion[12]; the one not involved in the isomerization (the dihydro derivatives can be isomerized) is first reduced, the one involved in the isomerization is next reduced, and the double bond in the 2,3-position is reduced only with considerable difficulty. The data of Table I[15] show that the reduction of the first double bond in osajin and pomiferin caused a shift in the ultraviolet absorption band toward

TABLE I

ABSORPTION CHARACTERISTICS OF OSAJIN AND POMIFERIN IN RELATION TO THEIR DEGREE OF UNSATURATION

Substance[a]	Molar concn. in abs. ethanol	Maximum: Wave length, Å.	Maximum: Extinction coefficient,[b] $\log_{10}(I_0/I)$
Osajin	2×10^{-5}	2750	1.07
Dihydro-osajin	2×10^{-5}	2720	0.75
Tetrahydro-osajin	2×10^{-5}	2710	0.70
Pomiferin	3×10^{-5}	2750	1.49[c]
Dihydropomiferin	3×10^{-5}	2680	1.06
Tetrahydropomiferin	3×10^{-5}	2680	1.02

[a] All exhibited fluorescence when irradiated by ultraviolet light, as did also iso-osajin and isopomiferin. This property was not exhibited by iso-osajetin monomethyl ether, isopomiferitin dimethyl ether or their dihydro derivatives at a molar concentration of 2×10^{-5}. [b] Cell length, 1 cm. [c] Calcd. from data for solution of 2×10^{-5} molar concentration.

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(8) E. Späth and K. Klager, *Ber.*, **66B**, 915 (1933); E. Späth and A. F. v. Christiani, *ibid.*, 1150 (1933).

(9) E. D. Walter, M. L. Wolfrom and W. W. Hess, THIS JOURNAL, **60**, 574 (1938).

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

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

(12) M. L. Wolfrom, P. W. Morgan and F. L. Benton, *ibid.*, **62**, 1484 (1940).

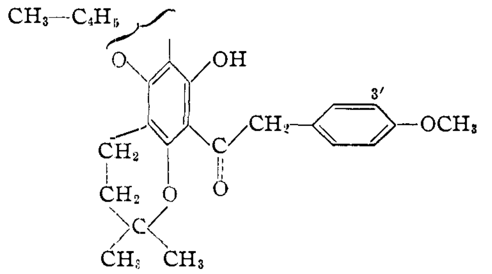
(13) M. L. Wolfrom, F. L. Benton, A. S. Gregory, W. W. Hess, J. E. Mahan and P. W. Morgan, *ibid.*, **63**, 422 (1941).

(14) (Mme.) Ramart-Lucas and (Mlle.) P. Amagat, *Bull. soc. chim.*, **51**, 965 (1932); M. Horio, *Mem. Coll. Eng. Kyoto Imp. Univ.*, **7**, 177 (1933) (reprint), *C. A.*, **27**, 4734 (1933); A. Smakula, *Angew. Chem.*, **47**, 657 (1934); J. D. Piper and W. R. Brode, THIS JOURNAL, **57**, 135 (1935).

(15) Unless otherwise noted, absorption measurements were made by Mr. B. Wildi.

the region of shorter wave lengths and lowered the height of the band. The reduction of the second bond, which is the one involved in the isomerization, produced no significant change in the ultraviolet absorption. This latter bond is therefore not conjugated to the benzene ring, in harmony with the formula previously presented.

The styrene–ethylbenzene effect is enhanced when the substituted benzyl phenyl ketones of the isomerized structures are subjected to examination, since in these derivatives there is much less general conjugation than in the original pigments. For this purpose, the ultraviolet absorption spectra of iso-osajetin monomethyl ether and iso-pomiferitin dimethyl ether were compared with those of their dihydro derivatives (Figs. 1 and 2).



Iso-osajetin monomethyl ether (isopomiferitin dimethyl ether, 3′ = OCH_3)

It is readily noted that the reduction of the double bond in the C_5H_8 residue of each of these substances caused a very pronounced shift of the bands toward the region of shorter wave lengths and also a very decided lowering in the intensity of the absorption.

It is therefore established that osajin and pomiferin are identical in all respects save for the position of an additional phenolic group in position 3′ of pomiferin and that the ethylenic linkage located in the C_5H_8 residue of both pigments is conjugate to position 8 of the isoflavone nucleus.

To complete the structural formulas of osajin and pomiferin, there remains the elaboration of the C_5H_8 residue attached at 7 (through oxygen) and 8 of the isoflavone nucleus. A number of facts pertinent to this problem are presently known and will be recapitulated. The substances are optically inactive.[9] This does not eliminate the remote possibility of naturally occurring racemic forms; D,L-galactose, for example, has been found in several natural products, as in agar.[16] Reduction experiments indicate the presence of only one olefinic linkage in the residue, which linkage is readily reduced, is not isomerized by acids and is conjugate to the phloroglucinol nucleus. The residue contains a $CH_3—C$ group. Acetone is formed on fusion of the iso compounds and no furan carboxylic acids are produced on oxidation with alkaline hydrogen peroxide. All of these facts are in harmony with the 2,2-dimethyl-chromene structure III. Robertson and co-workers[3,17,18] have shown that this ring yields acetone on alkaline fusion. It is known that the double bond is reducible to the chromane with great ease[3,18] and this reduction has been effected with hydriodic acid.[19]

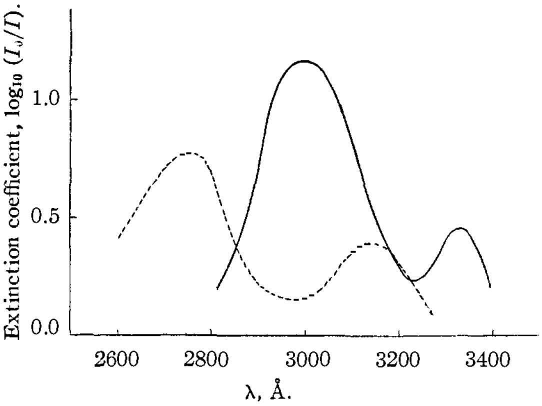


Fig. 1.—Absorption (2 × 10^{-5} molar) in absolute ethanol of iso-osajetin monomethyl ether (—) and dihydro-iso-osajetin monomethyl ether (- - -); cell length, 1 cm.; Bausch and Lomb medium quartz spectrometer.

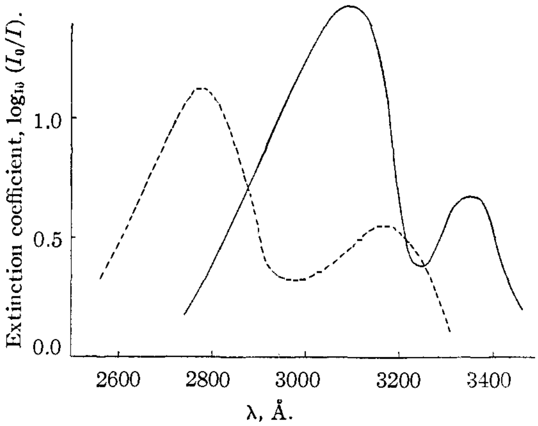
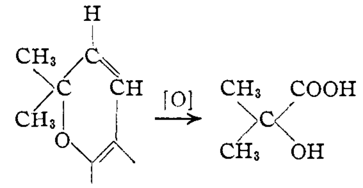


Fig. 2.—Absorption (3 × 10^{-5} molar) in absolute ethanol of isopomiferitin dimethyl ether (—) and dihydro-isopomiferitin dimethyl ether (- - -); cell length, 1 cm.; Bausch and Lomb medium quartz spectrometer.

Further experimental data have now been obtained which definitely establish this structure. α-Hydroxy-isobutyric acid has been formed on



III

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[16] N. W. Pirie, *Biochem. J.*, **30**, 369 (1936).

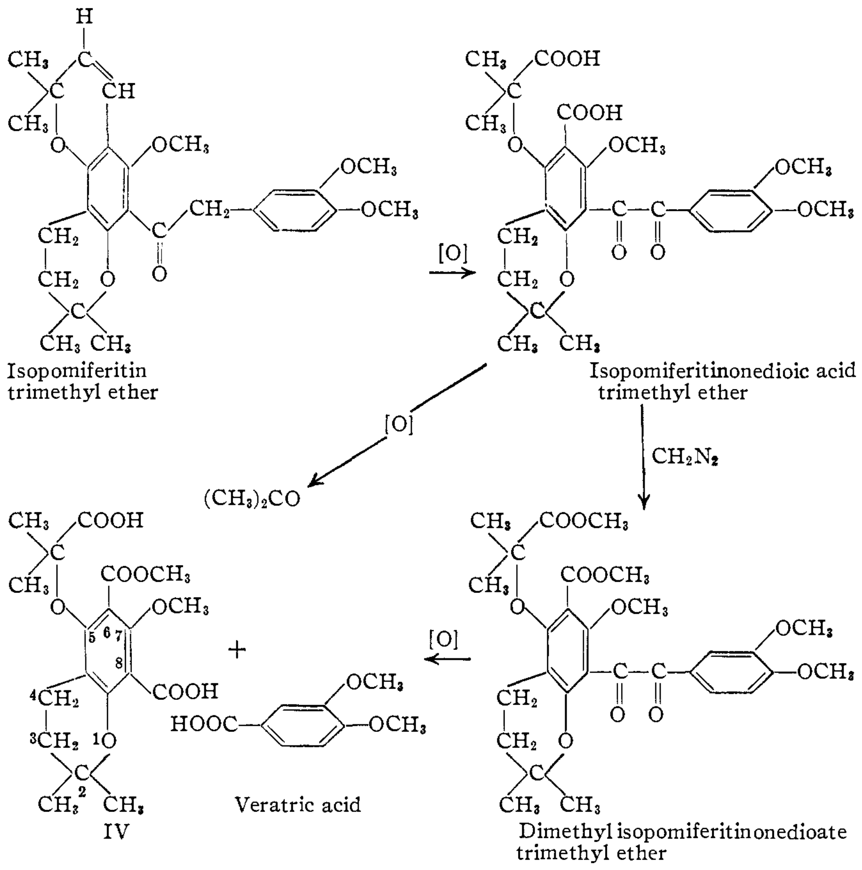
[17] R. G. Heyes and Alexander Robertson, *J. Chem. Soc.*, 681 (1935).

[18] Janet C. Bell, Alexander Robertson and T. S. Subramaniam, *ibid.*, 627 (1936); Janet C. Bell and Alexander Robertson, *ibid.*, 1828 (1936).

[19] H. M. Gordin, THIS JOURNAL, **28**, 1649 (1906).

oxidation of pomiferin with alkaline permanganate. The remainder of the unsubstituted molecule apparently undergoes complete oxidation. This type of behavior of the 2,2-dimethyl-chromene ring has been encountered previously in natural products, such as xanthoxyletin[3] and seselin.[20]

Isopomiferitin trimethyl ether was oxidized with potassium permanganate and an insoluble potassium salt separated from the reaction mixture. This salt was derived from a dibasic acid formed from the starting material by the addition of five atoms of oxygen without carbon loss. The free acid, designated isopomiferitinonedioic acid trimethyl ether, was a crystalline yellow substance that yielded acetone on oxidation with ammoniacal hydrogen peroxide. That it was a substituted benzil was proven by its transformation to the dimethyl ester with diazomethane and oxidation of this with hydrogen peroxide and potassium hydroxide. A typical benzil cleavage resulted with the production of veratric acid and a substance that by its elementary and methoxyl analysis was characterized as the expected product IV and may be designated 5-(1-carboxyisopropoxy)-3,4-dihydro-7-methoxy-2,2-dimethyl-6-methyl ester 1,2-benzopyran-6,8-dicarboxylic acid. It is to be noted that whereas the diortho-substituted aromatic acid on position 6 of IV was esterified by diazomethane it was not saponified under the alkaline oxidation conditions employed. This type of oxidation of the 2,2-dimethylchromene ring has been demonstrated by Clark for tephrosin[21] and isotephrosin[22] and was employed by Späth and co-workers in their work on seselin.[20]



The structures of osajin and pomiferin and of their iso-compounds are therefore established as shown (I and II). The systematic name for osajin (I) would be 5-hydroxy-3-(*p*-hydroxyphenyl)-8,8-dimethyl-6-(3-methyl-2-butenyl)-4,8-benzo[1,2-*b*;3,4-*b*′]dipyran-4-one (Ring Index No. 1989) and that for iso-osajin (II) would be 11-(*p*-hydroxyphenyl)-2,2,6,6-tetramethyl-2,6,12-benzo[1,2-*b*;3,4-*b*′;5,6-*b*″]tripyran-12-one (Ring Index No. 2761).

In confirmation of the final structure of osajin (and pomiferin), we note that osajin absorbed 9.7 equivalents of hydrogen when hydrogenated according to the procedure of Kuhn and Möller,[23] by which all points of unsaturation, including the aromatic nuclei, are reduced. The formula depicted requires six hydrogen equivalents for the two benzene nuclei, two for the γ-pyrone ring, one for the 2,2-dimethyl-chromene ring, and one for the 3-methyl-2-butenyl side-chain, or a total of ten equivalents, in agreement with experiment.

The substances are therefore isoflavones possessing the phloroglucinol structure in the central benzene ring and having two isoprene residues attached at positions 6 and 8 of the isoflavone nucleus. The one isoprene residue is present as a 2,2-dimethyl-chromene ring and the second as a 3-methyl-2-butenyl side-chain which can be isomerized to a 2,2-dimethyl-chromane system. The central benzene nucleus is rather unique among natural products in that it is of the hexa-substituted type.

Harper[24] has synthesized a number of furano-

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[20] E. Späth, P. K. Bose, J. Matzke and N. C. Guha, *Ber.*, **72B**, 21 (1939).

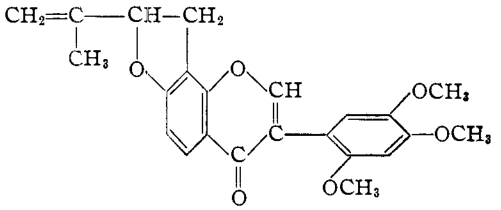
[21] E. P. Clark, This Journal, **53**, 729 (1931); **54**, 3000 (1932).

[22] E. P. Clark, *ibid.*, **55**, 759 (1933).

[23] R. Kuhn and E. F. Möller, *Angew. Chem.*, **47**, 145 (1934).

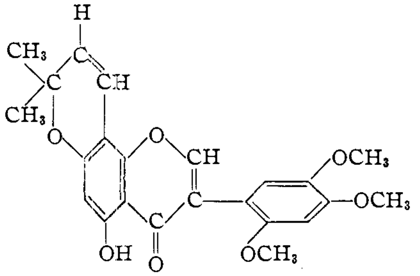
[24] S. H. Harper, *J. Chem. Soc.*, 595 (1942).

isoflavones from the substituted benzyl *o*-hydroxyphenyl ketones obtainable by the alkaline fusion of rotenone and related structures. Thus rotenone on reductive alkaline cleavage yields derritol and from derritol 2′-monomethyl ether there was obtained the optically active derritol isoflavone 2′-monomethyl ether on successive treatment with sodium and ethyl formate, and acetic acid.

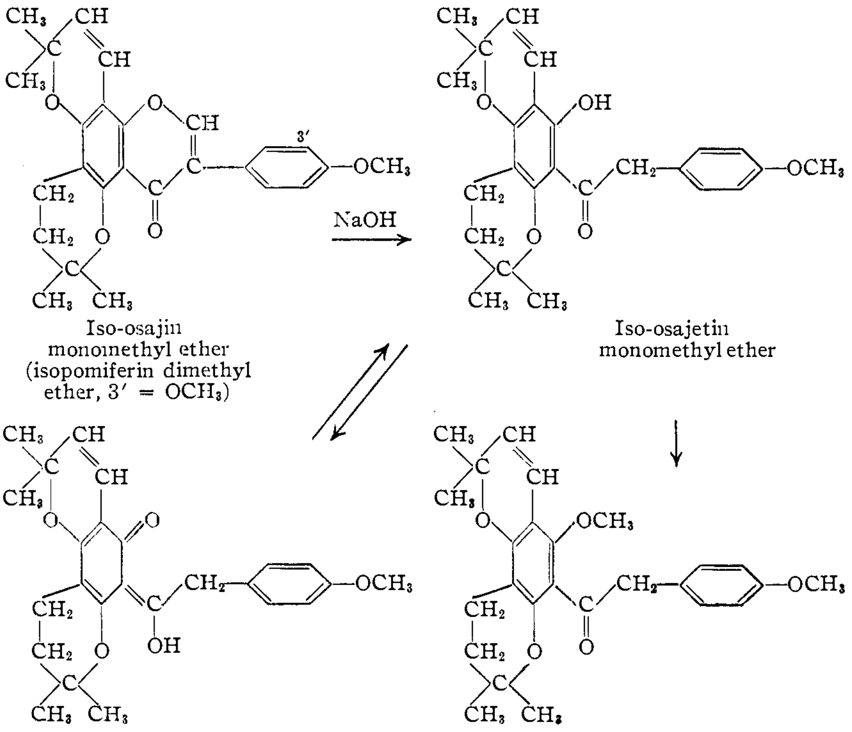


Derritol isoflavone 2′-monomethyl ether

Harper has isolated a substance from crude toxicarol fractions[25] which was proved to be an isoflavone[24] and for which the structure shown was postulated. If this formula is verified, the substance would be closely related to pomiferin.



Toxicarol isoflavone (possible structure)



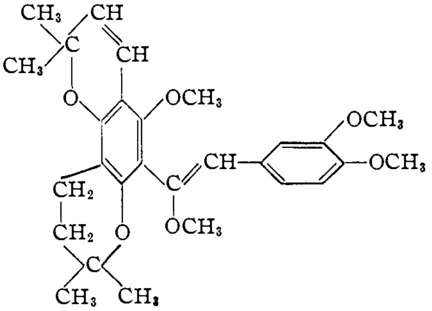
Iso-osajin monomethyl ether (isopomiferin dimethyl ether, 3′ = OCH_3)

Iso-osajetin monomethyl ether

Iso-osajetin dimethyl ether

Insecticidal assays on osajin and pomiferin and a number of their derivatives as well as on the crude meal made from the ground and dried fruit of the osage orange, showed no activity under conditions wherein rotenone is very active.

In the course of the above-described work, a number of derivatives of osajin and pomiferin were required. Improved preparative directions for iso-osajin and isopomiferin are recorded. The monomethyl ether of iso-osajin[13] was converted into iso-osajetin monomethyl ether by mild alkaline treatment. The yellow color of this derivative is explained by the formation of an orthoquinoid tautomer. Further methylation of the free phenolic group in iso-osajetin monomethyl ether is rendered difficult by the alkali sensitivity of the ketone and by the fact that this phenolic group is both diorthosubstituted and ortho to a carbonyl group. Iso-osajetin dimethyl ether, a colorless substance, was nevertheless prepared both by further methylation of its monomethyl ether and by the direct methylation of iso-osajetin.



Isopomiferitin enol tetramethyl ether

Similarly, isopomiferin dimethyl ether[13] yielded isopomiferitin dimethyl ether (lemon yellow) on mild alkaline treatment and this on further methylation gave isopomiferitin trimethyl ether (colorless). In one experiment, the alkaline methylation of isopomiferitin dimethyl ether produced a product (colorless) in low yield which by its analysis was considered to be isopomiferitin enol tetramethyl ether.

Methylation of dihydro-iso-osajin[13] with dimethyl sulfate and alkali yielded

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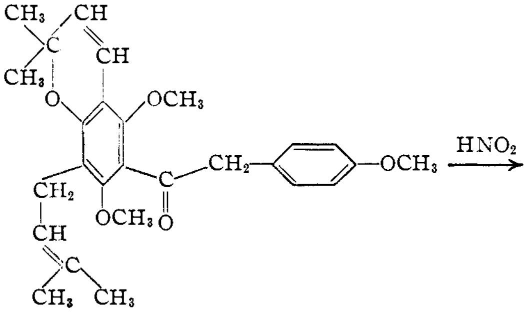
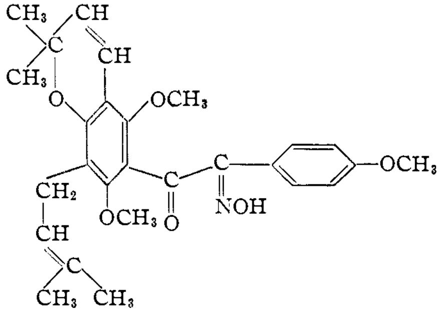
(25) S. H. Harper, *J. Chem. Soc.*, 1178 (1940).

its monomethyl ether (colorless) which on mild alkaline treatment produced dihydro-iso-osajetin monomethyl ether (lemon yellow). Similarly, methylation of dihydro-isopomiferin[13] with diazomethane yielded its dimethyl ether (colorless) which was converted to dihydro-isopomiferitin dimethyl ether (buff-colored) by mild alkaline "fusion." The latter was further methylated with dimethyl sulfate and alkali in the presence of acetone, to yield the colorless dihydroisopomiferitin trimethyl ether. These dihydro iso compounds of osajin and pomiferin differ from the normal iso compounds in that an olefinic linkage in the $CH_3—C_4H_5O$ residue has been reduced.

We record herein also the synthesis of osajetinone trimethyl ether monoxime by the nitration of osajetin trimethyl ether.[1] The corresponding pomiferitinone derivative was likewise prepared. Osajetinone trimethyl ether monoxime was isolated in two forms. These derivatives of osajin and pomiferin were very sensitive and were not amenable to further degradative reactions.

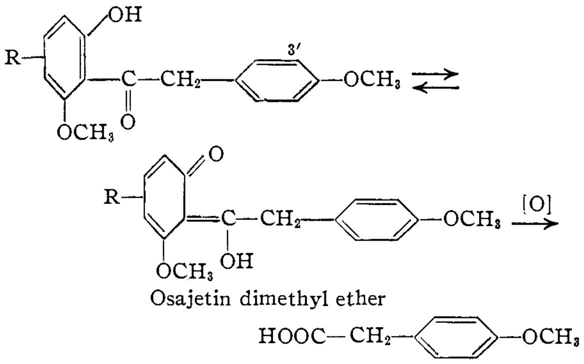


Osajetin trimethyl ether



Osajetinone trimethyl ether monoxime

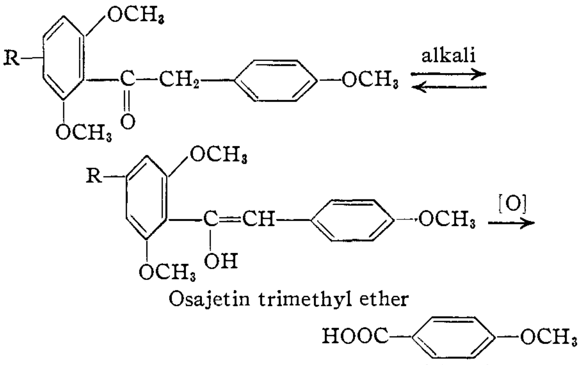
In a previous publication,[1] it was shown that osajetin dimethyl ether and pomiferitin trimethyl ether were cleaved by alkaline hydrogen peroxide to homoanisic and homoveratric acids, respectively, whereas under the same conditions, osajetin trimethyl ether and pomiferitin tetramethyl ether were cleaved to anisic and veratric acids, respectively. We wish to record herein what we consider to be a reasonable explanation of this behavior. Since the partially methylated derivatives are colored (yellow) while the completely methylated derivatives are colorless, it would seem that the phenolic group in the partially methylated derivatives must be part of a chromophoric system, depicted below for the osajetin derivative, as an orthoquinoid tautomer. Such an equilibrium would readily explain the formation of the substituted acetic acid on alkaline oxidation. The oxidation of pomiferitin trimethyl ether ($3' = OCH_3$) would be entirely analogous, with homoveratric acid being formed instead of homoanisic acid.



Osajetin dimethyl ether

Homoanisic acid

In the case of osajetin trimethyl ether, on the other hand, there is no free phenolic group ortho to the ketonic carbonyl and the above type of tautomerism could not exist. Instead, an enolization may be postulated involving one of the hydrogens of the methylene group adjacent to the carbonyl. Such a structure would readily explain the formation of the substituted benzoic acid on alkaline oxidation. The oxidation of pomiferitin trimethyl ether would be entirely analogous, with veratric acid being formed instead of anisic acid.



Osajetin trimethyl ether

Anisic acid

In a previous communication[5] from this Laboratory, a yield of 0.7 mole of acetone was obtained on the ozonization of osajin and pomiferin. The reasonable conclusion was thereupon drawn that each of these substances contained one isopropylidene group. It was later found of interest to extend these ozonization experiments to derivatives of osajin and pomiferin and the results are recorded in Table II.[26] These substances contain three olefinic linkages which may be hydrogenated in a stepwise fashion.[12] The third double

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(26) Measurements by J. E. Mahan and W. D. Harris.

TABLE II

ACETONE YIELDS ON OZONIZATION[a] OF OSAJIN AND POMIFERIN IN RELATION TO THEIR DEGREE OF UNSATURATION

Substance	Wt. subst., g.	Moles acetone per mole substance: 2,4-Dinitrophenylhydrazone method[b]	Moles acetone per mole substance: Volumetric method[c]
Pomiferin	2.0	0.69[d]	
Osajin	2.0	.68[d]	1.3
Iso-osajin	2.0		0.5
Iso-osajin monoacetate	2.3	.34	
Dihydro-osajin	2.0	.36	
Tetrahydro-osajin	2.0	.03	
Acetone control[e]	0.250	.65	

[a] *Cf.* ref. 5. [b] *Cf.* ref. 5; only pure hydrazone weighed. [c] Messinger method, *cf.* Scott–Furman, "Standard Methods of Chemical Analysis," 5th ed., D. Van Nostrand Co., Inc., New York, 1939, p. 2136. [d] Previously reported, ref. 5. [e] Ozonization omitted but hydrogen peroxide oxidation included.

bond is reduced with difficulty and is the one present in the isoflavone nucleus (positions 2,3). Thus tetrahydro-osajin yielded a negligible trace of acetone on ozonization. In dihydro-osajin, the olefinic linkage in the 9,10 positions is reduced whereas in iso-osajin monoacetate the olefinic linkage in the 3-methyl-2-butenyl side-chain has been saturated by isomerization. It is to be noted that both of these structures yield the same amount of acetone by the gravimetric procedure and this amount is very closely one-half of that produced from the original substances. On examining the gravimetric method used, it was found that an acetone control (subjected to hydrogen peroxide oxidation but not to ozonization) gave low results. A volumetric assay for the acetone formed from osajin was then employed and an amount of acetone in excess of one mole was found. By the same procedure iso-osajin was shown to yield an amount of acetone very closely equal to one-half of this amount.

It is therefore obvious that acetone was being formed from the 2,2-dimethyl-chromene ring system as well as from the isopropylidene group in the 3-methyl-2-butenyl side-chain. That the former ring system will yield acetone on ozonization has already been noted by Späth and coworkers.[20,27] Robertson and collaborators[4,18] have also demonstrated that the 2,2-dimethylchromene ring system behaves abnormally on ozonolysis. These results indicate again that caution must be exercised in drawing conclusions from ozonization experiments on easily oxidizable substances.

Absorption spectra of osajin and pomiferin, showing the main absorption band present in each, have been previously reported.[10] The maximum absorption shown by both osajin and pomiferin was a single strong band in the region 2700–2800 Å. The spectrum of pomiferin exhibited a minimum in the region 2400–2500 Å.

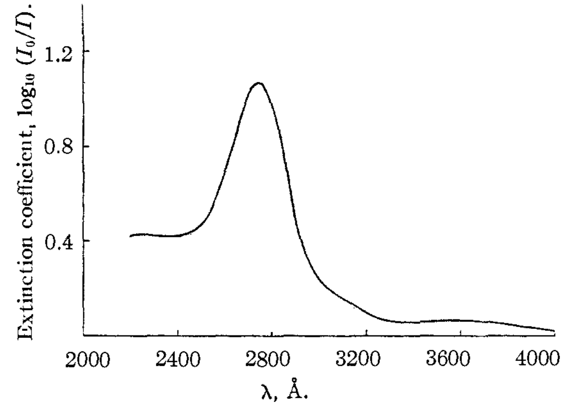

Fig. 3.—Absorption of osajin, 2 × 10^{-5} molar, in absolute ethanol; cell length 1 cm.; Beckman quartz spectrophotometer.

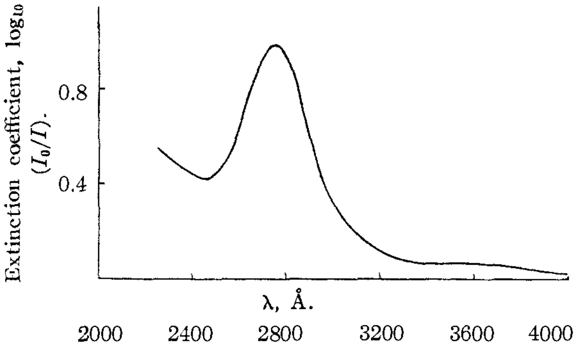

Fig. 4.—Absorption of pomiferin, 2 × 10^{-5} molar, in absolute ethanol; cell length, 1 cm.; Beckman quartz spectrophotometer.

The absorption spectra of these substances have now been extended to cover the entire region from 2200 to 4000 Å. (Figs. 3 and 4).[28] Small secondary bands were found for both pigments at *ca.* 3600 Å. Pomiferin exhibited a minimum at 2450 Å. but osajin did not. This divergence in spectra must be ascribed to the effect of the differing hydroxylation of the side chain benzene ring, since otherwise the two substances are identical. Reported[29] absorption spectra for known isoflavones (irigenin, tectorigenin and genistein) exhibit a minimum in the region 2300–2400 Å., a pronounced maximum at 2600–2700 Å. and a shelf in the region 3200–3300 Å.

Incidental to the absorption spectra measurements, it was observed that osajin and pomiferin, as well as their iso, dihydro and tetrahydro derivatives, exhibited fluorescence when irradiated with ultraviolet light in ethanol solution. This property was not shown by iso-osajetin monomethyl ether, isopomiferitin dimethyl ether or their dihydro derivatives.

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(27) E. Späth and K. Eiter, *Ber.*, **74B**, 1851 (1941).

(28) We are indebted to Mr. E. E. Pickett of this Laboratory for these measurements on osajin and pomiferin.

(29) Y. Asahina, B. Shibata and Z. Ogawa, *J. Pharm. Soc. Japan*, **48**, 150 (1928); E. D. Walter (and J. W. White, Jr.), THIS JOURNAL, **63**, 3273 (1941).

We report herein some tests made on the dyeing properties of osajin and pomiferin (Table III).[30] It is seen that these substances are acid dyes that require mordants. They have no effect upon cotton but dye wool and silk. Silk is not dyed as readily as wool, with pomiferin being the best on silk. Pomiferin tends to give a shade darker color than osajin. The employment of tin and chromium as mordants gives especially bright colors.

TABLE III

OSAJIN AND POMIFERIN AS MORDANT DYES[b]

Substance	Mordant	Color		
		Wool	Silk	Cotton
Osajin	Al	Yellow	Light yellow	..[a]
Pomiferin	Al	Yellow	Light yellow	...
Osajin	Cr[c]	Dark brown	Dark yellow	...
Osajin	Fe	Gray-brown	....	...
Pomiferin	Fe	Dark gray	Light gray	...
Osajin	Sn[c]	Chocolate brown	....	...
Pomiferin	Sn[c]	Dark brown	Yellow	...
Osajin	None	....	....	...
Pomiferin	None	....	....	...

[a] ..., indicates that cloth was unaffected. [b] The test cloth was heated at 100° (85° in the case of tin) in the mordant solution, removed and washed well with water. It was then rinsed with alcohol and added immediately to a methanolic solution of osajin and pomiferin (3 g. per 100 cc. soln.) and boiled therein for one hour. The cloth was then removed, washed successively with alcohol and water, and dried. [c] Brightest colors.

Experiments leading toward the possible verification of the structures of osajin and pomiferin by synthesis have been initiated in this Laboratory.

Experimental[31]

WITH SAM M. MOFFETT

Dimorphic Forms of Dihydro-iso-osajin Monoacetate.—On repetition of the preparation of dihydro-iso-osajin monoacetate,[13] the previously described product (m. p. 234°) was obtained in a slightly higher (235–236°) melting point. This material crystallized from methanol or ethanol in the form of small, white, cottony, elongated prisms. At other times large, colorless, elongated prisms of melting point 222.5–223° formed. On grinding the low-melting form to a powder, the melting point was raised to that (235–236°) of the high-melting form.

Dihydro-iso-osajin from the Hydriodic Acid Demethylation of Osajin Dimethyl Ether.[32]—Osajin dimethyl ether[11] (2 g.) was dissolved in warm glacial acetic acid (30 cc.) and acetic anhydride (10 cc.) and heated on a boiling water-bath. To the hot solution was added through a reflux condenser 24 cc. of 47% hydriodic acid containing approximately 1.5% hypophosphorous acid as a preservative. The mixture became dark brown almost immediately due to the liberation of iodine. After heating at 100° for six hours, the mixture was poured into 500 cc. of ice and water. The acid was neutralized by the addition of solid sodium bicarbonate and the iodine was removed by the addition of a saturated aqueous solution of sodium bisulfite. The crude precipitate (1.9 g.) was recrystallized several times from 95% ethanol, converted to the acetate with pyridine and acetic anhydride and identified as dihydro-iso-osajin monoacetate (see above); yield 1.0 g., m. p. 235–236° (mixed m. p. unchanged). When this product was dissolved in hot methanol, boiled to remove nuclei and nucleated with the low-melting form, this dimorph (m. p. 222–223°) was obtained. On grinding to a powder, the melting point was raised to 235–236°.

Dihydro-iso-osajin by Treatment of Osajin with Hydriodic Acid.—Osajin[5,9] (2.00 g.) was treated with hydriodic acid as described above for osajin dimethyl ether. The crude product was crystallized from 95% ethanol; 1.67 g., m. p. 276–278°. Pure material was obtained on further crystallization from acetone–water and ethanol and was identified as dihydro-iso-osajin; yield 0.31 g., m. p. 287–288° (dec.) unchanged on admixture with an authentic sample, for which the melting point of 287°[13] (dec.) has been recorded.

Dihydro-isopomiferin by Treatment of Pomiferin or Pomiferin Trimethyl Ether with Hydriodic Acid.—Pomiferin trimethyl ether[10] (500 mg.) was treated with hydriodic acid as described above for osajin dimethyl ether and the product, after acetylation, was identified as dihydro-isopomiferin diacetate; yield 146 mg., m. p. 221–222° unchanged on admixture with an authentic sample of the same melting point. The melting point of 218° had been previously recorded[13] for this substance.

Pomiferin[5,10] (2.00 g.) was treated in the same manner and there was isolated 1.53 g. of material melting at 255–256° (dec.) which on further purification yielded 0.65 g. of dihydro-isopomiferin of melting point 264.5–265° (dec.), unchanged on admixture with an authentic sample of this substance. A melting point of 258–259° (dec.) had been recorded[13] previously for this substance. Further identification was made by acetylation of the crude material from another experiment to form dihydro-isopomiferin diacetate; yield 1.3 g. from 2.0 g. of pomiferin, m. p. 221.5–222° (mixed m. p. unchanged).

Osajetinone Trimethyl Ether Monoxime.—Isoamyl nitrite (12 cc.) was added to a solution of 2.25 g. of sodium in 45 cc. of methanol. To this was added a solution of osajetin trimethyl ether[1] (3.0 g.) in 45 cc. of warm methanol. The mixture was allowed to stand at room temperature for twenty minutes and was then kept at icebox temperature for twelve hours, whereupon the solution was poured into ice and water (750 cc.) and made slightly acid with glacial acetic acid. The resultant oily suspension was removed by ether extraction and the residue obtained on solvent removal was crystallized from methanol; yield 1.1 g., pale yellow prisms, m. p. 149°, unchanged on further crystallization from methanol or ethanol–water.

Anal. Calcd. for $C_{27}H_{31}O_6N$: C, 69.66; H, 6.71; N, 3.01. Found: C, 69.72; H, 6.87; N, 2.91.

A lower-melting form (m. p. 134–135°; pale yellow) of this oxime was obtained from the mother liquors of the above preparation. It sometimes crystallized from a methanol solution of the purified higher-melting form. On grinding the lower-melting form in a mortar, it changed to the higher-melting form.

Pomiferitinone Tetramethyl Ether Monoxime.—Pomiferitin tetramethyl ether[1] (1.000 g.) was oximated with isoamyl nitrite and sodium as described above for the synthesis of osajetinone trimethyl ether monoxime and the product was isolated and crystallized in the same manner; yield 415 mg., m. p. 131–132°. Pure material was obtained in the form of clusters of pale yellow elongated prisms on recrystallization from methanol–water, methanol or benzene–petroleum ether; yield 168 mg., m. p. 133–133.5°.

Anal. Calcd. for $C_{28}H_{33}O_7N$: C, 67.86; H, 6.71; N, 2.83. Found: C, 67.86; H, 7.00; N, 2.87.

WITH WALTER D. HARRIS

Improved Preparation of Iso-osajin and Isopomiferin.—The crude material obtained on pouring the isomerization[10]

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(30) Experimental work by Mr. B. Wildi.

(31) All melting points are uncorrected and those below 210° were taken on a 360° thermometer with immersion to the −20° mark. Those above 210° were taken on a modified Berl–Kullmann block as described by F. W. Bergstrom, *Ind. Eng. Chem., Anal. Ed.*, **9**, 340 (1937).

(32) Preliminary experiments on the action of hydriodic acid on osajin and pomiferin were performed in this Laboratory by J. E. Mahan and Lee Randles.

reaction mixtures into water is difficult to bring to maximum melting point. It has been found that this purification is promoted by triturating the crude product with acetone, in which the material is not very soluble. In this manner, 42.5 g. of osajin yielded 41 g. of iso-osajin of m. p. *ca.* 220° (dec.) and 50 g. of pomiferin yielded 41 g. of isopomiferin of m. p. *ca.* 220° (dec.). For further preparative purposes, this material is generally of sufficient purity. Pure material (iso-osajin of m. p. 285° (dec.) and isopomiferin of m. p. 265° (dec.)) may be obtained on further crystallization from hot acetone.

Isopomiferitin Dimethyl Ether.—Isopomiferin dimethyl ether[13] (9.5 g.) was dissolved in 250 cc. of absolute ethanol. To this solution was added slowly, at reflux temperature, a hot alcoholic solution of sodium hydroxide (100 cc. of water, 110 cc. of 95% ethanol and 30 g. of sodium hydroxide). Heating was continued for nine minutes and the solution was immediately poured, in a thin stream and with rapid stirring, into 3 liters of ice and water. The mixture was made just acid with dilute sulfuric acid, allowed to stand for several hours and the light yellow solid removed by filtration. The crude and undried product was extracted successively with 150-cc. and 75-cc. portions of boiling ethanol. Bright lemon yellow rhombohedral prisms separated on cooling the combined alcoholic extracts and further quantities of material could be obtained by adding water to incipient turbidity; yield 8.1 g., m. p. 123–126°. Pure material was obtained on further crystallization from ethanol; yield 7.4 g. (80%), m. p. 126–126.5°, unchanged on further crystallization from benzene–petroleum ether. The substance gave a greenish-black coloration with a dilute alcoholic solution of ferric chloride. It was soluble in ethanol, acetone and benzene; slightly so in petroleum ether; and was practically insoluble in water and in 5% aqueous alkali.

Anal. Calcd. for $C_{24}H_{24}O_4(OCH_3)_2$: C, 71.21; H, 6.90; OCH_3, 14.16. Found: C, 71.41; H, 6.83; OCH_3, 14.02.

Dihydro-isopomiferin Dimethyl Ether.—Dihydro-isopomiferin (9.5 g.) was dissolved in 400 cc. of absolute ethanol and treated with a like volume of an ethereal solution of diazomethane (8 mg. per cc.). After standing overnight at *ca.* −20°, the solution was concentrated (hood) to one-fourth volume and crystallization was initiated; yield 9.0 g., m. p. 206–207°. Recrystallization from absolute ethanol (decolorizing charcoal) or benzene–petroleum ether raised the melting point slightly to 208–209°. The substance crystallized in colorless, slightly elongated prisms and gave no coloration with dilute alcoholic ferric chloride. It was soluble in benzene, ethanol, ether and acetone; slightly soluble in petroleum ether; and was insoluble in water and 5% aqueous alkali.

Anal. Calcd. for $C_{25}H_{24}O_4(OCH_3)_2$: C, 71.97; H, 6.71; OCH_3, 13.78. Found: C, 71.84; H, 6.50; OCH_3, 13.27.

Dihydro-isopomiferitin Dimethyl Ether.—Dihydro-isopomiferin dimethyl ether (2.0 g.) was treated with dilute alcoholic sodium hydroxide as described above for the synthesis of isopomiferitin dimethyl ether and the product was isolated in the same manner; yield 1.75 g., m. p. 114–115°. Pure material was obtained on crystallization from ethanol or benzene–petroleum ether; m. p. 116–116.5°. The substance crystallized in light buff-colored plates and gave a yellowish-brown coloration with dilute alcoholic ferric chloride. Its solubilities were similar to those of isopomiferitin dimethyl ether.

Anal. Calcd. for $C_{24}H_{28}O_4(OCH_3)_2$: C, 70.88; H, 7.32; OCH_3, 14.09. Found: C, 71.12; H, 7.17; OCH_3, 14.4.

Isopomiferitin Trimethyl Ether.—Isopomiferitin dimethyl ether (2 g.) was dissolved in 70 cc. of acetone in a 500-cc. three-necked flask equipped with two dropping funnels and a mercury-sealed stirrer. To the solution, heated to boiling on a water-bath, was added with rapid stirring, 3 cc. of dimethyl sulfate, and then 10 cc. each of dimethyl sulfate and of 42.5% aqueous potassium hydroxide solution were added dropwise at an equal rate during a period of about ten minutes. The nearly colorless solution was poured immediately, with stirring, into 1.5 liters of ice and water. The separated oil crystallized gradually to a nearly colorless solid and was immediately recrystallized from methanol (additional material was recoverable from the methanol mother liquor on the addition of water to incipient turbidity); yield 1.50 g., m. p. 92–93°. Pure material was obtained on recrystallization from ethanol; yield 1.25 g. (60%), m. p. 93–94°, unchanged on recrystallization from high-boiling petroleum ether. The substance crystallized in white rhombohedral prisms and gave no coloration with dilute alcoholic ferric chloride. It was very soluble in acetone, ether, ethanol and benzene; soluble in methanol and high-boiling petroleum ether; and was insoluble in water.

Anal. Calcd. for $C_{24}H_{23}O_3(OCH_3)_3$: C, 71.66; H, 7.13; OCH_3, 20.57. Found: C, 71.52; H, 6.79; OCH_3, 20.80.

Isopomiferitin Enol Tetramethyl Ether.—During one methylation of isopomiferitin dimethyl ether (10.00 g.), a very low yield of isopomiferitin trimethyl ether was obtained and from the methanol mother liquors there was isolated a new product; yield 0.45 g., m. p. 114–118°. Pure material was obtained on further crystallization from absolute ethanol; m. p. 120–121°, unchanged on further crystallization from benzene–petroleum ether; radial clusters of colorless, elongated prisms; ferric chloride–ethanol test negative; soluble in the common solvents (including ligroin) except water and 5% aqueous alkali.

Anal. Calcd. for $C_{24}H_{22}O_2(OCH_3)_4$: C, 72.08; H, 7.36; OCH_3, 26.60. Found: C, 72.10; H, 7.13; OCH_3, 26.88.

The only reasonable structure assignable to a substance with such a methoxyl content is that of the methylated enol of isopomiferitin trimethyl ether and the calculated analytical values shown above are based upon such a formula. The small amount of material obtained did not allow of further study.

Dihydro-isopomiferitin Trimethyl Ether.—Dihydro-isopomiferitin dimethyl ether (4.2 g.) was methylated as described above for the synthesis of isopomiferitin trimethyl ether. The crude product was isolated in the same manner and was crystallized from methanol–water; yield 3.6 g. (two crops), m. p. 100–103°. Pure material was obtained on further crystallization from methanol; m. p. 103.5–104°, unchanged on further crystallization from ligroin; colorless, elongated prisms; ferric chloride–ethanol test negative; soluble in the common solvents except water and 5% aqueous alkali.

Anal. Calcd. for $C_{24}H_{25}O_4(OCH_3)_3$: C, 71.34; H, 7.54; OCH_3, 20.48. Found: C, 71.35; H, 7.49; OCH_3, 20.47.

WITH BERNARD WILDI

Iso-osajetin Monomethyl Ether.—Iso-osajin monomethyl ether[13] (33 g.) was treated with dilute alcoholic sodium hydroxide as described above for the synthesis of isopomiferitin dimethyl ether except that 750 cc. of 85% ethanol was employed to dissolve the starting material and 330 cc. of the dilute alcoholic sodium hydroxide was used. The product was isolated in the same manner; yield 26.5 g. (80%), m. p. 111–112.5°. Recrystallization was effected from ethanol and from benzene; m. p. 111.5–112.5°. The substance formed yellow crystals and gave a green coloration with dilute alcoholic ferric chloride.

Anal. Calcd. for $C_{24}H_{25}O_4(OCH_3)$: C, 73.50; H, 6.91; OCH_3, 7.59. Found: C, 73.64; H, 6.97; OCH_3, 7.20.

Dihydro-iso-osajin Monomethyl Ether.—A solution of dihydro-iso-osajin (2.4 g.) in acetone (130 cc.) was heated under reflux and to the boiling solution was added 20 cc. of dimethyl sulfate, followed by 36 cc. of dimethyl sulfate and 48 cc. of 50% potassium hydroxide added dropwise and at an equal rate. Refluxing was maintained for thirty minutes following reagent addition and the cooled mixture was then poured into an excess of ice and water. The excess of dimethyl sulfate was neutralized with 50% potassium hydroxide. The colorless, curdy precipitate was removed by filtration and crystallized from 95% ethanol (decolorizing charcoal); yield 1.9 g., m. p. 198–199°, unchanged on further crystallization from ethanol or xylene.

The substance crystallized in colorless, fine needles and gave no coloration with ferric chloride (alcohol).

Anal. Calcd. for $C_{25}H_{25}O_4(OCH_3)$: C, 74.26; H, 6.71; OCH_3, 7.38. Found: C, 74.36; H, 6.78; OCH_3, 7.19.

Dihydro-iso-osajetin Monomethyl Ether.—A solution of dihydro-iso-osajin monomethyl ether (0.4 g.) in 10 cc. of 95% ethanol was added to a boiling solution containing 18 cc. of 20% sodium hydroxide and 12 cc. of 95% ethanol. Boiling was continued for about eighteen minutes whereupon the solution was cooled rapidly, acidified with phosphoric acid and extracted with ether. The residue obtained on solvent removal from the dried ethereal extract was crystallized from 95% ethanol; yield 0.2 g., m. p. 115–116°, unchanged on further crystallization from ethanol or xylene. The substance formed lemon-yellow crystals and gave a yellowish-brown coloration with ferric chloride (alcohol).

Anal. Calcd. for $C_{24}H_{27}O_4(OCH_3)$: C, 73.14; H, 7.36; OCH_3, 7.80. Found: C, 73.24; H, 7.39; OCH_3, 7.48.

WITH J. E. MAHAN

Iso-osajetin.—To a solution of iso-osajin (1.00 g.) in hot absolute ethanol (65 cc.) was added a solution of sodium hydroxide (11 g., 95%) in 50% ethanol (60 cc.) and the resultant mixture was boiled for twelve minutes. The cooled, yellow solution was acidified with dilute sulfuric acid and extracted with ether (200 cc.). Formic acid was detected in the aqueous layer by reduction to formaldehyde and application of the milk test.[33] The yellow oil obtained on solvent removal from the ethereal extract was crystallized from ethanol–water; yield 0.76 g., m. p. 145–146°. Pure material was obtained on further crystallization from ethanol–water; yield 0.69 g., yellow plates, m. p. 147–148°, unchanged on further crystallization from ethanol or benzene–petroleum ether.

The compound when dissolved in alcohol produced a dark green coloration with ferric chloride, and in glacial acetic acid no intensification of color was noted on the addition of one drop of concentrated sulfuric acid. The Wilson boric acid test [34,6] was positive. Iso-osajetin was practically insoluble in water and petroleum ether but was soluble in other common organic solvents.

Anal. Calcd. for $C_{24}H_{26}O_5$: C, 73.07; H, 6.65. Found: C, 72.87; H, 6.53.

WITH GEORGE F. JOHNSON

2,2-Dimethyl-5,7-chromandiol from Isopomiferin.—Isopomiferin (5 g.) was added to a hot solution of potassium hydroxide (62.5 g., 85%) in absolute ethanol (196 cc.) and the resultant solution was refluxed for twelve hours with a stream of nitrogen continually passing through the solution. The nitrogen was made essentially free of oxygen by the procedure of Fieser.[35] The reaction mixture was then cooled, diluted with water (100 cc.), made just acid with dilute sulfuric acid and extracted with ether. The ethereal extract (1000 cc.) was extracted with aqueous 5% sodium bicarbonate solution (four 200-cc. portions) to remove acidic constituents. The dark brown oil obtained on solvent removal from the dried ethereal solution was dissolved in benzene, filtered (decolorizing charcoal) and allowed to crystallize at icebox temperature; yield 253 mg., m. p. 155–157°. Pure material was obtained on crystallization from benzene; yield 153 mg., m. p. 160–160.5° (161.5–162°, cor.), unchanged on admixture with an authentic specimen of 2,2-dimethyl-5,7-chromandiol of like melting point, prepared according to the procedure of Bridge, Heyes and Robertson.[3] These authors recorded 162–163° for the melting point of this substance.

Anal. Calcd. for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.94; H, 7.09.

The above 2,2-dimethyl-5,7-chromandiol (69 mg.) was further identified by the preparation of its diacetate according to the procedure of Bridge, Heyes and Robertson.[3] The crude product was crystallized from ethanol–water; yield 62 mg., m. p. 84–85°, unchanged on admixture with an authentic specimen of 2,2-dimethyl-5,7-chromandiol diacetate (m. p. 85–85.5°). Bridge, Heyes and Robertson[3] recorded 86° for the melting point of this substance.

Anal. Calcd. for $C_{15}H_{18}O_5$: C, 64.73; H, 6.52. Found: C, 64.82; H, 6.28.

Acetone from Isopomiferin by Alkaline Degradation.—In the above alkaline degradation of isopomiferin (5 g.) with alcoholic potassium hydroxide, the vapors from the reaction mixture, swept through the reflux condenser by the nitrogen stream, were passed through two traps each containing 150 cc. of 2,4-dinitrophenylhydrazine reagent (4 g. of the hydrazine hydrochloride dissolved in 30 cc. of concentrated sulfuric acid and diluted with 400 cc. of methanol and 100 cc. of water). The combined contents of the traps was diluted with water to initiate crystallization and cooled at icebox temperature; yield 260 mg., m. p. 118–120°. Pure material was obtained on repeated crystallization from ethanol; m. p. 124–125°, unchanged on admixture with an authentic sample of acetone 2,4-dinitrophenylhydrazone of like melting point.

2,2-Dimethyl-5,7-chromandiol from Iso-osajin.—Iso-osajin (4 g.) was treated with alcoholic potassium hydroxide as described above for isopomiferin and 2,2-dimethyl-5,7-chromandiol was isolated from the reaction mixture in the same manner; yield 85 mg., m. p. 158–160.5°. Pure material was obtained on crystallization from benzene; yield 55 mg., m. p. 160–160.5° (161.5–162°, cor.), unchanged on admixture with an authentic specimen of 2,2-dimethyl-5,7-chromandiol; diacetate (prepared as described above), m. p. 83.5–84°, unchanged on admixture with an authentic specimen of 2,2-dimethyl-5,7-chromandiol diacetate (m. p. 85–85.5°).

Acetone from Iso-osajin by Alkaline Degradation.—Acetone was identified as its 2,4-dinitrophenylhydrazone in the above alkaline degradation of iso-osajin (4 g.) according to the procedure described for isopomiferin; yield 540 mg., m. p. 109–111°. Pure material was obtained on repeated crystallization from ethanol; yield 120 mg., m. p. 124–124.5°, unchanged on admixture with an authentic sample of acetone 2,4-dinitrophenylhydrazone of m. p. 124–125°.

***p*-Hydroxyphenylacetic Acid from Iso-osajin.**—The aqueous sodium bicarbonate washings from the processing of the alkaline fusion of iso-osajin (4 g.) were made just acid with dilute sulfuric acid and extracted with ether. The brown oil obtained after solvent removal from the dried ethereal extract was treated with hot water (50 cc.), filtered (decolorizing charcoal), concentrated to 10 cc. and allowed to crystallize at icebox temperature; yield 386 mg., m. p. 147–147.5°, unchanged on admixture with an authentic specimen of *p*-hydroxyphenylacetic acid of m. p. 148°.

Tetrahydro-osajinol 1-(3′,5′-Dinitrobenzoate) 3-Methyl Ether from Tetrahydro-osajin Dimethyl Ether.—In previous publications from this Laboratory,[6,7] a crystalline fraction (m. p. 65–70°) had been obtained from the alkaline fusion of both tetrahydro-osajin dimethyl ether and tetrahydropomiferin trimethyl ether. This material was found in low yield in the steam distillate from the neutral fraction. A re-investigation of this product showed that it was a complex mixture and was not always crystalline. In one such experiment, using tetrahydro-osajin dimethyl ether[6] (5 g.), the distillate from the steam distillation was extracted with ether and the residue obtained on solvent removal was refluxed for fifteen minutes with pyridine (4 cc.) and 3,5-dinitrobenzoyl chloride (1 g.). The solid material obtained on pouring the reaction mixture into an excess of ice and water was removed by decantation, washed with 50 cc. of 2% aqueous sodium carbonate solution, and obtained crystalline from ethanol–water; yield 810 mg., m. p. 124–125°. Pure material was formed on further crystallization from ethanol–water; yield 520

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

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

[35] L. F. Fieser, *ibid.*, **46**, 2639 (1924).

mg., golden plates, m. p. 128.5–129° unchanged on recrystallization from acetone–water.

Anal. Calcd. for $C_{23}H_{25}N_2O_7(OCH_3)$: C, 61.00; H, 5.97; N, 5.93; OCH_3, 6.57. Found: C, 60.99; H, 6.00; N, 5.89; OCH_3, 6.77.

Osajinol Dimethyl Ether from Pomiferin Trimethyl Ether.—Pomiferin trimethyl ether[10] (5 g.) was treated with alcoholic potassium hydroxide as described above for isopomiferin (except that the fusion was prolonged for a period of forty-eight hours) and the crude product, isolated in the same manner and obtained on solvent removal from the ether extract, was dissolved in acetone (75 cc.). The acetone solution was heated to boiling, 15 cc. of dimethyl sulfate added, and then 42.5% potassium hydroxide solution (130 cc.) and dimethyl sulfate (115 cc.) added dropwise and simultaneously to the hot solution. The solid obtained on pouring the cooled reaction mixture into an excess of ice and water was removed by filtration and obtained crystalline from ethanol–water; yield 635 mg., m. p. 60–61°. Further recrystallization (decolorizing charcoal) from ethanol–water yielded pure material in the form of colorless plates; m. p. 63.5–64°. This substance decolorized alkaline permanganate solution and readily absorbed hydrogen (platinum catalyst).

Anal. Calcd. for $C_{16}H_{18}O(OCH_3)_2$: C, 74.97; H, 8.39; OCH_3, 21.52. Found: C, 74.91; H, 8.48; OCH_3, 21.57.

Absence of Furan-carboxylic Acids as Oxidation Products of Osajin.—Osajin (1 g.) was oxidized with alkaline hydrogen peroxide according to the general procedure described by Späth and co-workers[8] but no final distillate of methyl esters of furan-carboxylic acids was obtained (distillation flask heated to 230° at 2–3 mm.).

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Iso-osajetin Dimethyl Ether.—Iso-osajetin (1.25 g.) was methylated as described above for the synthesis of isopomiferitin trimethyl ether and the product was isolated and crystallized from methanol in the same manner; yield 0.95 g., m. p. 79–80.5°. More material of lesser purity could be obtained on the addition of water to the methanolic mother liquor; yield 0.21 g., m. p. 68–74°. Pure material was obtained on further crystallization from absolute ethanol; m. p. 80.5–81°, unchanged on crystallization from benzene–petroleum ether; colorless plates or rhombohedral prisms; negative alcohol–ferric chloride test; soluble in benzene, ether, ethanol and acetone; less soluble in methanol and high-boiling petroleum ether; insoluble in water.

Anal. Calcd. for $C_{24}H_{24}O_3(OCH_3)_2$: C, 73.91; H, 7.16; OCH_3, 14.69. Found: C, 73.94; H, 7.10; OCH_3, 14.35.

This substance was also prepared in yields of 65–70% by the methylation of iso-osajetin monomethyl ether in a similar fashion.

α-Hydroxyisobutyric Acid from Pomiferin.—Pomiferin[5] (2 g.) was oxidized with alkaline permanganate and the acid fraction was isolated. After removal of oxalic acid, the residue was subjected to sublimation under reduced pressure. The detailed procedure followed was that of Späth and co-workers[20] for the isolation of α-hydroxyisobutyric acid from seselin by oxidative degradation. The colorless crystalline sublimate was twice crystallized from petroleum ether (b. p. 60–110°); yield 10 mg., m. p. 78–79°, unchanged on admixture with an authentic specimen of α-hydroxyisobutyric acid of like melting point.

Isopomiferitinonedioic Acid Trimethyl Ether from Isopomiferitin Trimethyl Ether.—To a solution of isopomiferitin trimethyl ether (13.5 g.) in acetone (75 cc.) was added rapidly under mechanical stirring a suspension of 27 g. of potassium permanganate in 300 cc. of acetone. The reaction was allowed to proceed at room temperature for twenty hours. The precipitate that formed was removed by filtration, extracted with boiling acetone and then with three 100-cc. portions of boiling water. The combined aqueous extracts were boiled with decolorizing charcoal, filtered, and to the boiling filtrate was slowly added dilute hydrochloric acid until a slight excess was present. Separation of yellow rhombohedral prisms was thus effected; yield 8.6 g., m. p. 180–200° (dec.). Pure material was obtained after several recrystallizations effected by dissolving in the minimum amount of dilute sodium hydroxide followed by acidification with 0.25 *N* hydrochloric acid; m. p. 205° (dec.).

Anal. Calcd. for $C_{22}H_{19}O_4(OCH_3)_3(COOH)_2$: C, 61.12; H, 5.70; OCH_3, 17.55; equiv. wt., 265.2. Found: C, 61.12; H, 5.74; OCH_3, 17.17; equiv. wt., 262.

Dimethyl Isopomiferitinonedioate Trimethyl Ether.—Isopomiferitinonedioic acid trimethyl ether (30 mg.) was suspended in 1 cc. of dry ether and treated with an ethereal solution of diazomethane (50% excess). After completion of the reaction (indicated by complete solution of the acid), the solvent was removed (hood) to yield a crystalline residue; yield 26 mg., m. p. 128–130°. Pure material was obtained on further crystallization from acetone–water; m. p. 133.5–134°, unchanged on crystallization from ethanol. The substance crystallized in light yellow plates that were soluble in the common organic solvents but were insoluble in water.

Anal. Calcd. for $C_{24}H_{19}O_6(OCH_3)_5$: C, 62.35; H, 6.14; OCH_3, 27.78. Found: C, 62.35; H, 5.93; OCH_3, 26.3.

Veratric Acid and 5-(1-Carboxyisopropoxy)-3,4-dihydro-7-methoxy-2,2-dimethyl-6-methyl Ester 1,2-Benzopyran-6,8-dicarboxylic Acid (IV) from Dimethyl Isopomiferitinonedioate Trimethyl Ether.—A solution of dimethyl isopomiferitinonedioate trimethyl ether (350 mg.) in 18 cc. of acetone was treated with 8 cc. of 5% aqueous potassium hydroxide and the whole heated to reflux temperature. To the refluxing solution there was added dropwise, under mechanical stirring, 15 cc. of aqueous hydrogen peroxide (30%) over a period of one hour. The initially yellow-colored solution became colorless during this addition. Excess hydrogen peroxide was destroyed with sodium bisulfite, a saturated aqueous solution of the latter being added until the reaction mixture no longer liberated iodine from a potassium iodide solution. The acidified (dilute sulfuric acid) solution was extracted with ether and the crystalline residue obtained on solvent removal from the dried ethereal extract was recrystallized from 35 cc. of hot water (decolorizing charcoal); yield 41 mg., m. p. 169°. Pure material was obtained on two further crystallizations from water; m. p. 177–178°, unchanged on admixture with an authentic sample of veratric acid of like melting point.

The aqueous solution remaining after extracting the oxidation mixture with ether was concentrated to 100 cc. and on standing overnight at room temperature, radiating clumps of colorless elongated prisms formed and were separated by hand from the more dense inorganic precipitate also present; yield 21 mg., m. p. 200° (dec.). Purification was effected by solution in 10% sodium hydroxide and crystallization by the addition of 0.25 *N* hydrochloric acid; m. p. 200° (dec.). The analysis and the previous knowledge of the structure of isopomiferitin characterized this substance as the expected 5-(1-carboxyisopropoxy)-3,4-dihydro-7-methoxy-2,2-dimethyl-6-methyl ester 1,2-benzopyran-6,8-dicarboxylic acid (IV).

Anal. Calcd. for $C_{17}H_{18}O_7(OCH_3)_2$: C, 57.57; H, 6.10; OCH_3, 15.66. Found: C, 57.57; H, 6.12; OCH_3, 15.0.

Acetone from Isopomiferitinonedioic Acid Trimethyl Ether.—Isopomiferitinonedioic acid trimethyl ether (500 mg.) was dissolved in ammonium hydroxide (3 cc. of 15 *N* ammonium hydroxide and 25 cc. of water) and to the boiling solution there was added successively, under mechanical stirring, 5 cc. and 10 cc. of aqueous hydrogen peroxide (30%), and refluxing was then maintained for one hour. The vapors from the reaction mixture were passed through two traps each containing 50 cc. of reagent (4 g. of 2,4-dinitrophenylhydrazine dissolved in 28 cc. of concentrated sulfuric acid to which was then added successively 100 cc. of methanol and 100 cc. of water). Yellow crystals formed in the reagent; yield 83 mg., m. p. 117°. Pure material was obtained on repeated crystallization from ethanol and methanol; m. p. 123.5–124.5°, unchanged on admixture with an authentic specimen of acetone 2,4-dinitrophenylhydrazone of m. p. 124–125°.

Degree of Total Unsaturation of Osajin.—This analysis was that described by Kuhn and Möller[23] and was carried out for us in 1938 by Dr. Kurt Wallenfels of the Laboratory of Professor Richard Kuhn in Heidelberg, Germany. An amount of 1.300 mg. of osajin in 2.0 cc. of acetic acid to which was added 7.2 mg. of platinic oxide (PtO_2) absorbed 700 cu. mm. of hydrogen at 0° and 760 mm. In a duplicate determination, 1.306 mg. of osajin with 8.1 mg. of platinic oxide and 2.0 cc. of acetic acid absorbed 559 cu. mm. of hydrogen at 0° and 760 mm.

Anal. Calcd. for osajin: 10 double bonds. Found: 9.72, 9.75.

Insecticidal Assays.[36]—Third and fourth instar Mexican bean beetle larvae were used as a test insect and application was made in an aqueous clay suspension at about 3 pounds to 100 gallons. Osajin, osajin monomethyl ether, iso-osajin, iso-osajin monomethyl ether, tetrahydro-osajin, pomiferin trimethyl ether, isopomiferin dimethyl ether, as well as the meal made from the ground and dried fruit of the osage orange (*Maclura pomifera* Raf.) were tested and were found to be non-toxic. Rotenone is highly toxic under these conditions.

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Summary

1. Hydriodic acid converts osajin or osajin dimethyl ether to dihydro-iso-osajin and similarly converts pomiferin or pomiferin trimethyl ether to dihydro-isopomiferin.

2. Dihydro-iso-osajin monoacetate is shown to be dimorphous.

3. Osajetinone trimethyl ether monoxime (dimorphous) and pomiferitinone tetramethyl ether monoxime have been prepared.

4. Some previously reported experimental results are interpreted by the assumption of tautomeric equilibria in the osajetin and pomiferitin structures.

5. Improved preparative directions for iso-osajin and isopomiferin are reported.

6. The following ethers are described: the monomethyl ethers of dihydro-iso-osajin, dihydro-iso-osajetin, and iso-osajetin; the dimethyl ethers of iso-osajetin, isopomiferitin, dihydro-isopomiferitin, and dihydro-isopomiferin; and the trimethyl ethers of isopomiferitin and dihydro-isopomiferitin.

7. A substance is described to which is assigned the structure isopomiferitin enol tetramethyl ether.

8. Iso-osajin, on alkaline fusion, has been degraded to iso-osajetin, formic acid, acetone, *p*-hydroxyphenylacetic acid and 2,2-dimethyl-5,7-chromandiol; 2,2-dimethyl-5,7-chromandiol and acetone have been isolated as alkaline fusion products of isopomiferin.

9. Two other alkaline fusion products have been shown to be very probably osajinol (from pomiferin trimethyl ether; isolated as the dimethyl ether) and tetrahydro-osajinol (from tetrahydro-osajin dimethyl ether; isolated as the 1-(3′,5′-dinitrobenzoate) 3-methyl ether).

10. No furan-carboxylic acids were obtained from osajin on oxidation with alkaline hydrogen peroxide.

11. The ultraviolet absorption spectra characteristics are recorded for osajin, pomiferin, dihydro-osajin, dihydropomiferin, tetrahydro-osajin, tetrahydropomiferin, iso-osajetin monomethyl ether, isopomiferitin dimethyl ether, dihydro-iso-osajetin monomethyl ether and dihydro-isopomiferitin dimethyl ether.

12. Interpretation of the above data in the light of previous knowledge, demonstrates that osajin and pomiferin are identical in all respects save for the position of an additional phenolic group in position 3′ of pomiferin and that the ethylenic linkage located in the C_5H_8 residue of both pigments is conjugate to position 8 of the isoflavone nucleus.

13. Osajin and pomiferin exhibit fluorescence when irradiated by ultraviolet light but this property disappears when the isoflavone ring is opened.

14. Osajin and pomiferin are mordant dyes for wool and silk.

15. Osajin and pomiferin (and a number of their derivatives tested) showed no insecticidal properties under conditions wherein rotenone is active.

16. Pomiferin yielded α-hydroxyisobutyric acid on permanganate oxidation.

17. Isopomiferitin trimethyl ether was oxidized with permanganate without carbon loss to yield isopomiferitinonedioic acid, characterized by the production of acetone on oxidation with ammoniacal hydrogen peroxide and by a dimethyl ester that yielded veratric acid and IV on further oxidation.

18. The above facts, together with those previously ascertained, definitely establish the structural formulas (I and II) of osajin, pomiferin and their iso compounds.

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[36] We are indebted for these tests to Doctors E. D. Witman, H. A. Waters and D. M. DeLong of the department of entomology of The Ohio State University.