COMPONENTS OF THE BARK OF *PRUNUS PUDDUM**

Part II. Padmakastin and Padmakastein

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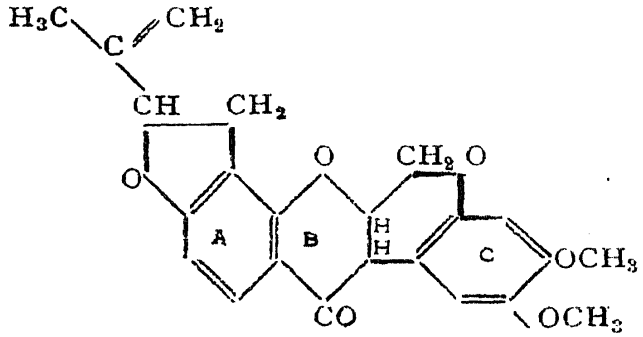
IN the complex molecular structure of rotenone (I) and other rotenoids in which there are five rings, the essential core would appear to be the isoflavanone structure (A, B and C). Though many isoflavones have been obtained from plant sources and by synthesis no simple dihydroderivatives (isoflavanones) have so far been recorded in the literature and characterised. In this paper are described the isolation of the first member representing this core of rotenoids. Padmakastin is the glycoside and Padmakastein (II) is the aglucone newly obtained from the bark of *Prunus puddum* and belonging to the abovementioned new type. The new names are derived from the Sanskrit name of this plant, *Padma kashta*. The more convenient names like puddumetin and prunusetin derived from the botanical name of the plant have already been employed[1] for other substances obtained from it though they are not new and have other names. Their use in the new context will create confusion.



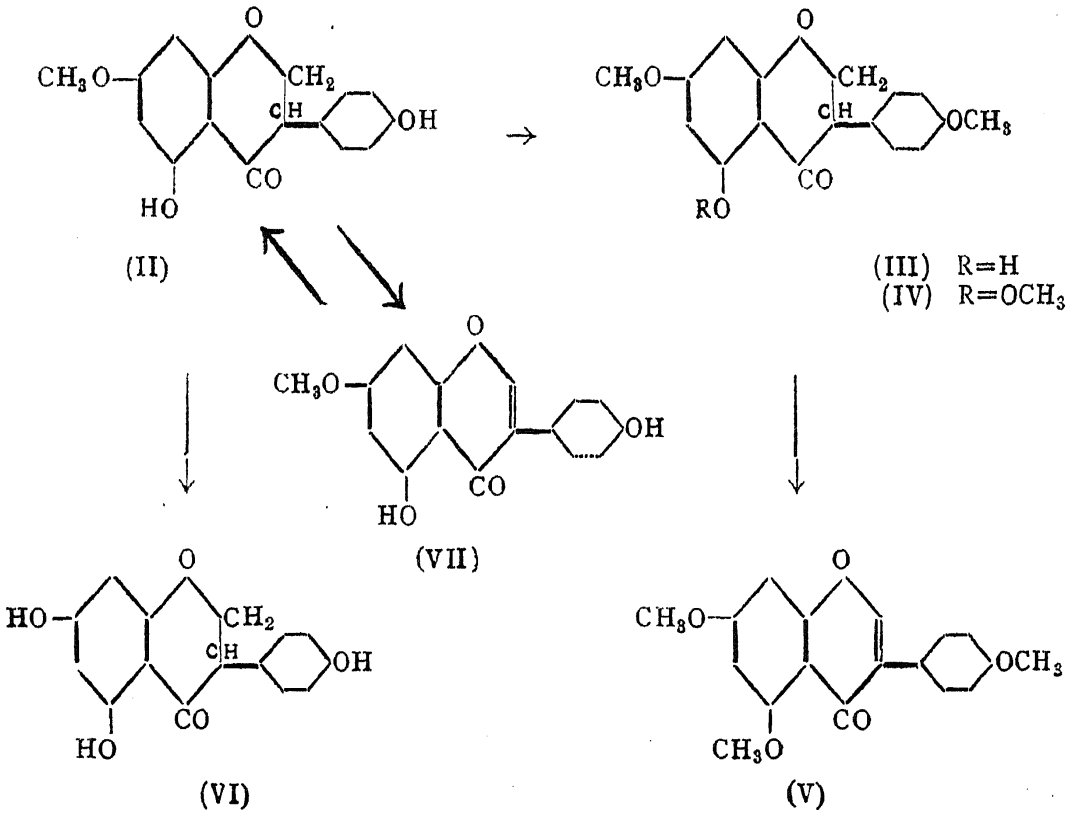
(I)

In our efforts to get prunetin, sakuranetin and genkwanin from the bark of *Prunus puddum*[2] we noticed there was considerable variation in the composition of samples obtained in various seasons. Two points are noteworthy. Samples collected in late summer (reported in our earlier publication[2]) contained very small quantities of glycosides whereas those obtained in late winter gave considerable yields of the glycosidic fraction. Further in the summer sample along with sakuranetin there was found another

* An earlier paper[2] on this subject entitled "A Note on the Components of the Bark of *Prunus puddum*" is considered to be Part I.

substance (not reported earlier) in very small quantity. This was present in larger quantities in the winter sample. Actually it has been possible to isolate this substance in adequate yield for a chemical examination from a sample of the bark obtained in February 1951. It is a non-glycosidic body identical with the aglucone of the new glycoside mentioned below. Further the glycoside fraction could be separated into two; the more soluble portion contained largely sakuranin whereas the less soluble fraction consisted of a new glycoside padmakastin. The aglucone padmakastein (II) has nothing in common with a substance reported by Chakravarti, Kundu and Ghosh[3] as found along with sakuranetin in the sample investigated by them. There is difference in melting point, colour reaction and composition. It is definite therefore that the new glycoside and its aglucone are now reported for the first time.

Padmakastein (II) has the molecular formula $C_{16}H_{14}O_5$, contains one methoxyl group and yields a diacetate. It undergoes partial methylation to form a monomethyl ether (III) and complete methylation to a dimethyl ether (IV). This along with the fact that it gives a prominent colour with ferric chloride would indicate the presence of a hydroxyl group situated ortho to a carbonyl group. The substance does not give the usual flavone or flavanone colour reaction with magnesium and hydrochloric acid but gives a green colour with nitric acid which slowly changes to red. This colour reaction is characteristic of certain hydroxy acetophenone derivatives (methyl ethers) and also of flavanone methyl ethers.[4] The red colour changes to green on addition of ammonia. This is positive Durham test. The sulphuric acid solution is colourless and on adding a few drops of conc. nitric acid develops a feeble yellowish red colour. Another significant feature is the capacity of the substance to reduce iodine. These reactions gave indications that padmakastein is in fact an isoflavanone having the essential features of the rotenone group. There seems to be no doubt that this part is the one responsible for the positive Durham test. The isoflavanone constitution has been confirmed by the dehydrogenation of padmakastein dimethyl ether (IV) with selenium dioxide in amyl alcohol solution yielding genistein trimethyl ether (V) which on demethylation yields genistein. Thus it is definite that padmakastein is a dihydrogenistein-monomethyl ether. The location of the methoxyl group became clear from the following considerations. The substance is insoluble in aqueous sodium carbonate but on demethylation with aluminium chloride it yields the nor compound (VI) which is soluble in this reagent. From considerations already explained elsewhere[5] it follows that the methoxyl group is located in the 7-position and that padmakastein is dihydroprunetin (II).



It has also been possible to reduce prunetin (VII) by means of sodium *meta*-bisulphite to the dihydro derivative which is found to be identical with the new aglucone padmakastein. The above reducing agent seems to provide a convenient method for the reduction of the pyrone ring to the corresponding pyranone. In this connection may be mentioned the recent report of Robertson, *et al.*[6] who studied catalytic hydrogenation of santal methyl ether and were able to obtain a non-ketonic product which they considered to be produced by the reduction of the CO group in addition to ethylenic double bond. Hence the *meta*-bisulphite method, has the distinct advantage of reduction of the ethylenic double bond only.

Another reaction which we have successfully carried out should also be mentioned. Earlier experiments on the use of selenium dioxide for dehydrogenation of flavanones or chalkones were found to proceed satisfactorily only for producing fully methylated flavones.[7] This method could not be successfully used for the preparation of partial methyl ethers of flavones.[3] It is now found that the acetate of padmakastein undergoes dehydrogenation with selenium dioxide in acetic anhydride solution very satisfactorily to yield prunetin acetate which on deacetylation yields prunetin (VII). This procedure is capable of being adopted in analogous cases of flavanone partial methyl ethers.

The association of sakuranetin and padmakastein is parallel to that of genkwanin and prunetin. As for the glycosidic fraction it seems to consist

entirely of the reduced compounds sakuranin and padmakastin. The nature of sugar and its position in padmakastin will be discussed later.

Experimental

Extraction.—The sun-dried bark of *Prunus puddum* (3 kg.) was powdered and extracted twice with cold alcohol by percolation. Each extraction was carried out for 48 hours. The deep red alcoholic extract was concentrated to about 500 c.c. and allowed to stand; after two days a yellow sticky solid separated out. This was filtered and the residue marked (fraction A). The alcoholic filtrate was then treated with excess of ether (1 litre) till it formed two layers and left in the ice chest. After a few days an almost colourless solid (very pale yellow) separated out. It was filtered, washed with ether and marked (fraction B). After separating the ether layer the mother-liquor was twice extracted with hot benzene, and the ether and benzene fractions combined (fraction C). The residual viscous liquid (marked D) was directly hydrolysed with aqueous alcoholic sulphuric acid (7%).

Fraction A (Genkwanin, prunetin and padmakastein).—The solid was boiled with benzene (10 c.c.) twice and filtered. The residue was then boiled with excess of alcohol (60 c.c.) and the clear solution filtered. On cooling it slowly deposited a yellow solid which when recrystallised from alcohol melted at 285° and was identical with genkwanin. When the alcoholic filtrate was concentrated (to 40 c.c.) and then cooled a small quantity of a pale yellow solid separated. After two crystallisations the compound melted at 236–8° and was found to be identical with prunetin. On completely distilling off alcohol from the mother-liquor an almost colourless solid separated. After two crystallisations from alcohol it melted between 225–30° and further crystallisations did not improve the melting point. The product was acetylated with acetic anhydride and pyridine and the dry acetate crystallised from ethyl acetate. The fraction that separated on cooling melted at 218–20°. A recrystallisation from ethyl acetate yielded glistening colourless rhombic prisms having the same melting point. It slowly developed a green colour with conc. nitric acid (Found: C, 65·3; H, 5·0; $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9%). By further concentration of the ethyl acetate solution and crystallisation of the product a little more of the above acetate was obtained. Complete removal of the ethyl acetate gave some impure substance melting between 190–95° and was not worked up.

When the acetate was deacetylated by heating in alcohol (10 c.c.) with conc. hydrochloric acid (10 c.c.) on a water-bath for 15 minutes the hydroxy compound separated as colourless needles. The mixture was diluted with water (20 c.c.) and the solid product filtered and washed with water. On

crystallising from alcohol the compound separated as colourless rectangular plates and prisms melting at 236–8°; a mixed m.p. with prunetin was depressed. It gave a green colour with conc. nitric acid; the colour changed red on standing, but the green colour was again produced on adding strong ammonia. The substance formed a reddish violet colour with ferric chloride. It was insoluble in aqueous sodium carbonate but dissolved readily in aqueous sodium hydroxide giving a yellow solution. An alcoholic solution of iodine and sodium acetate was readily decolourised when added to an alcoholic solution of this substance. It gave no colour with magnesium and hydrochloric acid. It differed in its properties and reactions from the other components of the bark and is given the name padmakastein (Found: C, 66·7; H, 5·0; $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%). Yield, genkwanin, 0·5 g.; prunetin, 1 g. and padmakastein, 1 g.

Fraction B (*padmakastin and sakuranin*).—The crude glycoside was boiled with alcohol (15 c.c.) twice and filtered when only part of it went into solution. The residue was repeatedly crystallised from alcohol and then it melted at 225–7° (padmakastin). It gave a red colour with ferric chloride and a green colour with conc. nitric acid. On hydrolysis with 7% aqueous alcoholic sulphuric acid it gave padmakastein. When the original alcoholic solution was cooled it yielded some more of the new glycoside. On concentrating it further and crystallising the product from aqueous alcohol a colourless crystalline solid was obtained which melted at 210–12° and agreed in all its properties with the description of sakuranin. Yield: new glycoside (padmakastin), 2·0 g. and sakuranin, 1 g.

Fraction C.—This was distilled to remove benzene and worked up as described earlier.[2] Yield of sakuranetin, 3 g.

Fraction D.—The viscous liquid (marked D) was diluted with water (400 c.c.), treated with conc. sulphuric acid (16 c.c.) and the solution refluxed for 2 hours. It was then cooled and left in the refrigerator for 24 hours. The brown solid that separated was filtered; it was boiled twice with benzene (20 c.c.) to remove sakuranetin and filtered. The residue on crystallisation from alcohol melted at 234–36°. It was directly acetylated and the acetate crystallised from dry ethyl acetate from which it came out as colourless rhombic prisms melting at 218–20°. It gave a green colour with conc. nitric acid. On deacetylation with alcoholic hydrochloric acid it gave padmakastein which crystallised from alcohol as colourless rectangular prisms and plates melting at 236–8°. Both the acetate and the hydroxy compound were identical with the samples obtained from the fraction A. Yield of padmakastein, 2·5 g.

Partial methylation (*padmakastein monomethyl ether*) (*III*)

Padmakastein (0·3 g.) was refluxed in acetone (30 c.c.) solution with dimethylsulphate (0·15 c.c.) and anhydrous potassium carbonate (0·5 g.) for 6 hours. On filtering and distilling off acetone from the filtrate a pale yellow solid separated. It crystallised from alcohol as colourless stout prisms melting at 131–32°. It gave a red colour with ferric chloride in alcohol and a green colour with conc. nitric acid (Found: C, 68·4; H, 5·4; $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·3%).

Complete methylation (*padmakastein dimethyl ether*) (*IV*)

Padmakastein (0·5 g.) was refluxed for 24 hours in acetone solution with excess dimethyl sulphate (0·7 c.c.) and anhydrous potassium carbonate (1·5 g.). Acetone was then distilled off, water added to the residue and the solid filtered. The methyl ether crystallised from alcohol as colourless thin leaf-like crystals melting at 146–7°. It gave no colour with ferric chloride and was insoluble in aqueous alkali. It developed a green colour with conc. nitric acid (Found: C, 68·4; H, 5·5; $C_{18}H_{18}O_5$ requires C, 68·8; H, 5·7%).

Demethylation with anhydrous aluminium chloride (*nor-padmakastein*) (*VI*)

Padmakastein (80 mg.) was heated with anhydrous aluminium chloride (0·5 g.) in benzene (10 c.c.) for 2 hours. Benzene was then removed by evaporation and the organo metallic complex decomposed with ice and hydrochloric acid. The solid that separated was filtered, washed with dilute hydrochloric acid and water. It was then dissolved in aqueous sodium carbonate, the solution filtered and the clear filtrate acidified. The colourless product was filtered, washed with water and crystallised from alcohol when it came out as colourless short prisms melting at 270–2°. It gave a reddish violet colour with ferric chloride and was easily soluble in aqueous sodium carbonate (Found: C, 63·6; H, 4·5; loss on drying *in vacuo* at 110°, 3·1; $C_{15}H_{12}O_5$, $\frac{1}{2}H_2O$ requires C, 64·0; H, 4·6; loss on drying 3·2%).

Selenium dioxide oxidation (*O-trimethyl genistein*) (*V*)

Padmakastein dimethyl ether (0·2 g.) was dissolved in amyl alcohol (10 c.c.), selenium dioxide (0·25 g.) added and the mixture heated at 140° for 12 hours. Metallic selenium that was formed in the reaction was then filtered off and from the filtrate amyl alcohol was removed by distillation on a water-bath under reduced pressure. The last traces of amyl alcohol were removed by passing a current of steam. The solid residue was filtered and crystallised from alcohol. It was obtained as colourless needles melting

at 161–2° and was identical with genistein trimethyl ether, the mixed melting point with an authentic sample being undepressed. Yield, 0·12 g.

It was demethylated by heating with anhydrous aluminium chloride in benzene solution. The product which crystallised from aqueous alcohol as colourless tiny prisms melted at 290° and was identical with genistein the mixed melting point being undepressed.

Selenium dioxide oxidation of padmakastein acetate (prunetin acetate)

A mixture of padmakastein acetate (0·2 g.) and selenium dioxide (0·2 g.) in acetic anhydride (7 c.c.) was heated in an oil-bath at 140° for 12 hours. Metallic selenium was filtered off and the acetic anhydride solution poured into ice water. The colurless solid that separated was filtered and washed with water. After drying it was crystallised first from absolute alcohol and again from ethyl acetate when it separated as small rectangular prisms and needles melting at 222–4°. A mixed melting point with an authentic sample of prunetin acetate did not show any depression. Deacetylation of this acetate gave prunetin (VII) (m.p. and mixed m.p. 238–40°).

Reduction of prunetin (VII) to padmakastein (II)

A solution of prunetin (0·5 g.) in aqueous sodium hydroxide (2·5 g. in 50 c.c. of water) was treated with solid sodium *meta*-bisulphite (5 g.) in small quantities with boiling. After the addition was over the solution was boiled for further 5 minutes. It was then cooled in ice and acidified with hydrochloric acid. The colourless solid that separated was filtered, washed with water and crystallised from alcohol. The product separated as colourless rectangular plates and prisms melting at 236–8° with sintering at about 228°. It gave a green colour with conc. nitric acid and decolourised iodine and sodium acetate in alcohol. It was directly acetylated by heating with acetic anhydride and pyridine. The acetate crystallised from ethyl acetate as colourless glistening rectangular plates and prisms melting at 218–20° and mixture with padmakastein acetate described earlier melted at the same temperature.

SUMMARY

Two new substances, padmakastin, a glycoside and padmakastein, the corresponding aglucone have been isolated from the bark of *Prunus puddum*. The properties and reactions of padmakastein indicate that it is dihydroprunetin. This has been confirmed by its oxidation to prunetin and by its preparation from prunetin by reduction. Padmakastein is the first

isoflavanone to be isolated and it gives the well-known Durham test characteristic of rotenoids.

References

1. Chakravarti, *et al.* .. *J. I. C. S.*, 1944, 171 ; 1945, 301.
2. Narasimhachari and Seshadri .. *Proc. Ind. Acad. Sci.*, 1949 A, **30**, 271.
3. Chakravarti, Kundu and Ghosh .. *J. I. C. S.*, 1948, 329.
4. Rao and Seshadri .. *Proc. Ind. Acad. Sci.*, 1949 A, **30**, 30.
5. Narasimhachari and Seshadri .. *Ibid.*, 1950, 32, 256.
6. Robertson, Suckling and Whalley *J. C. S.*, 1949, 1571.
7. Mahal, Rai and Venkataraman .. *Ibid.*, 1935, 866.