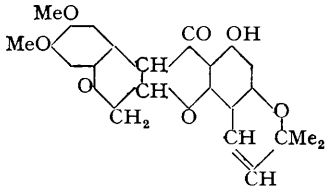
122. *The Active Principles of Leguminous Fish-poison Plants. Part IX. The Synthesis of Furano*isoflavones related to Rotenone.

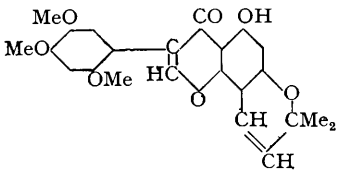
By Stanley H. Harper.

By utilising the method of Venkataraman *et al.* (J., 1934, 513, 1120, 1769) *iso*flavones have been prepared from the methyl ethers of derritol, *iso*derritol, and elliptol. In the case of the last, by the isolation of a crystalline intermediate, it is shown that the reaction does not proceed directly to the *iso*flavone but occurs through the formation of an *iso*flavanonol and its subsequent dehydration. *iso*Flavones of this type have been postulated as likely to occur in *Derris* resin along with rotenone and elliptone, with which they are isomeric.

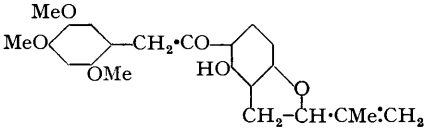
These *iso*flavones are remarkable in giving a positive Durham test, previously regarded as specific for the rotenoids. Their reactions have been studied, and a method devised for the detection of the formic acid formed in their hydrolysis. This method has been applied to the " toxicarol *iso*flavone " isolated from crude toxicarol to establish conclusively its *iso*flavone nature and hence support for the formula previously suggested (J., 1940, 1178).

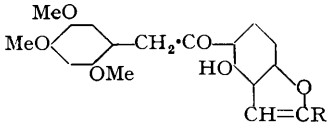
In Part V (J., 1940, 1178) a substance was isolated from crude *l*-α-toxicarol (I), isomeric with the latter but containing an additional methoxyl group. As a working hypothesis the *iso*flavone structure (II) was advanced for this substance, which can be referred to as " toxicarol *iso*flavone." If this type of structure could be substantiated, the possibility was realised of there being a series of *iso*flavones isomeric with rotenone, etc., in *Derris* resin.

(I.) 



(II.)

(III.) 

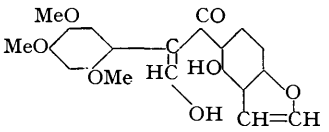


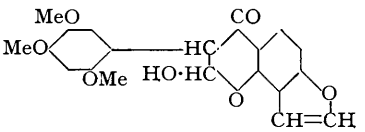
(IV.)

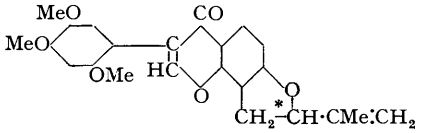
Owing to the comparatively ready availability of the substituted benzyl *o*-hydroxyphenyl ketones, derritol (III), *iso*derritol (IV, R = Pr^{β}), and elliptol (IV, R = H) methyl ethers, it was decided to use the *iso*flavone synthesis by Venkataraman *et al.* (J., 1934, 513, 1120, 1769) in an approach to these problems. Späth and Lederer (*Ber.*, 1930, **63**, 743) postulated an open-chain hydroxymethylene intermediate of type (V), but Venkataraman *et al.* (*loc. cit.*) regarded the condensation with sodium and ethyl formate as proceeding directly to the *iso*flavone. However, recently Wolfrom *et al.* (*J. Amer. Chem. Soc.*, 1941, **63**, 1248) have shown that with osajetin dimethyl ether an intermediate hydroxy-compound is formed, probably an *iso*flavanonol, which loses a molecule of water on subsequent treatment with acetic acid. This formation of a hydroxy-compound has now been observed in the preparation of *iso*flavones from derritol and elliptol methyl ethers. It is therefore probable that in the compounds studied by Venkataraman *et al.* (*loc. cit.*) intermediate *iso*flavanonols were actually formed but dehydrated in the subsequent crystallisation from acetic acid.

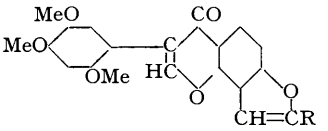
Elliptol methyl ether readily condensed with sodium and ethyl formate at 0°. The product obtained by evaporation of the ethyl formate crystallised in contact with methyl alcohol and so enabled the intermediate to be characterised. This substance contains an additional molecule of water to the *iso*flavone and hence two formulæ appear possible (V and VI). As the substance gives neither a ferric chloride colour nor the Wilson boric acid test (*J. Amer. Chem. Soc.*, 1939, **61**, 2303), the open-chain formula (V) is eliminated and the substance is without doubt represented by the *iso*flavanonol formula (VI). Dehydration of this intermediate was readily effected by refluxing in acetic acid to give *elliptol* iso*flavone* (VIII, R = H). In like manner derritol and *iso*derritol methyl ethers condensed with sodium and ethyl formate to give non-crystalline intermediates which readily dehydrated to the iso*flavones* (VII) and (VIII, R = Pr^{β}) respectively. The former is optically active

owing to the asymmetric carbon atom marked with an asterisk, and this substance is believed to be the first optically active *iso*flavone described.

(V.) 

 (VI.)

(VII.) 

 (VIII.)

As the *iso*flavone from derritol methyl ether contains the *iso*propenyldihydrofuran system characteristic of rotenone, it would be expected to undergo the reactions characteristic of that system. The *iso*flavone readily isomerised to *iso*derritol *iso*flavone (VIII, R = Pr^{β}) in sulphuric–acetic acids with consequent loss of optical activity, a change closely paralleled by the rotenone ⟶ *iso*rotenone change (Wright, *J. Amer. Chem. Soc.*, 1928, **50**, 3355). Hydrogenation of the exocyclic double bond was readily effected in the presence of a palladium–barium sulphate catalyst to give *dihydroderritol* iso*flavone*, but there was no sign of a product analogous to rotenonic acid in which the dihydrofuran ring had suffered fission.

These synthetic *iso*flavones give a positive Durham test when carried out by the spot plate method but not by the procedure of Jones (*Ind. Eng. Chem.*, 1933, **5**, 75), whereas rotenone gives a positive result by both techniques. Nevertheless the fact that these substances with markedly different molecular structures give a positive result considerably lessens the diagnostic value of this test for the rotenoids.

The identity of these substances as *iso*flavones has been fully confirmed by examination of the products of their hydrolysis with aqueous alcoholic alkali. In the case of derritol *iso*flavone, from the acidified hydrolysis solution the open-chain benzyl *o*-hydroxyphenyl ketone, derritol methyl ether, separated in practically quantitative yield, and from the filtrate one mole of formic acid was isolated by steam distillation. In agreement with the observations of Wolfrom *et al.* (*loc. cit.*) these *iso*flavones give the same reduction colour tests described by Asahina *et al.* (*Ber.*, 1928, **61**, 1646; 1929, **62**, 3016; 1931, **64**, 1256) as characteristic of flavones. Hence the detection of formic acid as a hydrolysis product is an essential step in the characterisation of an *iso*flavone.

As a step in the characterisation of the " toxicarol *iso*flavone " isolated from crude toxicarol, the sodium amalgam and magnesium reduction tests for flavones and *iso*flavones have been applied. As with the synthetic *iso*flavones described above, the first test was positive and the second negative. To establish the *iso*flavone nature of this substance it was then necessary to identify formic acid as a hydrolysis product. The above synthetic *iso*flavones being used, a semimicromethod was devised in which the formic acid liberated was separated by steam-distillation and estimated by precipitation of mercurous chloride. This was applied to " toxicarol *iso*flavone " with a clear-cut positive result. Hence the *iso*flavone structure (II) postulated previously is given considerable support.

TABLE I.

(For test insect, carrying medium, and other details, see Table I, this vol., p. 590.) Rel. humidity, 37%.

Substance.	Concn., mg./l.	% Kill.	Substance.	Concn., mg./l.	% Kill.	Substance.	Concn., mg./l.	% Kill.
Derritol *iso*flavone	20	7	Dihydroderritol *iso*flavone	20	5	Rotenone	2	98
	2	9		2	14		1	56
*iso*Derritol *iso*flavone	20	2	Elliptol *iso*flavone	20	24		0·5	33
	2	22		10	27	Control	—	20
				2	20			

With the co-operation of Dr. C. Potter these synthetic *iso*flavones have been compared with rotenone for their possible value as insecticides. The general technique has been described by Potter (*Ann. Appl. Biol.*, 1941, **28**, 142). The kill in the control is unusually large owing probably to the low relative humidity, but the results clearly show that derritol, *iso*derritol, and dihydroderritol *iso*flavones have no significant toxicity even at concentrations ten times that at which rotenone is completely toxic. Elliptol *iso*flavone showed a definite paralytic effect, but the concentration necessary for an adequate kill would render it of no practical interest.

EXPERIMENTAL.

Synthesis of Derritol iso*Flavone* (2′ : 4′ : 5′-*Trimethoxy*-5″-iso*propenyl*-4″ : 5″-*dihydro*-2″ : 3″ : 7 : 8-*furano*iso*flavone*). —Sodium (2·25 g.) was powdered under toluene, and the latter replaced by ether. Dry nitrogen was passed while the flask was cooled in ice and salt. After the ether had evaporated, derritol methyl ether (LaForge and Smith, *J. Amer. Chem. Soc.*, 1930, **52**, 1089) (4·5 g.) was added to the sodium (no reaction), followed by redistilled ethyl formate (45 c.c.) in three portions at 5 minute intervals. The reaction mixture was left in the freezing mixture to warm to room temperature overnight. Water (250 c.c.) was added, and unreacted ethyl formate removed in a current of air. The gum was taken out in ether, washed with water, and evaporated (in a pilot experiment this gummy intermediate could not be crystallised) and the residue refluxed in glacial acetic acid (50 c.c.) for 30 mins. Acetic acid (30 c.c.) was distilled off,

and water added to the residue to give a precipitate of *derritol* iso*flavone*, which crystallised from alcohol (sparingly soluble) in feathery needles, or from benzene in prisms (3·8 g.), m. p. 215°, $[\alpha]_D^{17}$ −37° in chloroform (c = 1·0) and −60° in benzene (c = 1·0) (Found: C, 70·0; H, 5·6; OMe, 23·4. $C_{23}H_{22}O_6$ requires C, 70·0; H, 5·6; 3OMe, 23·6%). The *iso*flavone was insoluble in 5% aqueous potassium hydroxide and gave no colour with ferric chloride.

Hydrolysis of Derritol iso*Flavone*.—Sodium hydroxide (2·5 g.) was dissolved by warming in water (12·5 c.c.)–absolute alcohol (12·5 c.c.), and derritol *iso*flavone (500 mg.) added. After refluxing for 15 mins., the yellow solution was cooled and acidified with 10% sulphuric acid. Immediate crystallisation occurred and after 2 hours the crop of needles was filtered off, washed free from acid, dried, and recrystallised from methyl alcohol. The derritol methyl ether (451 mg.) so obtained had m. p. 120—122°, not depressed by an authentic specimen (m. p. 122°). Derritol methyl ether gave a deep red colour with alcoholic ferric chloride.

The combined aqueous filtrates (about 250 c.c.) were steam-distilled, the volume being reduced to 25 c.c. and maintained at this level until no further acid distilled over. The free acid in the distillate required 12·75 c.c. of 0·1N-sodium hydroxide (calc. for 1 mol. of formic acid liberated, 12·70 c.c.). The neutralised distillate was evaporated to small bulk, strongly acidified with hydrochloric acid, and reduced with magnesium metal; the solution was then buffered with sodium acetate, and an aqueous solution of dimedon added. Next day the crystalline dimedon derivative of formaldehyde was collected and dried at 60°; m. p. 187°, not depressed by an authentic specimen (m. p. 190°).

Isomerisation to iso*Derritol* iso*Flavone* (2′ : 4′ : 5′-*Trimethoxy*-5″-iso*propyl*-2″ : 3″ : 7 : 8-*furano*iso*flavone*).—Derritol *iso*flavone was not isomerised by sulphuric acid in acetic acid at room temperature. However, it was successfully synthesised under the following conditions: Derritol *iso*flavone (500 mg.) was dissolved in acetic acid (5 c.c.), and sulphuric acid (2·5 c.c.) added without cooling. After 1 hour on the steam-bath, ice was added to the red solution, and the precipitated *iso*flavone collected, dried in a vacuum, and crystallised from methyl alcohol (charcoal). iso*Derritol* iso*flavone* separated in fine needles (337 mg.), m. p. 160°, α_D ±0° in chloroform (Found: C, 69·65; H, 5·7; OMe, 23·4. $C_{23}H_{22}O_6$ requires C, 70·0; H, 5·6; 3OMe, 23·6%).

Hydrolysis of iso*Derritol* iso*Flavone*.—*iso*Derritol *iso*flavone (250 mg.) was hydrolysed with alcoholic sodium hydroxide as described under derritol *iso*flavone. iso*Derritol methyl ether*, obtained by crystallising the precipitate from methyl alcohol, separated in needles (200 mg.), m. p. 125° (Found: C, 68·8; H, 6·4; OMe, 24·2. $C_{22}H_{24}O_6$ requires C, 68·8; H, 6·3; 3OMe, 24·2%). The aqueous filtrate was not examined for the presence of formic acid.

Isomerisation of Derritol Methyl Ether.—Sulphuric acid (0·5 c.c.) was added to derritol methyl ether (105 mg.) in acetic acid (1 c.c.) at room temperature. Next day water was added, and the solid collected and, after drying, crystallised from methyl alcohol (5 c.c.). Needles and nodules separated, but on slight warming the needles dissolved and filtration gave the nodules (27 mg.). This crop had m. p. 200°, was therefore presumably an anhydroderritol and was not further examined. Dilution of the filtrate led to the separation of *iso*derritol methyl ether (38 mg.) in needles, m. p. 125°, not depressed by the specimen prepared above. In an attempt to effect isomerisation on the steam-bath extensive decomposition occurred with the production of water-soluble substances.

Synthesis of iso*Derritol* iso*Flavone*.—*iso*Derritol methyl ether (200 mg.) and sodium (100 mg.) were treated with ethyl formate (2 c.c.) as described under derritol *iso*flavone above. Crystallisation of the product from methyl alcohol gave *iso*derritol *iso*flavone (55 mg.), m. p. 160°, identical with that prepared by the isomerisation of derritol *iso*flavone.

Dihydroderritol iso*Flavone* (2′ : 4′ : 5′-*Trimethoxy*-5″-iso*propyl*-4″ : 5″-*dihydro*-2″ : 3″ : 7 : 8-*furano*iso*flavone*).—Derritol *iso*flavone (1 g.) in ethyl acetate (100 c.c.) was stirred under hydrogen with palladium–barium sulphate catalyst (0·5 g.) (Houben–Weyl, 2nd Edtn., II, p. 270). Absorption of hydrogen corresponding to one double bond was rapid and towards the end of the reduction the product separated. By filtration of the hot solution and crystallisation *dihydroderritol* iso*flavone* (0·83 g.) was obtained; it crystallised from alcohol in needles, m. p. 193°, $[\alpha]_D^{21}$ −52° in chloroform (c = 1·0) and −67° in benzene (c = 1·0) (Found: C, 69·6; H, 6·25; OMe, 23·4. $C_{23}H_{24}O_6$ requires C, 69·7; H, 6·1; 3OMe, 23·5%). The *iso*flavone was insoluble in 5% aqueous potassium hydroxide.

Synthesis of Elliptol iso*Flavanonol* (2′ : 4′ : 5′-*Trimethoxy*-2″ : 3″ : 7 : 8-*furano*iso*flavanonol*).—Elliptol methyl ether (650 mg.) (this vol., p. 592) and sodium (325 mg.) were treated with ethyl formate (6·5 c.c.) as described under derritol iso*flavone* above. The product obtained by evaporation of the ethyl formate was, however, sparingly soluble in ether and crystallised readily from methyl alcohol in nodules, m. p. 165°. It did not lose water on prolonged drying in a vacuum (Found: C, 65·1; H, 4·9; OMe, 25·0. $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9; 3OMe, 25·1%). Above the m. p. evolution of gas occurred, probably water vapour.

Synthesis of Elliptol iso*Flavone* (2′ : 4′ : 5′-*Trimethoxy*-2″ : 3″ : 7 : 8-*furano*iso*flavone*).—Elliptol *iso*flavanonol (250 mg.) was refluxed in acetic acid (5 c.c.) for 30 mins.; after dilution with water, the precipitate was collected and crystallised from methyl alcohol, in which it was sparingly soluble, giving *elliptol* iso*flavone* in plates (205 mg.), m. p. 185° (Found: C, 67·7; H, 4·8; OMe, 26·6. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·6; 3OMe, 26·4%).

TABLE II.

Colour Reactions.

	Rotenone.	Derritol *iso*flavone.	*iso*Derritol *iso*flavone.	Dihydro-derritol *iso*flavone.	Elliptol *iso*flavone.	Elliptol *iso*-flavanonol.	Toxicarol *iso*flavone.
Durham test (procedure that of Harper, J., 1939, 1100)	Blue	Blue-green	Blue-green	Green	Blue-green	Blue-green	Brick-red
Durham test (procedure that of Jones, *Ind. Eng. Chem.*, 1933, **5**, 75)	Blue	—	—	—	—	Blue-green	—
Goodhue test (*J. Assoc. Off. Agric. Chem.*, 1936, **19**, 118)	Red	—	—	—	—	—	—
Sodium amalgam reduction test (cf. *J. Amer. Chem. Soc.*, 1940, **62**, 1488)	—	Red solution, purple ppte.	Red solution	Red solution	Red solution	Red solution	Purple solution, purple ppt.
Magnesium reduction test (cf. *J. Amer. Chem. Soc.*, 1940, **62**, 1488)	—	—	—	—	—	—	—
Sulphuric acid in acetic acid	—	Yellow	Yellow	Yellow	Yellow	Yellow on heating	Yellow

Durham Test.—The following additional substances gave a positive colour reaction with either procedure (*i.e.*, a blue or green colour): Derritol methyl ether, *iso*derritol methyl ether, elliptol methyl ether, derritol, elliptol, derrisic acid, and elliptic acid.

Semimicro-detection of Formic Acid as a Hydrolysis Product of iso*Flavones*.—The synthetic *iso*flavones described above being used, the following method was devised for the detection of formic acid in the alkaline hydrolysis of *iso*flavones and then applied to the "toxicarol *iso*flavone" described earlier (J., 1940, 1178).

10—15 Mg. of the substance were refluxed in 1 c.c. of alkali solution (10% sodium hydroxide in 1 : 1 alcohol–water) for 15 minutes in a flask with a ground joint. A steam distillation head was attached and 25 c.c. of water were distilled over, the contents of the flask being kept at constant volume. After acidification with 10% phosphoric acid (2 c.c.)

the steam-distillation was continued at constant volume until a further 50 c.c. of distillate had been obtained. This distillate was made alkaline with 0·5N-sodium carbonate (0·5 c.c.) and evaporated to dryness in a 50 c.c. flask. The residue was acidified with 10% acetic acid (0·5 c.c.), 10 c.c. of saturated mercuric chloride in 2% acetic acid added, and the flask stoppered and heated in a boiling water-bath for 2 hours. The precipitated mercurous chloride was collected in a small Gooch crucible, washed with N-hydrochloric acid, water, and 95% alcohol, and dried at 100° for 30 mins.

Formic acid (1 mg.) quantitatively precipitated mercurous chloride (10·2 mg.) with this reagent (cf. Miles and Pirie, *Biochem. J.*, 1939, **33**, 1709).

Derritol *iso*flavone (11·9 mg.) gave 6·55 mg. of mercurous chloride (Found: formic acid, 5·4. Calc. for $C_{23}H_{22}O_6$, 11·7%. Recovery, 46%).

Toxicarol *iso*flavone (13·7 mg.) gave 8·20 mg of mercurous chloride (Found: formic acid, 5·8. Calc. for $C_{23}H_{22}O_7$, 11·2%. Recovery, 56%).

Rotenone and *dl*-*a*-toxicarol gave no formic acid under the above conditions. The recovery of formic acid by steam-distillation of a limited volume is incomplete, but in the two cases the percentage recovery is of an order consistent with the formulæ given to them.

I am indebted to Dr. C. Potter for carrying out the biological trials recorded in this and the previous paper and to the Ministry of Agriculture and the Colonial Development Fund for grants.

ROTHAMSTED EXPERIMENTAL STATION, HARPENDEN, HERTS. [*Received, June 3rd*, 1942.]