COMPONENTS OF THE BARK OF *PRUNUS PUDDUM**

Part III. Synthesis of Padmakastein and its Derivatives

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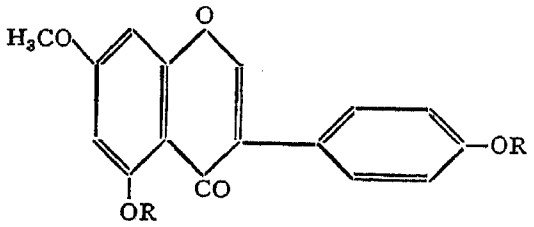
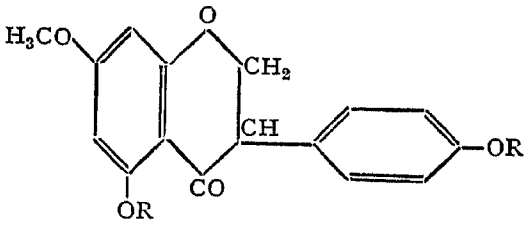
PADMAKASTEIN (I)[1] was the first isoflavanone to be isolated from plant materials. Its constitution was based mainly on the dehydrogenation of the complete methyl ether (II) by means of selenium dioxide to yield prunetin dimethyl ether (III) and of the acetate (IV) to prunetin diacetate (V). It was also reported that prunetin (VI) could be reduced by means of sodium meta-bisulphite to give padmakastein (I). Later experiments carried out in this laboratory have shown that sodium hydrosulphite and sodium meta-bisulphite are not suitable for reducing isoflavones. Prunetin (VI) is unaffected when a weakly basic medium (aqueous alcoholic sodium carbonate) is employed. In strongly alkaline solution (aqueous sodium hydroxide) a product is obtained which has melting point, colour reactions and analytical values somewhat near those of the isoflavanone (I). But the difference was noted when its derivatives were prepared and it could now be identified as 2: 6: 4′-trihydroxy-4-methoxy desoxybenzoin arising out of the fission of the isoflavone.



(III) $R=CH_3$
(V) $R=COCH_3$
(VI) $R=H$



(I) $R=H$
(II) $R=CH_3$
(IV) $R=COCH_3$

Other methods of reduction were therefore attempted. Catalytic hydrogenation using platinum oxide catalyst was reported by Robertson *et al.*[2] to carry the reduction of santal methyl ether too far, affecting the carbonyl group also. But by suitably restricting the time, Bradbury and White[3]

* Part II. Narasimhachari and Seshadri, *Proc. Ind. Acad. Sci.*, 1952, 35 A, 202.

succeeded in reducing prunetin dimethyl ether (III) to the dihydro derivative (II). But the palladised charcoal catalyst employed by King and his co-workers[4–6] for isoflavones appeared to be better and we used it for the reduction of prunetin dimethyl ether (III) quite satisfactorily. The work was completed in 1956 but owing to lack of the natural sample for comparison it was not published. Meanwhile Gilbert *et al.*[7] in connection with their work on iso-shekkangenin also reduced prunetin dimethyl ether (III) in this way.

This method of reduction has also been applied for the synthesis of padmakastein (I) itself. Prunetin diacetate (V) was subjected to this reduction and the dihydro compound (IV) (padmakastein diacetate) was deacetylated with alcoholic hydrochloric acid. The product agreed with padmakastein (I) in every respect. Comparison was also made between the acetates and the methyl ethers of the natural and synthetic compounds.

For the above mentioned comparison a fresh sample of padmakastein (I) was obtained from the bark of *Prunus puddum* using a modified procedure. In the course of this work it was found that a small amount of taxifolin was also present along with other components already reported.[1] No glycoside of padmakastein (I) could be obtained and the yields of the components were poor except for sakuranin which was the major product.

EXPERIMENTAL

Extraction of the bark of Prunus puddum.—The sample was collected in the winter of 1956 and supplied by Messrs. Ghose and Co., Darjeeling.

The extraction of the bark (1·2 kg.) was carried out with cold alcohol as given in an earlier paper.[1] The alcoholic extract was distilled under reduced pressure to remove the solvent, water (1 litre) added, the mixture shaken up with ether (800 c.c.) and left in the refrigerator for a month. The crystallised deposit was separated, the ether layer removed and the aqueous solution shaken up and allowed to stand with more ether for another week. More of the colourless solid was obtained. The total yield was 22 g. and it was found to be almost pure sakuranin.

The ether solution was fractionally extracted by means of aqueous sodium carbonate (20%) and aqueous sodium hydroxide (20%). The final ether solution contained very little material. The carbonate solution when acidified and extracted repeatedly with ether, gave a small amount (0·1 g.) of a colourless substance which was fairly soluble in hot water. It gave a brown colour with alcoholic ferric chloride and a bright pink colour with magnesium and hydrochloric acid or with zinc and hydrochloric acid. It

crystallised from dilute methyl alcohol yielding colourless prisms, m.p. 232–34°. It was identified as taxifolin and the mixed m.p. with an authentic sample was undepressed. The sodium hydroxide extract yielded a mixture which was separated into genkwanin (0·2 g.), prunetin (0·2 g.) and sakuranetin (0·5 g.) by the method already described.[1]

When the aqueous solution was subjected to continuous extraction using ethyl acetate, the extracted solid (1 g.) was found to be largely soluble in ether. This ether-soluble portion was repeatedly crystallised from ethyl alcohol when it gave colourless plates, m.p. 230–32°. Further purification was effected by sublimation in vacuum and a final crystallisation from alcohol yielded colourless rectangular plates and prisms, m.p. 238–40°. It gave no colour with magnesium and hydrochloric acid, gave a reddish violet ferric reaction, was insoluble in aqueous sodium carbonate and soluble in aqueous sodium hydroxide to give a yellow solution (Found: C, 66·6; H, 5·1; $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%). It agreed closely in its properties with padmakastein. The identity was further confirmed by preparing its diacetate which crystallised from ethyl acetate as colourless rhombic prisms, m.p. 220–22° (Found: C, 64·6; H, 4·7; $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9%). Its glycoside could not be detected in this sample of the bark.

Padmakastein dimethyl ether (*II*).—This was prepared by the dimethyl sulphate (excess), potassium carbonate method. The product crystallised from alcohol as colourless thin laminæ, m.p. 152–54° (Narasimhachari and Seshadri,[1] 146–47°). It was insoluble in aqueous alkali, gave no colour with ferric chloride but developed a green colour with concentrated nitric acid (Found: C, 68·3; H, 5·7; $C_{18}H_{18}O_5$ requires C, 68·3; H, 5·7%). It formed a dinitrophenylhydrazone which crystallised from glacial acetic acid as red flakes, m.p. 227°.

Prunetin dimethyl ether (0·5 g.), methyl alcohol (200 c.c.), palladised charcoal (0·15 g., 10%) and a drop of concentrated hydrochloric acid were used for the hydrogenation which was carried out in Paar's low pressure hydrogenation apparatus at 36 lb. per square inch. The hydrogen absorption was over in less than an hour. The product crystallised from alcohol as colourless plates, m.p. 152–54° (Bradbury and White,[3] 156°; Gilbert *et al.*,[7] 154–55°). Mixed m.p. with padmakastein dimethyl ether was undepressed and with prunetin dimethyl ether was considerably depressed. The reduction product gave a green colour with nitric acid identical with that of padmakastein dimethyl ether and a DNP, m.p. 227°, undepressed by the

DNP prepared from the natural sample. The ether underwent smooth oxidation with selenium dioxide to yield prunetin dimethyl ether.

The natural and synthetic samples were also compared using infra-red spectra. Both gave identical spectra. For comparison prunetin dimethyl ether also was studied; its infra-red spectrum differed markedly. Padmakastein dimethyl ether gives a strong peak at 5·95 μ, which corresponds to a carbonyl group of the acetophenone type and this is absent in prunetin dimethyl ether; the peaks at 6·08 μ (shoulder) and 6·10 μ (shoulder) in prunetin dimethyl ether represent the frequency of the carbonyl in conjugation with double bonds on either side.

Padmakastein diacetate (*IV*).—Prunetin diacetate (0·6 g.) dissolved in glacial acetic acid (250 c.c.) was hydrogenated at a pressure of 49 lb. per square inch using palladised charcoal (0·2 g.; 10%). Hydrogenation was complete in eight hours. The catalyst was filtered and the filtrate after removal of acetic acid under reduced pressure gave a product that crystallised from ethyl acetate as glistening colourless rhombic prisms (0·38 g.), m.p. 220–22°, depressed by prunetin diacetate and undepressed by natural padmakastein diacetate (Found: C, 64·6; H, 4·7; $C_{20}H_{18}O_7$ requires C, 64·9; H, 4·9%). The substance underwent oxidation with selenium dioxide in acetic anhydride solution to yield prunetin diacetate.[1]

Padmakastein (*I*).—The synthetic diacetate was boiled under reflux with alcoholic hydrochloric acid for half an hour and the product that separated on cooling and dilution with water, was crystallised from alcohol, m.p. 234–36°. After sublimation in vacuum it was crystallised twice from alcohol when it yielded colourless rectangular plates and prisms, m.p. 238–40°, undepressed by natural padmakastein and considerably depressed by prunetin. It gave an intense green colour with concentrated nitric acid (Found: C, 66·9; H, 4·8; $C_{16}H_{14}O_5$ requires C, 67·1; H, 4·9%). Yield 0·12 g.

The natural and synthetic samples of padmakastein were also compared using infra-red spectra. Both gave identical spectra. For comparison the infra-red spectrum of prunetin was also taken and it differed markedly. The isoflavanone has the carbonyl frequency at 5·98 μ and the isoflavone at 6·06 μ agreeing with the explanation given already in the case of the methyl ethers.

SUMMARY

The synthesis of padmakastein has been effected by the catalytic reduction of prunetin diacetate and subsequent deacetylation. Similarly

prunetin dimethyl ether yields padmakastein dimethyl ether. The natural and synthetic samples have been compared.

Acknowledgement

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References

1. Narasimhachari and Seshadri .. *Proc. Ind. Acad. Sci.*, 1952, **35 A**, 202.
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4. King, King and Warwick .. *Ibid.*, 1952, 96.
5. King and Neill .. *Ibid.*, 1952, 4752.
6. Neill .. *Ibid.*, 1953, 3454.
7. Gilbert, McGookin and Robertson *Ibid.*, 1957, 3740.