

# NUCLEAR OXIDATION IN FLAVONES AND RELATED COMPOUNDS

## Part XXXVI. Oxidation of Some Hydroxy Isoflavones

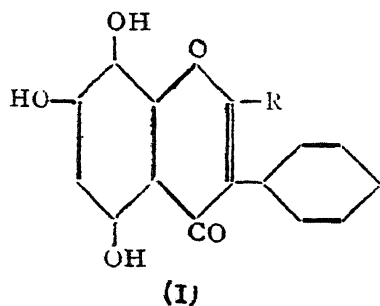
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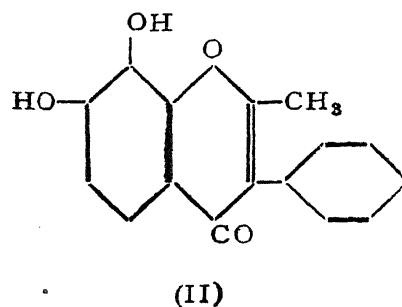
IN the earlier publications of this series<sup>1</sup> the application of two processes of nuclear oxidation to the flavone group and certain related compounds was described in detail. The first is the single stage process using alkaline persulphate and it introduces the hydroxyl group satisfactorily in the position para to an activating hydroxyl group. It is however not satisfactory for ortho oxidation. The two-stage process involving an ortho-hydroxy-aldehyde as an intermediate and its oxidation with hydrogen peroxide is quite satisfactory. Though the preparation of *p*-hydroxy-aldehyde is easy, its oxidation with hydrogen peroxide to yield the corresponding para dihydroxy compound is not satisfactory<sup>2</sup> and the two stage process is not therefore efficient for para oxidation. The two processes would therefore appear to be complimentary to each other.

As far as our present knowledge goes, isoflavones do not exhibit such a large range of structural variations as the flavones and flavonols. Still there are indications that nuclear oxidation plays an important part in the evolution of certain members of these groups also, *e.g.*, 5:6:7-trihydroxy type is found in irigenin and tectorigenin. In this paper are described experiments testing the applicability of nuclear oxidation methods to isoflavones. They are found to work satisfactorily and 5:7:8-trihydroxy-2-methyl isoflavone (I *a*), 5:7:8-trihydroxy isoflavone (I *b*) and 7:8-dihydroxy-2-methyl isoflavone (II) and their derivatives are prepared and described.



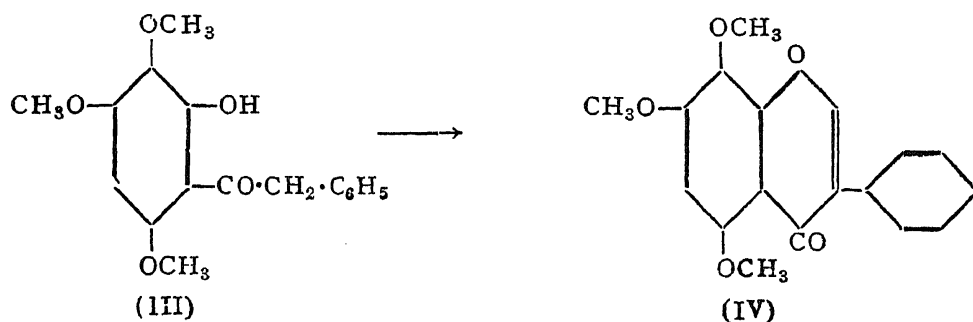
*a*, R = CH<sub>3</sub>

*b*, R = H



Another object of the present work is to test the possibility of the isomerisation of the 5:7:8-trihydroxy isoflavone into the isomeric 5:6:7-trihydroxy compound by the action of hydriodic acid. This type of isomeric change is known to take place very readily with flavones having 5:7:8-trihydroxy grouping and not with flavonol derivatives.<sup>3</sup> The position is similar in 2-methyl chromone derivatives. It has recently been shown that the methyl ether of 2-methyl-5:7:8-trihydroxy chromone undergoes isomeric change on boiling with hydriodic acid whereas the corresponding 3-methoxy compound does not.<sup>4</sup> It is now found that 2-methyl-5:7:8-trimethoxy isoflavone yields the corresponding trihydroxy compound on demethylation with hydriodic acid. The result has been confirmed by remethylation whereby the original trimethyl ether is obtained. Consequently it is clear that even a phenyl group in the 3-position is capable of preventing this isomeric change.

The above experiments do not however provide information about the influence of substitution in the 2-position. In all the hitherto known cases where the isomeric change was prevented, this position contained a phenyl or methyl group in addition to substitution in the 3-position. In order to clarify this point 5:7:8-trimethoxy isoflavone (IV) has now been prepared from 2-hydroxy-3:4:6-trimethoxy-phenyl-benzyl ketone (III) by condensation with ethyl formate and demethylated with hydriodic acid and with anhydrous aluminium chloride. Both methods yield the same trihydroxy isoflavone giving reactions agreeing with the expected behaviour of 5:7:8-trihydroxy compounds. Further, persulphate oxidation of 5:7-dihydroxy isoflavone yields the same product and both samples give identical derivatives. Hence there is no doubt that substitution in the 2-position has no influence and that the 3-position alone is important.



#### EXPERIMENTAL

##### 5:7:8-Trihydroxy-2-methyl-isoflavone (Ia)

5:7-Dihydroxy-2-methyl isoflavone (1.4 g.) was dissolved in 4% aqueous sodium hydroxide (50 c.c.) and potassium persulphate solution (2 g. in 50 c.c.) was added dropwise with stirring during the course of two hours. The

solution was kept between 15–20° throughout the addition. After 24 hours, the solution was neutralised to litmus with dilute hydrochloric acid. A pale yellow precipitate of the unreacted dihydroxy isoflavone separated out. It was filtered and the filtrate extracted with ether twice to remove the last traces of it. The aqueous solution was then treated with concentrated hydrochloric acid (25 c.c.) and sodium sulphite (1 g.) and heated on the water-bath for 20 minutes. A pale brown crystalline solid separated out on cooling. It was crystallised from alcohol when the 5:7:8-trihydroxy-2-methyl isoflavone separated out in the form of pale yellowish brown elongated needles melting at 204–5°. Yield 0.4 g. (Found in a sample dried at 110° for 3 hours: C, 67.7; H, 4.2;  $C_{16}H_{12}O_5$  requires C, 67.6; H, 4.2%). Its alcoholic solution developed dark olive green colour with ferric chloride changing slowly to dark brown and a brown red colour with *p*-benzoquinone. It dissolved in aqueous alkali producing a bright yellow colour changing rapidly into brown, pink, bluish violet and finally to pale yellow after 15 minutes. It readily dissolved in 5% aqueous sodium carbonate giving a reddish brown solution changing to pale yellow. In concentrated sulphuric acid it gave a yellowish red colour with no fluorescence.

The triacetate obtained by heating the isoflavone (Ia) with acetic anhydride and sodium acetate crystallised from alcohol as colourless prismatic rods melting at 220–21°. (Found in sample dried at 100° for 3 hours: C, 64.5; H, 4.1;  $C_{22}H_{18}O_8$  requires C, 64.4; H, 4.4%.)

*Methylation.*—The trihydroxy-2-methyl isoflavone (0.3 g.) was methylated by refluxing with dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (5 g.) in dry acetone medium (100 c.c.). After 15 hours another lot of dimethyl sulphate (1 c.c.) was added and refluxing continued for 15 hours more. The acetone was then distilled off and water added to the residue. A semi-solid mass separated out which gradually solidified in the course of a few hours. The solid was filtered and crystallised from alcohol. The trimethyl ether separated in the form of transparent stout rectangular prismatic rods melting at 92–3°. (Found: C, 69.9; H, 5.8;  $C_{19}H_{18}O_5$  requires C, 69.9; H, 5.5%.) It was insoluble in alkali and gave no colour with alcoholic ferric chloride.

*Demethylation of 5:7:8-trimethoxy-2-methyl isoflavone*

The trimethyl ether described above (0.3 g.) was demethylated with hydriodic acid and the product worked up as given later for an analogous case. It crystallised from alcohol as needles melting at 204–205° (0.15 g.). It was identical in every respect with 5:7:8-trihydroxy-2-methyl isoflavone des-

cribed above and the mixed melting point was undepressed. On remethylation it produced the original trimethyl ether, m.p. and mixed m.p. 92–93°.

*7:8-Dihydroxy-2-methyl isoflavone (II)*

(i) *Oxidation of 7-hydroxy-2-methyl-isoflavone-8-aldehyde*.—A solution of the aldehyde<sup>5</sup> (0.7 g.) in aqueous alkali (N/2, 6 c.c.) was treated dropwise with hydrogen peroxide (5%, 3 c.c.) in the course of 15 minutes with stirring. During the addition, the solution was kept at the room temperature by cooling in water. Within half an hour a pale yellow solid began to separate out. After 3 hours the mixture was acidified with hydrochloric acid while cooling. The solid product was then filtered and crystallised twice from alcohol when the dihydroxy isoflavone came out in the form of pale yellow rectangular plates melting at 227–8°. Yield 0.5 g. (Found: C, 66.8; H, 4.7;  $C_{16}H_{12}O_4$ ,  $H_2O$  requires C, 67.1; H, 4.9%). It dissolved in aqueous alkali yielding a stable yellow solution. Ferric chloride developed a bright green colour in alcoholic solution. Its solution in concentrated sulphuric acid was yellow without fluorescence. It was easily soluble in acetone and alcohol.

(ii) *Oxidation of 7-hydroxy-2-methyl isoflavone*.—To a continuously stirred solution of 7-hydroxy-2-methyl isoflavone (3.5 g.) in aqueous alkali (4%, 100 c.c.), a solution of potassium persulphate (4.2 g. in 75 c.c. of water) was added dropwise in the course of 3 hours. After 24 hours, the product was worked up as in similar cases. It crystallised from alcohol in the form of pale yellow prismatic plates melting at 227–8°. Yield 0.2 g. It was identical with 7:8-dihydroxy-2-methyl isoflavone obtained by method (i) and the mixed melting point was undepressed.

*Methylation*.—The dihydroxy isoflavone (0.4 g.) was refluxed in dry acetone (60 c.c.) with dimethyl sulphate (0.8 c.c.) and anhydrous potassium carbonate (2 g.) for 10 hours and the product worked up. The dimethyl ether crystallised from alcohol as colourless hexagonal prisms melting at 133–4°. Yield 0.3 g. (Found: C, 72.8; H, 5.0;  $C_{18}H_{16}O_4$  requires C, 73.0; H, 5.4%). It gave no colour with ferric chloride and was insoluble in aqueous alkali.

*2-Hydroxy-3:4:6-trimethoxy-phenyl-benzyl ketone (III)*

Anhydrous aluminium chloride (15 g.) was dissolved in dry ether (200 c.c.) with cooling and to the solution was added 1:2:3:5-tetramethoxy benzene (8 g.) in dry ether. The mixture was then treated with phenyl acetyl chloride (8 g.) while cooling in ice and the reaction mixture was kept in the ice-bath for 2 hours and at room temperature for 12 hours. Ether was

removed on a warm water-bath, the remaining organo-aluminium complex first treated with ice and hydrochloric acid and subsequently heated on a water-bath to complete the hydrolysis. The solid that separated on cooling was filtered and washed with water. It was purified by dissolving it in aqueous sodium hydroxide, filtering the solution and reprecipitating it by acidification. 2-Hydroxy-3:4:6-trimethoxy-phenyl-benzyl ketone crystallised from alcohol in the form of colourless broad rectangular plates melting at 86-7°. It gave a deep red colour with ferric chloride. (Found: C, 67.6; H, 6.2;  $C_{17}H_{18}O_5$  requires C, 67.5; H, 6.0%.)

*5:7:8-Trimethoxy isoflavone (IV)*

Pulverised sodium (1 g.) cooled in ice, was treated with a solution of 2-hydroxy-3:4:6-trimethoxy-phenyl-benzyl ketone (2 g.) in dry ethyl formate (10 c.c.). The mixture was kept in the refrigerator for 24 hours at the end of which pieces of ice were added to the reaction mixture. On removing the excess of ethyl formate under reduced pressure a colourless crystalline solid separated. Acidification of the aqueous solution gave a little more of it. It was filtered, washed with water and crystallised from alcohol. On recrystallising from ethyl acetate, 5:7:8-trimethoxy isoflavone separated as colourless stout rectangular prisms melting at 146-7°. It gave no colour with ferric chloride and was insoluble in aqueous sodium hydroxide. Yield, 1.2 g. (Found: C, 65.7; H, 5.7;  $C_{18}H_{16}O_5$ ,  $H_2O$  requires C, 65.4; H, 5.7%.)

*5:7:8-Trihydroxy isoflavone (Ib)*

(i) *By demethylation with hydriodic acid.*—An acetic anhydride solution of 5:7:8-trimethoxy isoflavone (1 g. in 5 c.c.) was treated with cooling with hydriodic acid (sp. gr. 1.7, 10 c.c.) and the mixture heated in an oil bath at 140° for 3 hours. It was then cooled and diluted with sulphur dioxide water. The bright yellow solid that separated was filtered, washed with water and crystallised from alcohol. On recrystallising from ethyl acetate it was obtained as shining yellow thin plates melting at 280-2°. It gave a green colour changing to brown slowly with ferric chloride in alcohol and a yellow solution changing to brown with aqueous sodium hydroxide. It was identical in its properties with the samples of 5:7:8-trihydroxy isoflavone described below and the mixed melting points were undepressed, (Found: C, 66.2; H, 4.0;  $C_{15}H_{10}O_5$  requires C, 66.7; H, 3.7%.)

The triacetate crystallised from alcohol as colourless needles melting at 206-8° and the mixed melting point with the acetate sample described

later (expt. iii) was undepressed. (Found: C, 64.0; H, 4.4;  $C_{21}H_{16}O_8$  requires C, 63.6; H, 4.0%.)

(ii) *By persulphate oxidation.*—A stirred solution of 5:7-dihydroxy isoflavone (1.7 g.) in sodium hydroxide (20 c.c., 1 N) was treated with sodium persulphate solution (4 g. in 30 c.c. water) during the course of 3 hours the temperature being maintained between 15 and 20°. After 24 hours the solution was acidified to congo-red and the unchanged dihydroxy isoflavone filtered off and finally extracted with ether. The aqueous solution was then treated with sodium sulphite (1 g.) and concentrated hydrochloric acid (20 c.c.), heated to 90° on a water-bath and allowed to cool gradually when a yellow solid separated. After leaving overnight the product was filtered, washed with water and crystallised from alcohol when it was obtained in the form of bright yellow plates melting at 280–2°. It gave a green colour changing to brown with alcoholic ferric chloride and agreed in all its properties with samples obtained by other methods. Yield, 0.5 g.

(iii) *By demethylation with aluminium chloride.*—The trimethoxy isoflavone (0.2 g.) was dissolved in dry benzene (10 c.c.), treated with anhydrous aluminium chloride (1 g.) and the mixture refluxed on a water-bath for 2 hours. Benzene was then removed on a boiling water-bath and the residue treated with ice and hydrochloric acid. The mixture was warmed on a water-bath for a few minutes, cooled and the solid product filtered. It crystallised from alcohol as yellow thin plates melting at 280–2° and agreed in its properties with the compound obtained in the above experiments and the mixed melting point showed no depression. On acetylation the triacetate was obtained which crystallised from alcohol in the form of colourless needles melting at 206–8°.

#### 5-Hydroxy-7:8-dimethoxy isoflavone

5:7:8-Trihydroxy isoflavone (obtained by demethylation with hydriodic acid in expt. i) (0.3 g.) was partially methylated by heating with dimethyl sulphate (0.25 c.c.) and anhydrous potassium carbonate (1 g.) in acetone solution for 6 hours. On filtering and removing the solvent from the filtrate the product separated as a yellow solid. It crystallised from alcohol as pale yellow prisms melting at 156–7°. It gave a green colour with ferric chloride and was sparingly soluble in aqueous alkali. It was identical with a similar derivative prepared from the sample obtained by oxidation with persulphate (in exp. ii). (Found: C, 68.0; H, 4.7;  $C_{17}H_{14}O_5$  requires C, 68.5; H, 4.7%.)

## SUMMARY

Using methods of nuclear oxidation 7:8-dihydroxy-2-methyl isoflavone, 5:7:8-trihydroxyisoflavone and 5:7:8-trihydroxy-2-methyl isoflavone and their derivatives have been prepared. Demethylation of the 5:7:8-trimethoxy isoflavones with or without a 2-methyl group does not produce isomeric change in the trihydroxy product. It could therefore be concluded that a phenyl group in the 3-position prevents this isomeric change just like a methoxyl (hydroxyl) in the same position and that substitution in the 2-position has no influence.

## REFERENCES

1. Seshadri .. *Proc. Ind. Acad. Sci., A*, 1948, **28**, 1, 1949, **30**, 333.
2. ——— and Varadarajan .. *Ibid.*, 1949, **30**, 342.
3. Sastri and Seshadri .. *Ibid.*, 1946, **24**, 243.  
Rao, Seshadri and Viswanadham .. *Ibid.*, 1949, **29**, 72.
4. Chakravorty, Mukherjee, Murti .. *Ibid.*, 1952, **35**, 34.  
and Seshadri
5. Row and Seshadri .. *Ibid.*, 1951, **34**, 192.