THE *ISO*FLAVONES

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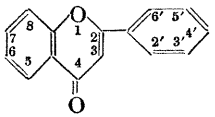
1. Structural Relation between Flavones, *iso*Flavones, and Anthocyanidins

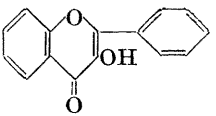
THERE occur in plants several classes of compound having the same or similar fundamental nuclei, sometimes called "C_6–C_3–C_6" compounds. The most widespread and numerous of these are the flavones, which occur in almost all plants. They are frequently responsible for the colour of plants, but flavones occur in the colourless parts of plants, such as white flowers.

The naturally occurring flavones are hydroxylated derivatives of flavone (2-phenylbenzo-4-pyrone) which may be partially alkylated.[1] Positions 5 and 7 are almost invariably hydroxylated, and also frequently one or more of positions 3′, 4′, and 5′. However, the unsubstituted parent, flavone (I), also occurs in Nature. Most of the flavones occur as glycosides of glucose or rhamnose, but a few occur in the free state.

The flavone nucleus occurs in various states of oxidation. Thus, flavonol (3-hydroxyflavone) (II) is found in several botanical species as its polyhydroxy-derivatives.[2] Flavanone (III) is represented by, among other compounds, the rutinoside hesperidin (IV). Catechin (V) is a derivative of 2 : 3-dihydro-3-hydroxy-2-phenylbenzopyran, in which there are opportunities for optical isomerism.



(I)



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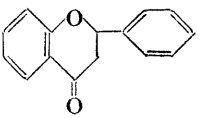

(III)

The anthocyanins form a particularly important group of colouring matters which are responsible for the mauve, blue, purple, and red colours in all parts of most coloured plants, and they are always found as glycosides. The aglycones, the anthocyanidins, are oxonium (or carbonium or carbenium [3]) salts derived from 2-phenylbenzopyrylium chloride (VI). Different anions occur in the cell sap.

The structural similarity of anthocyanidins to flavones leads to the question whether one is derived from the other by an oxidation or reduction

[1] Perkin and Everest, "The Natural Organic Colouring Matters", Longmans, Green & Co., London, 1918.

[2] Link, in Gilman's "Organic Chemistry", John Wiley & Sons, New York, 2nd Edn., 1943, Vol. II, p. 1331.

[3] Ref. 2, p. 1317.

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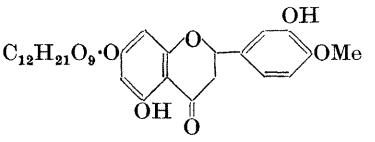
effected in the plant, and the same question may be asked about the other states of oxidation. Quercetin (VII) has been converted into cyanidin, the corresponding anthocyanidin (VIII),[4] by reduction with magnesium and hydrochloric acid, and cyanidin into catechin (V) by catalytic hydrogenation,[5] but no comparable conversion has ever been achieved *in vitro* under conditions tolerable to living cells, and the converse oxidation has never been achieved. Robinson and Robinson [6] have expressed the opinion that the flavones and anthocyanidins are not interconverted as such in Nature, but have a common starting-point, and that subsequently there occurs an oxidation rather than a reduction.



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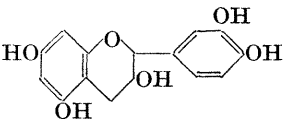

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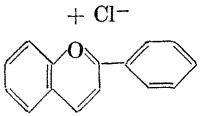

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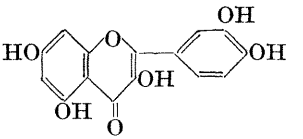

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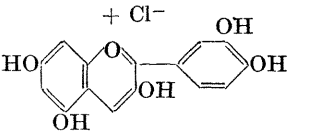
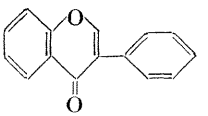


(VIII)

The compounds so far mentioned all bear a phenyl substituent in the 2-position. The isomer of flavone with this substituent in the 3-position, 3-phenylbenzo-4-pyrone (IX), is called *iso*flavone. *iso*Flavones occur in Nature in widely differing botanical species, sometimes as the glycosides and sometimes in the free state. They are less widespread or numerous than the flavones, and the number whose structures have been established with reasonable certainty is less than 15. By contrast, there are more than 50 known flavones.

The migration of the phenyl substituent from position 2 to 3, or the reverse migration, has never been accomplished *in vitro* with a flavone or *iso*flavone, and there is no reason to believe that such a reaction occurs in living material. However, dehydration of catechin tetramethyl ether,



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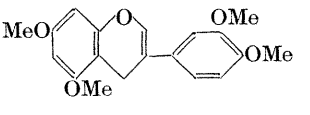
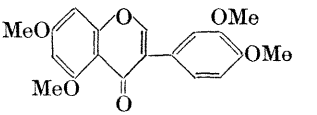


(X)



(XI)

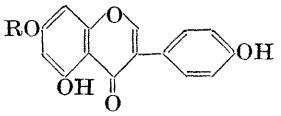
in which the 2-H and 3-OH * are *trans*, is accompanied by migration of the 3 : 4-dimethoxyphenyl residue to give the anhydro-derivative (X). Baker [7] attempted to oxidise the methylene group in anhydrocatechin

[4] Willstätter, *Ber.*, 1914, **47**, 2873. [5] Freudenberg, *Annalen*, 1925, **444**, 135.
[6] Robinson and Robinson, *J.*, 1935, 744. [7] Baker, *J.*, 1929, 1593.
* In the *epi*catechin series the H and OH are *cis* and a ready dehydration occurs without migration.

tetramethyl ether to a carbonyl group in order to produce 5 : 7 : 3′ : 4′-tetramethoxy*iso*flavone (XI), but the 4-position proved resistant to oxidation. Baker expressed the opinion that the *iso*flavones were not in Nature derived from catechins.

2. Proof of the *iso*Flavone Structure

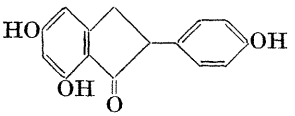
The first compound to be regarded as a derivative of 3-phenylbenzo-4-pyrone was prunetin (XII ; R = Me), 5 : 4′-dihydroxy-7-methoxy*iso*-flavone. Finnemore [8] suggested that this compound, isolated from the bark of an unidentified species of *Prunus* as the glycoside prunetrin, was



(XII)

a monomethyl ether of 5 : 7 : 4′-trihydroxy*iso*flavone (XII ; R = H). He based this suggestion on the molecular formula $C_{16}H_{12}O_5$, and on the fact that fusion with potassium hydroxide gave *p*-hydroxyphenylacetic acid and phloroglucinol. Prunetin contained one methoxyl group, and on demethylation with hydriodic acid gave a trihydric phenol, prunetol. Since the methyl group was lost in the alkaline degradation, Finnemore was unable to determine its position. This type of degradation, which is the basis of most structural determinations in the *iso*flavone series, is dealt with more fully on p. 80.

A. G. Perkin [9] realized that prunetol was possibly identical with genistein, a colourless constituent of dyer's broom (*Genista tinctoria*) which had earlier been isolated by Perkin and Newbury [10] and investigated by them and by Perkin and Horsfall.[11] Perkin and Newbury assigned the incorrect formula $C_{14}H_{10}O_5$ to genistein. They showed the presence of three hydroxyl groups by acetylation, and the production of phloroglucinol and *p*-hydroxyphenylacetic acid on alkaline hydrolysis. They concluded that genistein was probably a phenylketocoumaran (XIII). Perkin and Horsfall also studied various ethers of genistein and their degradation products, which



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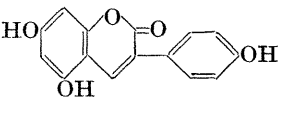


(XIV)

appeared to confirm the structure (XIII). It was unfortunate that Perkin did not detect the presence of formic acid during the alkaline degradation. Another incorrect formula for genistein was suggested by Bargellini,[12]

[8] Finnemore, *Pharm. J.*, 1910, **31**, 604.
[9] A. G. Perkin, quoted by Baker and Robinson, *J.*, 1925, 1981.
[10] Perkin and Newbury, *J.*, 1899, 830.
[11] Perkin and Horsfall, *J.*, 1900, 1310.
[12] Bargellini, *Gazzetta*, 1925, **55**, 949.

namely, that of the coumarin (XIV). However, in 1925 Baker and Robinson [9] succeeded in synthesising a 5-hydroxy-7 : 4′-dimethoxy-*C*-methyl*iso*flavone and showed that it was identical with material derived from naturally occurring genistein by methylation. This synthesis afforded the first proof that the 3-phenylbenzo-4-pyrone structure occurs in Nature, and also showed that genistein must be 5 : 7 : 4′-trihydroxy*iso*flavone (XII ; R = H).

While synthetic methods have advanced considerably since the *iso*flavone structure was first proved, the basic methods of degradation are still substantially those mentioned above. The former will be described more fully later, and an account will now be given of the more important methods by which new *iso*flavones have been recognised and their structures determined.

There are valuable colour tests by means of which a flavone may be recognised early during an investigation. Until it was realised that *iso*flavones usually give the same colour reactions, investigators sometimes mistook *iso*flavones for flavones.[13] Flavanones are reduced by sodium amalgam or by magnesium in hydrochloric acid, whereas flavones are reduced by sodium amalgam and not by magnesium–hydrochloric acid. Flavonols with a free hydroxyl group in the 3-position are reduced by magnesium–hydrochloric acid and not by sodium amalgam. However, flavonols having a substituted hydroxyl group in position 3, *e.g.*, a methoxyl group or a sugar residue, are reduced by both methods. All the reduction products exist in, or separate from, hydrochloric acid solution as red substances of a flavylium salt nature.[14]

Most of the *iso*flavones isolated from plant material have been recognised without the aid of spectroscopy, but the ultra-violet absorption is sufficiently characteristic to make it a useful physical property. There is one strong absorption band which usually lies between 262 and 270 mμ and sometimes a band of very much less intense absorption at 320—360 mμ. In *iso*flavone itself, both bands are at a much shorter wave-length,[15] and in the only 5-methoxy*iso*flavone studied, the position of the principal band is between that of *iso*flavone and of its 5-hydroxy-derivatives.

The spectra of the flavones have been more extensively studied.[16] In general, flavones absorb strongly in the region 240—270 mμ, with another strong band in the region 300—350 mμ. The variation in the positions of both maxima with substitution is quite large, but the magnitude of the band of longer wave-length should serve to distinguish these compounds from *iso*flavones. In the anthocyanidins the principal maximum is in the visible region. Pelargonidin, cyanidin, and delphinidin all show strong maxima at 520—540 mμ, with another band of about the same intensity near 270 mμ.[17] The glycosides do not differ greatly in absorption from the anthocyanidins.

Oxidation of *iso*flavones does not usually give recognisable fragments

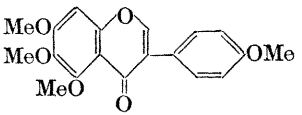
[13] Wolfrom, Morgan, and Benton, *J. Amer. Chem. Soc.*, 1940, **62**, 1484.
[14] Asahina and Inubuse, *Ber.*, 1928, **61**, 1646 ; 1931, **64**, 1256 ; Briggs and Locker, *J.*, 1949, 2157.
[15] J. B. Harborne, personal communication.
[16] Aronoff, *J. Org. Chem.*, 1940, **5**, 561 ; Skarzyński, *Biochem. Z.*, 1939, **301**, 150.
[17] Schou, *Helv. Chim. Acta*, 1927, **10**, 907.

of the benzo-4-pyrone portion of the molecule, but it may assist in identifying the substituted 3-phenyl component. Degradation of genistein with alkaline hydrogen peroxide gives *p*-hydroxybenzoic acid. In the same way permanganate oxidation sometimes gives a substituted benzoic acid derived

TABLE 1. *Ultra-violet absorption of some* iso*flavones* [15, 18, 19]

Compound	Max (mμ)	$\text{Log}_{10}\,\varepsilon$
Osajin and pomiferin	275, 360	
Irigenin and tectorigenin	268, 320	
Genistein	263	4·50
Prunetin	262·5	4·57
Biochanin A	262·5	4·56
Genistein 5-methyl ether	256	4·51
*iso*Flavone	245, 307	4·41, 3·82

from the 3-phenyl nucleus. By far the most valuable method of degradation, however, is alkaline hydrolysis. As an example, the hydrolysis of dimethylmuningin (XV) may be considered.[20] When dimethylmuningin is boiled for 30 minutes with 4% ethanolic potassium hydroxide, the products are formic acid and 6-hydroxy-2 : 3 : 4-trimethoxyphenyl 4-methoxybenzyl



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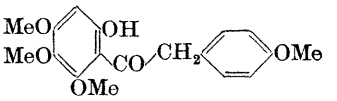
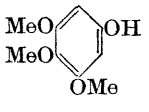


(XVI)

ketone (XVI). Further degradation is effected by fusion of dimethylmuningin with potassium hydroxide, the deoxybenzoin (XVI) being hydrolysed to *p*-methoxyphenylacetic acid and antiarol (XVII). The hydroxyl and methoxyl groups in the original *iso*flavone can be located by degradation of other alkyl ethers, and comparison of the resulting alkylated phenol with synthetic material of known structure. In this way muningin has been shown to be 6 : 4′-dihydroxy-5 : 7-dimethoxy*iso*flavone (XVIII). The methods illustrated in this example are very generally applicable and the production of formic acid is usually quantitative.



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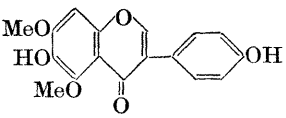


(XVIII)

3. Naturally Occurring *iso*Flavones

Many reports of new *iso*flavones have appeared in recent years, but at present the number of known naturally occurring compounds of this

[18] Wolfrom, Harris, Johnson, Mahan, Moffett, and Wildi, *J. Amer. Chem. Soc.*, 1946, **68**, 406. [19] Baker, Chadderton, Harborne, and Ollis, *J.*, 1953, 1852.
[20] King, King, and Warwick, *J.*, 1952, 96.

class tends to diminish rather than increase. The use of improved methods of synthesis, to be described later, has shown that structures based solely on identification of degradation products cannot always be accepted after comparison with compounds prepared by unambiguous synthetic methods. Among the compounds of doubtful structure are *iso*genistein (5 : 7 : 2′-trihydroxy*iso*flavone), 8-methylgenistein, and 8-methyl*iso*genistein, all of which were reported by Okano and Beppu [21] to occur in soya bean but are probably genistein of varying degrees of purity.[22] 5 : 7 : 2′-Trihydroxy-*iso*flavone prepared by synthesis differs from *iso*genistein, and synthetic 8-methylgenistein [23] is not identical with the material isolated by Okano and Beppu. Okano and Beppu also described tatoin, to which they gave the structure 5 : 4′-dihydroxy-8-methyl*iso*flavone, as a constituent of soya bean, but it may well prove to be daidzein (7 : 4′-dihydroxy*iso*flavone).[22] Recent chromatographic work on soya-bean extracts has so far not shown any *iso*flavones except genistein and daidzein.[24]

The simple natural *iso*flavones (as distinct from their glycosides) whose structures may be accepted with reasonable certainty at present number

TABLE 2. *Naturally occurring* iso*flavones*

Compound	*iso*Flavone	Source	Ref
Daidzein	7 : 4′-Dihydroxy-	*Soja hispida* (soya bean) (as daidzin)	25—28
Formononetin	7-Hydroxy-4′-methoxy-	*Ononis spinosa* L. (as ononin); subterranean clover; *Trifolium pratense*, L. (red clover) *	25, 27, 29
ψ-Baptigenin	7-Hydroxy-3′ : 4′-methylenedioxy-	*Baptisia tinctoria* (as ψ-baptisin)	25, 30
Genistein	5 : 7 : 4′-Trihydroxy-	*Trifolium subterraneum* L., (subterranean clover); *Genista tinctoria* L. (miller's broom); *Soja hispida* (as genistin); *Sophora japonica* (as sophoricoside and sophoricobioside)	10, 26, 28, 29, 31
Prunetin	5 : 4′-Dihydroxy-7-methoxy-	*Prunus puddum*	8
Biochanin A	5 : 7-Dihydroxy-4′-methoxy-	Germinated *Chana* grain, *Ferreirea spectabilis*	32
Orobol	5 : 7 : 3′ : 4′-Tetrahydroxy	*Orobus tuberosus* L. (vetch), (as oroboside)	33
Santal	5 : 3′ : 4′-Trihydroxy-7-methoxy-	*Pterocarpus santalinus* L. (sandalwood); *Baphia nitida* (barwood)	33
Muningin	6 : 4′-Dihydroxy-5 : 7-dimethoxy-	*Pterocarpus angolensis* (wood)	20
Tectorigenin	5 : 7 : 4′-Trihydroxy-6-methoxy-	*Iris tectorum* (root) (as tectoridin)	34
Irigenin	5 : 7 : 3′-Trihydroxy-6 : 4′ : 5′-trimethoxy-	*Iris florentia* (orris) (root); (as iridin)	35

* This substance was originally thought to be 7-hydroxy-4′-methoxyflavone, but it has been shown by Bate-Smith, Swain, and Pope (*Chem. and Ind.*, 1953, 1127) that it is, in fact, the corresponding *iso*flavone.

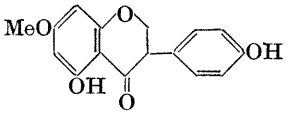
eleven. These, together with their botanical sources, are set out in Table 2. There are also more complex molecules containing the *iso*flavone nucleus. These will be treated later.

Flavanones [type (III)] have long been known to occur in Nature, but it is only recently that *iso*flavanones have been discovered. In 1952 Narasimhachari and Seshadri [36] isolated from the bark of *Prunus puddum* a glycoside which they called padmakastin, and the aglycone padmakastein. The latter compound formed a dimethyl ether which was oxidised with selenium dioxide to trimethylgenistein. Moreover, padmakastein diacetate on similar treatment gave prunetin diacetate. Since only the hydrogen content changed during these experiments, padmakastein is dihydroprunetin (XIX). More recently King and Neill [37] isolated from the heartwood of *Ferreirea spectabilis*, a South American hardwood, two *iso*flavanones, ferreirin and homoferreirin. These gave the colour reactions of *iso*flavones, but contained two extra hydrogen atoms. Trimethylferreirin gave no identifiable products when fused with alkali but, after dehydrogenation with palladium–charcoal or selenium dioxide, alkaline degradation yielded formic acid and a deoxybenzoin. The positions of the substituents were shown by degrading the die hyl ether of ferreirin, and catalytic reduction of synthetic 5 : 7 : 2′ : 4′-tetramethoxy*iso*flavone gave trimethylferreirin. Ferreirin is thus 5 : 7 : 2′-trihydroxy-4′-methoxy*iso*flavanone (XX ; R = H), and homoferreirin is the closely related (XX ; R = Me). The structure of homoferreirin has been confirmed by synthesis.[38]

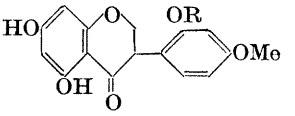
Many of the *iso*flavones occur as glycosides as well as free. The glycosides are usually degraded by boiling dilute mineral acid, but this procedure destroys the evidence showing where the sugar residue was attached. The glycosides have not in general been studied as extensively as the *iso*flavones themselves, so that the point of attachment of the sugar residue is not known in every case. However the glycosides of genistein have been

[21] Okano and Beppu, *J. Agric. Chem. Soc. Japan*, 1939, **15**, 645.
[22] Baker, Harborne, and Ollis, *J.*, 1953, 1860.
[23] Whalley, *J. Amer. Chem. Soc.*, 1953, **75**, 1059 ; Seshadri and Varadarajan, *Proc. Indian Acad. Sci.*, 1953, **37**, *A*, 145, 508, 514, 526.
[24] A. J. Hamblin, personal communication.
[25] Mahal, Rai, and Venkataraman, *J.*, 1934, 1120, 1769.
[26] Walz, *Annalen*, 1931, **489**, 118.
[27] Wessely, Kornfeld, and Lechner, *Ber.*, 1933, **66**, 685.
[28] Baker, Robinson, and Simpson, *J.*, 1933, 274.
[29] Bradbury and White, *J.*, 1951, 3447.
[30] (*a*) Späth and Lederer, *Ber.*, 1930, **63**, 743 ; (*b*) Baker, Robinson, and Simpson, *J.*, 1937, 805.
[31] Zemplén, Bognár, and Farkas, *Ber.*, 1943, **76**, 267.
[32] Siddiqui, *J. Ind. Sci. Res. India*, 1945, **3**, 68 ; Bose and Siddiqui, *ibid.*, p. 231.
[33] Robertson, Suckling, and Whalley, *J.*, 1949, 1571.
[34] Shibata, *J. Pharm. Soc. Japan*, 1927, **47**, 380 ; Shriner and Stephenson, *J. Amer. Chem. Soc.*, 1942, **64**, 2737.
[35] Baker, *J.*, 1928, 1022 ; Baker and Robinson, *J.*, 1929, 152.
[36] Narasimhachari and Seshadri, *Proc. Indian Acad. Sci.*, 1952, **35**, *A*, 202.
[37] King and Neill, *J.*, 1952, 4752.
[38] K. G. Neill, personal communication.

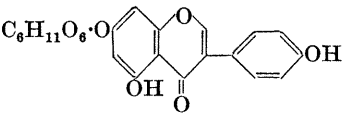
studied and will be taken as examples. Genistin (XXI) is a D-glucoside of genistein. It was first studied by Walz,[26] who found that dilute acid liberated the sugar, which he identified as glucose. Acid hydrolysis of trimethylgenistin, prepared by the action of methyl iodide, gave an aglycone with only two methoxyl groups, showing that one methyl group had entered the sugar residue. This was shown to be genistein 5 : 4′-dimethyl ether, and genistin is therefore the 7-glucoside of genistein. Genistin was also



(XIX)



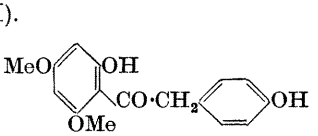
(XX)



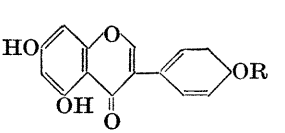
(XXI)

isolated from soya bean in 1941 by Walter,[39] who observed the same position of absorption maxima in both it and genistein, the absorption of the aglycone being more intense. By methods similar to those used with genistin, Walz showed that daidzin from soya bean is the 7-D-glucoside of daidzein.

Zemplén, Bognár, and Farkas [31] showed that the *iso*flavone glycoside sophoricoside, found in *Sophora japonica* L., was also a D-glucoside of genistein. Degradation of dimethylsophoricoside gave an *iso*flavone, identified by its hydrolysis with alkali to 4-hydroxybenzyl 2-hydroxy-4 : 6-dimethoxyphenyl ketone (XXII), and by the production of *p*-hydroxybenzoic acid on oxidation, as genistein 5 : 7-dimethyl ether. The ketone was identical with material prepared by the Hoesch synthesis [40] from phloroglucinol dimethyl ether and 4-hydroxybenzyl cyanide. Sophoricoside is therefore the 4′-glucoside. There is also in *Sophora japonica* L. a second 4′-glycoside, sophoricobioside, which the same workers showed to be (XXIII).



(XXII)



R = L-rhamnosyl-D-glucosyl

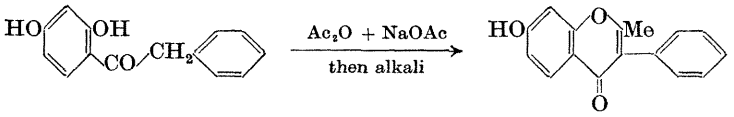
(XXIII)

4. The Synthesis of *iso*Flavones

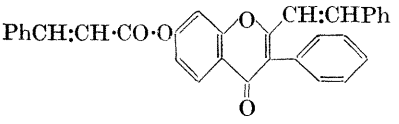
It is not difficult to synthesise *iso*flavones with an alkyl or aryl substituent in the 2-position, but since this position is never substituted in

[39] Walter, *J. Amer. Chem. Soc.*, 1941, **63**, 3273.
[40] Spoerri and DuBois, *Org. Reactions*, 1945, **5**, 387.

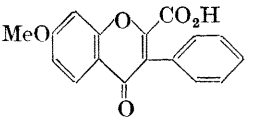
naturally occurring compounds, such synthetic methods are of value only if the substituent can be subsequently removed. Baker and Robinson [9] showed in 1925 that condensation of benzyl 2 : 4-dihydroxyphenyl ketone with acetic anhydride and sodium acetate followed by hydrolysis gave 7-hydroxy-2-methyl*iso*flavone :



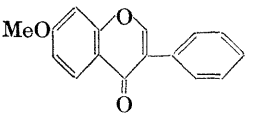
Deoxybenzoins derived from phloroglucinol gave 5 : 7-dihydroxy*iso*flavones, and the reaction also proceeded with benzoic anhydride and sodium benzoate to give 2-phenyl*iso*flavones. Cinnamic anhydride and sodium cinnamate with benzyl 2 : 4-dihydroxyphenyl ketone gave 7-cinnamoyloxy-2-styryl-*iso*flavone (XXIV). This compound was converted into the 7-methoxy-compound, which was then degraded by permanganate in pyridine to the 2-carboxylic acid (XXV) and decarboxylated to 7-methoxy*iso*flavone (XXVI). It then seemed feasible to synthesise genistein (XII ; R = H) by the same method, and this was undertaken in 1926.[41] Cinnamoylation



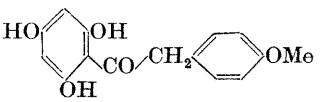
(XXIV)



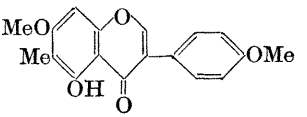
(XXV)



(XXVI)



(XXVII)



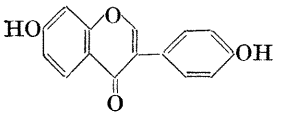
(XXVIII)

of 4-methoxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone (XXVII) under closely defined conditions gave the 7-cinnamoyloxy-5-hydroxy-4′-methoxy-2-styryl*iso*flavone and the cinnamoyl group was removed, but methylation of the product introduced a *C*-methyl group, while only the 7-hydroxyl group was methylated. Acetylation of the remaining hydroxyl group, followed by permanganate oxidation, gave a small amount of an acid which, after decarboxylation and hydrolysis, gave 6-methylgenistein dimethyl ether (XXVIII).[23] The poor yields and experimental difficulty of this method make it now of little more than historical interest, but it should be noted that in 1928 Baker and Robinson [42] succeeded in preparing

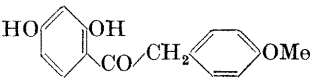
[41] Baker and Robinson, *J.*, 1926, 2713. [42] *Idem*, *J.*, 1928, 3115.

genistein itself by selecting conditions under which nuclear methylation did not occur.

The difficulty of preparing 2-styryl*iso*flavones can be reduced by condensing 2-methyl*iso*flavones with benzaldehyde. This method avoids the experimental difficulties arising from the use of derivatives of cinnamic acid and is due to Baker, Robinson, and Simpson,[28] who in 1933 prepared

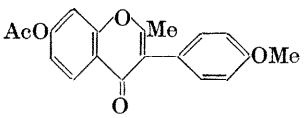


(XXIX)

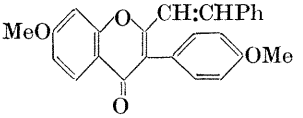


(XXX)

daidzein (XXIX) by condensing 2 : 4-dihydroxyphenyl 4-methoxybenzyl ketone (XXX) with acetic anhydride to give 7-acetoxy-4′-methoxy-2-methyl-*iso*flavone (XXXI), which after deacetylation and methylation was condensed with benzaldehyde in the presence of sodium ethoxide ; the resulting



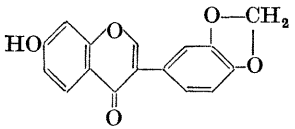
(XXXI)



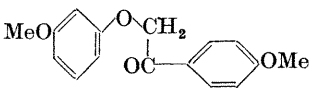
(XXXII)

2-styryl derivative (XXXII) was degraded in the usual way and demethylation then gave daidzein in 5% overall yield from (XXX). The method was also successfully employed in the preparation of ψ-baptigenin (XXXIII) by Baker, Robinson, and Simpson,[30b] but it has the disadvantage of the original method that the yields in the oxidation are small, and since hydroxyl groups must be protected before oxidation, it is limited to compounds in which the final demethylation will not cleave required alkoxy-groups.

In 1929 Baker, Pollard, and Robinson [43] evolved a synthesis of 7-methoxy*iso*flavone which avoided oxidation altogether. They condensed *m*-methoxyphenol with phenacyl bromide to give ω-*m*-methoxyphenoxyacetophenone (XXXIV), which was converted into the cyanohydrin. This compound when treated with zinc chloride and hydrogen chloride in ether



(XXXIII)



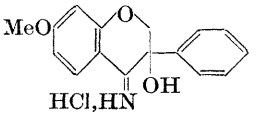
(XXXIV)

underwent an intramolecular Hoesch reaction, and the ketimine hydrochloride (XXXV) on hydrolysis yielded 3-hydroxy-7-methoxy*iso*flavanone, which was dehydrated to 7-methoxy*iso*flavone by sulphuric acid. This method, in which the yields are good at all stages, was later employed by Späth and Lederer [30a] to prepare ψ-baptigenin, but an attempt by Baker,

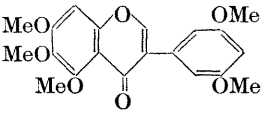
[43] Baker, Pollard, and Robinson, *J.*, 1929, 1468.

Morgans, and Robinson [44] to prepare irigenin trimethyl ether (XXXVI) in this way failed. The scope and limitations of this synthetic method have not been fully explored.

Strangely enough, it was not until 1930 that any synthesis based on reversal of the alkaline hydrolysis of *iso*flavones was successfully carried

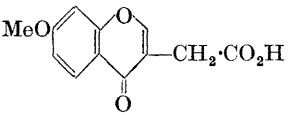


(XXXV)

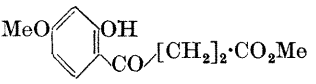


(XXXVI)

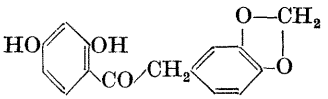
out, although a 3-substituted benzo-1 : 4-pyrone had been prepared as early as 1908 by Perkin and Robinson.[45] They had prepared anhydrobrazilic acid (XXXVII) by condensing methyl β-(2-hydroxy-4-methoxybenzoyl)propionate (XXXVIII) with ethyl formate at 53° in the presence of sodium, thus introducing the 2-carbon atom. Hydrolysis of the ester gave anhydrobrazilic acid. Although the yield was not stated, it was undoubtedly small. In 1930, Späth and Lederer [30a] condensed 2 : 4-dihydroxyphenyl 3 : 4-methylenedioxybenzyl ketone (XXXIX) with ethyl formate in the presence of sodium at 100° in a sealed tube and obtained



(XXXVII)



(XXXVIII)



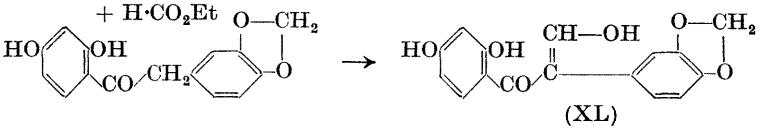
(XXXIX)

a very small yield of ψ-baptigenin. The method was shortly after applied by Wessely, Kornfeld, and Lechner [27] to the synthesis of daidzein and formononetin, but in each case in poor yield. The ethyl formate synthesis was greatly improved in 1934 by Mahal, Rai, and Venkataraman [25] who carried out the condensation at 0° and obtained daidzein and ψ-baptigenin in yields of about 30% each from the deoxybenzoins. In these and other cases reported by Venkataraman, the only free hydroxyl group in the deoxybenzoin was that required for the ring closure, but there are reports of the successful preparation of *iso*flavones from deoxybenzoins in which other hydroxyl groups were also left free. Shriner and Hull [46] described the preparation of 8-methylgenistein from a trihydroxydeoxybenzoin, but later workers have not been able to repeat their results.

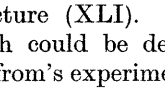
The mechanism of the ring closure between a deoxybenzoin and ethyl

[44] Baker, Morgans, and Robinson, *J.*, 1933, 374.
[45] Perkin and Robinson, *J.*, 1908, **93**, 489.
[46] Shriner and Hull, *J. Org. Chem.*, 1945, **10**, 228.

formate has been much discussed. In 1930 Späth and Lederer [30a] suggested that the reaction followed the course :



(XL)

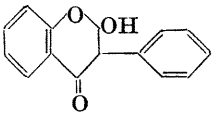
in which the hydrogen atoms of the active methylene group, together with an oxygen atom from the ester, were eliminated as water, and the unsaturated triol (XL) lost water to form an ether linkage on treatment with mineral acid. However, no such intermediates were isolated by them, or by Mahal, Rai, and Venkataraman,[25] who considered that the reaction proceeded directly to the *iso*flavone. There are cases, however, in which intermediates of the molecular composition postulated have been isolated. Wolfrom, Mahan, Morgan, and Johnson [47] in 1941 isolated four such compounds, to which they gave the 2-hydroxy*iso*flavanone structure (XLI), and they showed that these compounds in every case lost a molecule of water on treatment with glacial acetic acid, to yield an *iso*flavone. However, the usual methods of removing water of crystallisation did not expel any water. Since the intermediates did not develop a colour with ferric chloride, or with boric acid (which indicates the absence of the group  [48]), the evidence supported the hydroxy*iso*flavanone structure (XLI). Harper,[49] in 1942, also prepared hydroxy-compounds which could be dehydrated to *iso*flavones. The evidence of his and of Wolfrom's experiments strongly suggests the hydroxy*iso*flavanone structure.

Study of the conditions used by Venkataraman *et al.*, however, shows that he probably obtained the *iso*flavones directly from the condensation. More recently, Robertson, Suckling, and Whalley [33] prepared santal trimethyl ether (XLII) and other *iso*flavones under conditions which might have been expected to give the intermediate hydroxy*iso*flavanones if they had been formed, but isolated *iso*flavones directly from the reaction mixtures. Consideration of the actual structures of the compounds involved in these apparently inconsistent experiments does not greatly help in explaining why only in some cases stable intermediates are formed, and it may be that there are two available reaction paths.

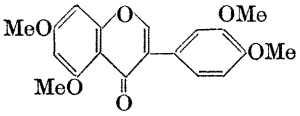
Other sources of the 2-carbon atom have been explored. Sathe and Venkataraman [50] in 1949 condensed benzyl 2 : 4-dihydroxyphenyl ketone with ethyl orthoformate in pyridine containing a little piperidine, obtaining 7-hydroxy*iso*flavone. They also obtained prunetin by treating 2-hydroxy-4 : 6-dimethoxyphenyl 4-nitrobenzyl ketone with the same ester to give 5 : 7-dimethoxy-4′-nitro*iso*flavone, from which prunetin was prepared by

[47] Wolfrom, Mahan, Morgan, and Johnson, *J. Amer. Chem. Soc.*, 1941, **63**, 1248.
[48] Wilson, *ibid.*, 1939, **61**, 2303.
[49] Harper, *J.*, 1942, 595.
[50] Sathe and Venkataraman, *Current Sci.*, 1949, **18**, 373.

conversion of the nitro-group into a hydroxyl group, followed by partial demethylation. This method, also, suffers from the disadvantage that it is not generally applicable to polyhydroxydeoxybenzoins.

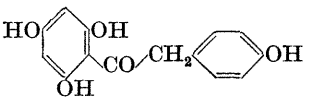


(XLI)

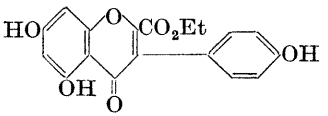


(XLII)

Although the ethyl formate synthesis has been of great value, its failure with deoxybenzoins containing several free hydroxyl groups * has been a severe limitation, since many of the natural *iso*flavones contain such substituents. In 1949 a method of considerable importance was discovered, in which ring closure can be carried out with compounds containing several free hydroxyl groups. Baker and Ollis, with Binns, Chadderton, Dunstan, Harborne, and Weight,[52] reported that the 2-carbon atom could be supplied by ethoxalyl chloride, $COCl{\cdot}CO_2Et$, and that *iso*flavone-2-carboxylic esters were formed in good yield. In 1953 the first of a series of papers appeared describing the use of this method.[19, 22] Benzyl *o*-hydroxyphenyl ketones [*e.g.*, 4-hydroxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone (XLIII)] reacted with ethoxalyl chloride in pyridine at room temperature, to give ethyl *iso*flavone-2-carboxylates [*e.g.*, (XLIV)], hydrolysis and decarboxylation of



(XLIII)



(XLIV)

which gave high overall yields of *iso*flavones. The new synthesis was well suited to the preparation of highly substituted *iso*flavones. The synthesis of twelve *iso*flavones, six of them naturally occurring (ψ-baptigenin, daidzein, formononetin, genistein, biochanin A, and prunetin), was reported. Synthesis of 5 : 7 : 2′-trihydroxy*iso*flavone [22] showed that the "*iso*genistein" of Okano and Beppu [21] did not have this structure.

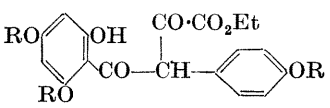
The ring closure is easily carried out. The ketone, containing in all n free phenolic hydroxyl groups, is treated with $n + 1$ equivalents of ethoxalyl chloride in pyridine and left overnight; water then precipitates the ethyl *iso*flavone-2-carboxylate. Hydrolysis with sodium carbonate is followed by decarboxylation, which occurs at a little above the melting point. The value of the method was shown by the preparation at the Middlesex Hospital of 120 grams of genistein in 50% yield from the deoxybenzoin.

[51] R. B. Bradbury, personal cummunication.

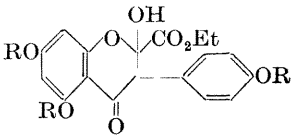
[52] Baker and Ollis, with Binns, Chadderton, Dunstan, Harborne, and Weight, *Nature*, 1952, **169**, 706.

* There are reports to the contrary (see, *e.g.*, ref. 46). But Bradbury and White [29] were unable to obtain 4′-*O*-methylgenistein from 4-methoxybenzyl 2 : 4 : 6-trihydroxyphenyl ketone with sodium and ethyl formate.[51]

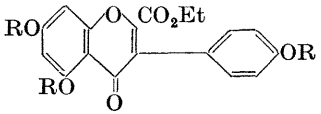
Baker, Chadderton, Harborne, and Ollis [19] studied the mechanism of this reaction and presented evidence that it proceeded by the following steps: (1) Ethoxalylation of all phenolic hydroxyl groups except one *ortho* to the carbonyl group. (2) *C*-Ethoxalylation of the reactive methylene group of the deoxybenzoin to give, *e.g.*, (XLV). (3) Cyclisation to the hydroxy*iso*flavanonecarboxylic ester, *e.g.*, (XLVI). (4) Loss of a molecule



(XLV)



(XLVI)



(XLVII)

of water to give the *iso*flavonecarboxylic ester (XLVII). (5) Removal of the ethoxalyl groups by reaction with dilute acid. The principal evidence for this course is as follows: It is necessary to use $n + 1$ equivalents of the acid chloride with a deoxybenzoin containing n hydroxyl groups. Phenols are rapidly ethoxalylated under the conditions employed, but a single hydroxyl group *ortho* to a carbonyl group is relatively unreactive. Moreover, preliminary experiments had shown that direct *C*-ethoxalylation of deoxybenzoins occurred in pyridine to give 1 : 3-diketones.

The postulated 2-hydroxy*iso*flavanone-2-carboxylic esters were isolated in several cases. Thus, benzyl *o*-hydroxyphenyl ketone gave, under the usual conditions, the non-phenolic ethyl 2-hydroxy*iso*flavanone-2-carboxylate. These intermediates were readily dehydrated by acid or alkali. When the ethoxalylation was carried out in pyridine and boiling benzene, the *iso*flavone esters were in most cases isolated directly, but in very low yields.

No useful synthesis of *iso*flavones has appeared which does not require a deoxybenzoin. Such compounds may be made in three ways, of which the most useful is the Hoesch reaction.[40] A phloroglucinol or resorcinol reacts with a phenylacetonitrile and hydrogen chloride in dry ether, to give a ketimine hydrochloride, which is hydrolysed to the deoxybenzoin by boiling with dilute mineral acid. Yields usually exceed 40% and are sometimes much higher. The Friedel–Crafts reaction of a methoxy-compound with a phenylacetyl chloride and the Fries rearrangement of a phenylacetoxy-compound may also sometimes be used.

5. Reactions of *iso*Flavones

Until recently *iso*flavones were rare compounds and reports of their reactions were usually confined to those which had been undertaken to prove the structure of natural products. By far the most fruitful of these have been alkaline degradation, either with dilute alkali or by the more

drastic method of alkaline fusion. Oxidation has usually given nothing recognisable except an acid derived from the 3-aryl group. The carbonyl group is not reactive to the usual carbonyl reagents.

Exploitation of the Elbs persulphate oxidation of phenols has enabled Seshadri to introduce an additional hydroxyl group *para* to existing hydroxyl groups in the *iso*flavone nucleus. For example, the persulphate oxidation of 5 : 7-dihydroxy*iso*flavone gives 5 : 7 : 8-trihydroxy*iso*flavone [53a] and 5 : 8-dihydroxy-7-methoxy*iso*flavone [53b] is obtained from 8-hydroxy-7-methoxy*iso*flavone.

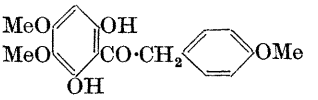
The phenolic hydroxyl groups of *iso*flavones behave normally and can be alkylated and acetylated, provided that they are not in the 5-position. It is well known that a phenolic hydroxyl group *ortho* to a carbonyl group is affected by hydrogen bonding and is relatively unreactive. A 5-hydroxyl group in a flavone or *iso*flavone is similarly affected by chelation. It can be methylated by the prolonged action of methyl sulphate in alkali, but other hydroxyl groups are readily methylated by the same reagent in hot acetone in the presence of potassium carbonate. Partial methylation of an *iso*flavone or deoxybenzoin is therefore not difficult. The use of methyl iodide is usually avoided because of the risk of nuclear methylation, which sometimes occurs with an excess of the halide under vigorous conditions. Recently Seshadri [53c] has taken advantage of nuclear methylation of a deoxybenzoin to synthesise the trimethyl ether of 8-methylgenistein ; complete demethylation of the latter was achieved by using aluminium chloride in benzene, whilst partial demethylation by the action of hydriodic acid in acetic anhydride under controlled conditions left the 7-methoxyl group unaffected and gave 8-methylgenistein 7-methyl ether.

Demethylation of methylated *iso*flavones is usually carried out with hydriodic acid or hydrobromic acid, and occasionally with aluminium chloride. Partial demethylation (in the 5-position) can be effected under mild conditions without affecting other methoxyl groups. In some cases there is an apparent migration of a substituent during the reaction and products of unexpected structure may be obtained. This rearrangement is well known in the flavone series, in which 5 : 8-dihydroxy-compounds often give 5 : 6-dihydroxy-compounds under the conditions of demethylation with hydriodic or hydrobromic acid. It is due to the hydrolytic opening of the pyrone ring and closure in the alternative direction involving the hydroxyl group initially in position 5. Baker, Dunstan, Harborne, Ollis, and Winter [54] observed that 5 : 7 : 8-trihydroxy-2-methyl*iso*flavone when boiled for eight hours in acetic acid containing hydrogen bromide gave the 5 : 6 : 7-isomer. Demethylation of 5 : 7-dihydroxy-8 : 3′ : 4′ : 5′-tetramethoxy*iso*flavone similarly gave irigenol, 5 : 6 : 7 : 3′ : 4′ : 5′-hexahydroxy*iso*flavone. This effect may be used to rearrange products arising from the ethoxalyl chloride synthesis. With sodium and ethyl formate,

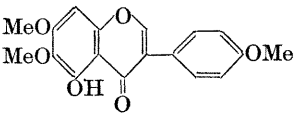
[53] (*a*) Narasimhachari, Row, and Seshadri, *Proc. Indian Acad. Sci.*, 1952, **35**, *A*, 46 ; (*b*) Ishwar-Dass, Narasimhachari, and Seshadri, *ibid.*, 1953, **37**, *A*, 599 ; (*c*) Seshadri and Varadarajan, *ibid.*, p. 145.

[54] Baker, Dunstan, Harborne, Ollis, and Winter, *Chem. and Ind.*, 1953, 277.

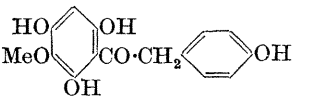
2 : 6-dihydroxy-3 : 4-dimethoxyphenyl 4-methoxybenzyl ketone (XLVIII) gives 5-hydroxy-6 : 7 : 4′-trimethoxy*iso*flavone (XLIX), but with ethoxalyl chloride and 4-hydroxybenzyl 2 : 4 : 6-trihydroxy-3-methoxyphenyl ketone (L) the final product is 5 : 7 : 4′-trihydroxy-8-methoxy*iso*flavone (LI).



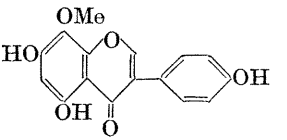
(XLVIII)



(XLIX)



(L)



(LI)

Hence in such cases, while the ethyl formate method gives compounds with 5 : 6 : 7-orientation, the ethoxalylation method gives 5 : 7 : 8-orientation. Products with the 5 : 7 : 8-orientation may be converted into products with the 5 : 6 : 7-orientation by the rearrangement already mentioned.

Whalley [55] also observed that 5 : 7 : 2′-trimethoxy-8-methyl*iso*flavone when treated with aluminium chloride in dry benzene gave some 5 : 7 : 2′-trihydroxy-6-methyl*iso*flavone. This observation could hardly be explained by hydrolytic ring fission, but, as Donnelly, Philbin, and Wheeler [56] have pointed out, it may be due to the simple migration of the methyl group under the influence of aluminium chloride, a reagent well known to cause migration of alkyl groups. If this is so, there are two possible modes of rearrangement during demethylation.

6. Isolation and Separation of *iso*Flavones

The usual methods of extraction by solvents from dried and cut or ground plant material have been used to obtain crude extracts of *iso*flavones or their glycosides. The glycosides are usually extracted with water, while the *iso*flavones themselves are soluble in ethanol. Fats are conveniently removed with light petroleum. Use may be made of complex formation with lead salts if the compound has a 3′ : 4′-dihydroxy-configuration. Further purification may be difficult and wasteful.

It is only recently that the use of chromatography to purify and separate natural *iso*flavones has been described. An interesting case is the separation of formononetin from genistein. Bradbury and White [29] found that

[55] Whalley, *Chem. and Ind.*, 1953, 277.
[56] Donnelly, Philbin, and Wheeler, *ibid.*, p. 567.

the ethanolic extract from subterranean clover, after removal of fats, deposited some formononetin, but it was not until the mother-liquors were chromatographed on alumina that they isolated genistein. Adsorption was made from ethanol–ether, and elution with ethanol–ether containing increasing amounts of ethanol eluted formononetin more readily than genistein. *iso*Flavones may also be separated by paper chromatography, either as glycosides or free. The glycosides genistin and daidzin in soya-bean extract are separated on paper if suitable solvents are used, and acid hydrolysis of eluted material gives compounds which have been identified as the *iso*flavones.[24] The position of the *iso*flavones on the chromatogram may be found by fluorescence in ultra-violet light or by spraying with colour reagents.

7. The Biological Activity of *iso*Flavones

Until fairly recently there was no reason to suspect that any *iso*flavone was of particular biological interest, and it was known that osajin and pomiferin, as well as several synthetic *iso*flavones related to rotenone, had no value as insecticides.[18, 49, 57] In 1941 there appeared with spectacular suddenness an outbreak of infertility in sheep in a large part of Western Australia. The areas affected, in all over 8000 square miles, were all of low rainfall and in all of them the predominant pasture was subterranean clover of the Dwalganup strain. Since this clover had been the predominant pasture for 15 years, the sudden outbreak was difficult to explain on the basis that the clover was responsible, but this proved to be the case. A combination of war-time factors (*e.g.*, shortage of fertiliser and of bulk feed) and climatic conditions had caused a much greater intake of clover per sheep for a long period. Isolated outbreaks of infertility were also observed at the same time in other parts of the continent.

The symptoms included failure of ewes to conceive, stillbirth or early death of lambs, and various disorders of the female reproductive system. Rams were not affected. The lambing percentage sometimes fell to as low as 8%, and transfer of ewes to good non-clover grazing areas for three successive seasons did not restore fertility. The only measures which proved effective were based on reduction of the amount of clover grazed, and on the observation that the clover was most potent in early spring.

Early attempts to isolate the substance responsible were handicapped by the lack of suitable methods of assay. This problem was finally solved in 1948 by Curnow, Robinson, and Underwood,[58] who measured the increase in uterine weight of ovariectomised mice when fed on a diet containing the dried ether-soluble material from a known amount of clover. Robinson [59] subsequently improved the method by injecting ether-soluble material into immature whole mice. He showed that the potency of the leaves was much greater than that of the rest of the plant. At this stage the only knowledge of the nature of the œstrogen or pro-œstrogen was that

[57] Harper, *J.*, 1940, 1178.
[58] Curnow, Robinson, and Underwood, *Austral. J. Exp. Biol.*, 1948, **26**, 171.
[59] Robinson, *ibid.*, 1949, **27**, 297.

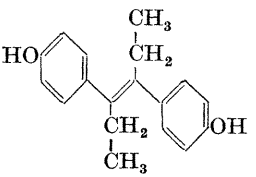
it was not steam-volatile, not extracted from alkali by ether or by water from ether, and soluble in ethanol but not in light petroleum.

Bradbury and White [29] finally isolated from 4020 kg. of clover formononetin (6·2 g.) and genistein (24·4 g.). The latter was shown to be the principal œstrogen and to be about 10^{-5} times as active as œstrone. The same workers [60] also prepared a number of related compounds, some of which had much higher potency than genistein, and they expressed the opinion that genistein was a pro-œstrogen rather than an œstrogen. By catalytic reduction they converted *iso*flavones into *iso*flavanones and *iso*flav-3-enes, the intermediate alcohols not being isolated. The œstrogenic activity of a few of the more important compounds is set out in Table 3. 4-(7 : 4′-Dimethoxyphenyl-) and -(*p*-methoxyphenyl-)*iso*flav-3-ene show in mice activity of the same order as that of the triphenylethylenes.[51]

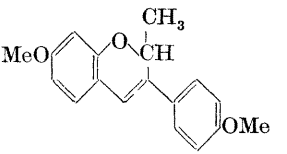
TABLE 3. *Œstrogenic activity of various compounds*

Compound	Active at mg /mouse	Inactive at mg /mouse
iso*Flavones*		
5 : 7 : 4′-Trihydroxy-	1 0	
5 : 4′-Dihydroxy-7-methoxy-	2 1	
iso*Flav*-2-*ene*		
5 : 7 : 3′ : 4′-Tetramethoxy-		5·6
iso*Flav*-3-*enes*		
7 : 4′-Dimethoxy-		4 1
7 : 4′-Dimethoxy-2-methyl-	0·07	
7 : 4′-Dimethoxy-4-methyl-	0·35	
4-Ethyl-7 : 4′-dimethoxy-	0·07	

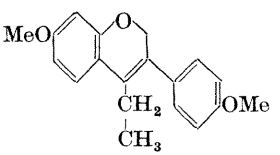
Bradbury and White remarked that the œstrogenic activity of the *iso*flavones is not analogous to that of the stilbene series. A 5-hydroxy-group appears to be essential while 2-alkyl substituents greatly reduce activity, probably by distortion of the planar system of genistein. How-



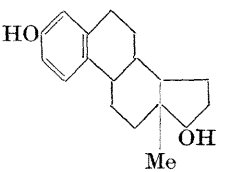
(LII)



(LIII)



(LIV)



(LV)

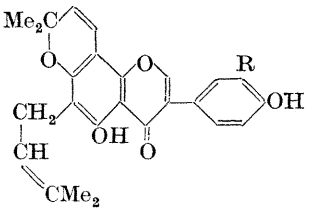
[60] Bradbury and White, *J.*, 1953, 871.

ever the *iso*flav-3-enes are œstrogenic only if they carry a 2- or a 4-alkyl substituent, and are then much more active than genistein. The active *iso*flav-3-enes have no 5-hydroxyl groups and appear to be analogous to compounds of the stilbœstrol series in œstrogenic activity. It is instructive to compare the structures of diethylstilbœstrol (LII) and 7 : 4′-dimethoxy-2-methyl- (LIII) and -4-ethyl-*iso*flav-3-ene (LIV). Diethylstilbœstrol was originally [61] prepared because of its structural similarity to the steroid sex hormones, *e.g.*, œstradiol (LV), and the activity of the *iso*flavones is not surprising if it be assumed that they are converted by the body into compounds of the type (LIII) or (LIV).

A survey of English pastures has shown the presence of œstrogens in various grasses and in clover, but not in lucerne.[62]

8. *iso*Flavones with Isoprene Side Chains

*iso*Flavones with isoprene side chains are also known, and these may cyclise with hydroxyl groups. Two particularly interesting compounds are osajin and pomiferin (LVI ; R = H and OH respectively). In a series of researches begun in 1934, Wolfrom and his co-workers [63, 13, 18] determined their structures, and finally in 1951 achieved a synthesis of a compound closely related to osajin itself. The Osaje orange tree (*Maclura pomifera* Raf.) is widely distributed in the United States and the large greenish-yellow fruit yields, when dry, as much as 6% of yellow, crystalline material, which is soluble in most organic solvents. The more readily isolated compound is osajin, and it was not at first realised that a second constituent was present. The formula $C_{25}H_{24}O_5$ was assigned to osajin, which was shown to form a diacetate and to give the colour reactions of a phenol.[63a]



(LVI)

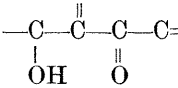
Titration with alkali indicated a lactone ring, but the lactone theory was later abandoned. The presence of pomiferin in the extract was discovered in 1939, and pomiferin was shown to be $C_{25}H_{24}O_6$ and to form a triacetate.[63b] It was later found that pomiferin, a catechol derivative, could be removed by formation of its lead salt, but osajin, which is not a catechol, does not form such a complex.[63e] Both osajin and pomiferin were isomerised by

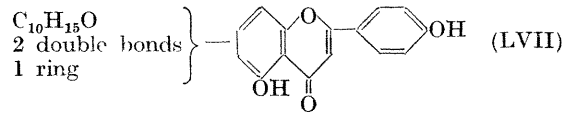
[61] Solmssen, *Chem. Reviews*, 1945, **37**, 481.

[62] Legg, Curnow, and Simpson, *Biochem. J.*, 1950, **46**, xix.

[63] (*a*) Walter, Wolfrom, and Hess, *J. Amer. Chem. Soc.*, 1938, **60**, 574 ; (*b*) Wolfrom, Benton, Gregory, Hess, Mahan, and Morgan, *ibid.*, 1939, **61**, 2832 ; (*c*) Wolfrom and Gregory, *ibid.*, 1940, **62**, 651 ; (*d*) Wolfrom, Benton, Gregory, Hess, Mahan, and Morgan, *ibid.*, 1941, **63**, 422 ; (*e*) Wolfrom and Mahan, *ibid.*, 1942, **64**, 308 ; (*f*) Wolfrom and Moffett, *ibid.*, p. 311 ; (*g*) Wolfrom and Wildi, *ibid.*, 1951, **73**, 235.

sulphuric acid to high-melting, colourless products, *iso*osajin and *iso*pomiferin. *iso*Osajin no longer gave phenolic colour reactions. Osajin dimethyl ether on oxidation with hydrogen peroxide gave anisic acid, while pomiferin trimethyl ether gave veratric acid.[63c] Reduction of osajin and of pomiferin with sodium amalgam and acidification gave red colours, and a flavone structure was provisionally assigned to both compounds.[13]

Hydrogenation of osajin gave dihydro-, tetrahydro-, and hexahydro-osajin, but no fresh hydroxyl groups were formed. Moreover both compounds gave a positive colour reaction with boric acid. This test, according to Wilson,[48] indicates the grouping  . On the basis of these results the provisional partial structure (LVII) was given to osajin. However the flavone structure was abandoned when it was shown[63c] that alkaline degradation of osajin dimethyl ether gave one mole of formic acid.

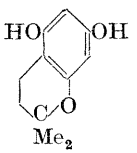


(LVII)

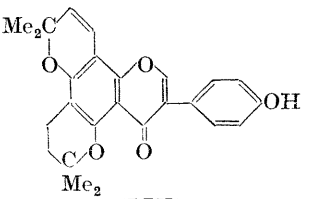
Osajin was therefore an *iso*flavone. Methylation under conditions of varying severity showed that osajin had one relatively unreactive hydroxyl group. This group disappeared when *iso*osajin was formed and an extra ring was produced.

The appearance of acetone on ozonolysis (0·7 mole) established the presence of an *iso*propylidene group in the side-chain,[63e] and Kuhn–Roth determinations showed two *C*-methyl groups.[63f]

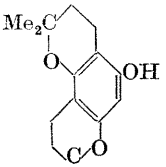
Four years later the isolation of 2 : 2-dimethylchroman-5 : 7-diol (LVIII) enabled the complete structures to be assigned.[18] The formula (LVI; R = H) for osajin accounts for the ring closure to *iso*osajin (LIX), for the formation from this compound of the chromandiol, for the *C*-methyl content, and for all the other experimental observations.



(LVIII)



(LIX)

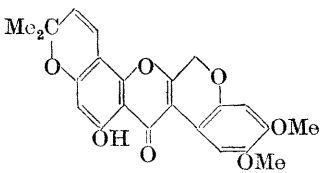


(LX)

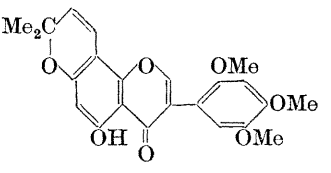
In 1951 the synthesis of dihydro*iso*osajin and dihydro*iso*pomiferin was announced.[63g] Dihydro*iso*osajinol (LX) was prepared by dialkylation of phloroglucinol with $\gamma\gamma$-dimethylallyl bromide, and this compound underwent a Hoesch reaction with homoanisonitrile to give a deoxybenzoin which when condensed with ethyl formate in the presence of sodium (p. 76) gave the methyl ether of dihydro*iso*osajin. Demethylation with hydriodic acid completed the synthesis.

Another compound with an isoprene side-chain cyclised with a hydroxyl group probably exists in the root of *Derris malaccensis*, a rich source of compounds of chromone structure.

While studying the active principles of leguminous fish-poison plants, and in particular toxicarol, Harper [57] isolated a small amount of a related substance of phenolic nature, optically inactive and insoluble in alkali. These and other properties, and the assumption that the compound was closely related to dehydrotoxicarol (LXI), led Harper to propose the provisional *iso*flavone structure (LXII). Harper remarked : " Such a formula, if substantiated, is of great biochemical interest as suggesting a link in the biogenesis of toxicarol in the plant. Moreover the possibility is present of there being a series of *iso*flavones in the plant corresponding to rotenone,



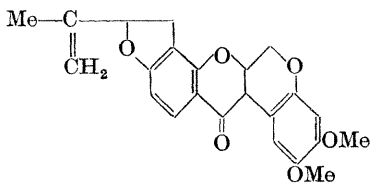
(LXI)



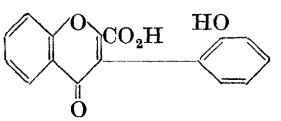
(LXII)

etc., and similarly constituted." Harper later showed that formic acid was produced on alkaline degradation and observed colour reactions consistent with an *iso*flavone structure.

Rotenone (LXIII), a potent insecticide, is abundantly available from derris root. It is not an *iso*flavone, but the relation of its skeleton to the *iso*flavone nucleus deserves comment. An *iso*flavone-2-carbyoxlic acid of the type (LXIV) readily cyclises to give a lactone,[22] and the product, except in its state of oxidation, has the basic rotenone skeleton. There is, however, no evidence that rotenone is made in the plant by this route.



(LXIII)



(LXIV)

The author is deeply grateful to Professor Wilson Baker, F.R.S., for several discussions and for advice during the preparation of the manuscript.