A NOTE ON THE COMPONENTS OF THE BARK OF *PRUNUS PUDDUM*

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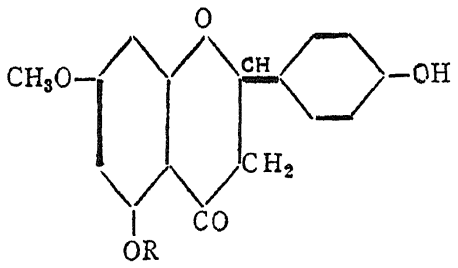
IN connection with our work on the synthesis of sakuranetin[1] we needed some quantity of the natural sample for purposes of comparison. It was prepared by the extraction of the bark of *Prunus puddum* which had been earlier shown to contain it.[2] In the course of their examination of this bark Chakravarti, *et al.* recorded the presence of puddumetin which was later shown to be the same as genkwanin,[3] sakuranetin, and also an isoflavone which they named prunusetin.[4] In our experiments described in this paper using a modified method of extraction improved yields of these substances have been obtained. Sakuranetin (I *a*) occurs in the largest amount (1% of the air-dried bark) along with small quantities of its glucoside sakuranin (I *b*).[5] Genkwanin (II) and prunusetin are obtained in yields of 0·2% and 0·6% respectively. In their study of sakuranetin obtained from this source Chakravarti and co-workers[2] recorded that they could not get the colourless diacetate melting at 98°. This derivative was originally prepared by Asahina and co-workers[6] and also by Zemplen,[7] *et al.* and inability to obtain it would throw some doubt about the identity of the natural flavanone. We have therefore examined this reaction and are able to record that the particular diacetate melting at 98° could be prepared from this sample of sakuranetin obtained from *Prunus puddum* bark thus confirming the identification. Further this sample of sakuranetin could also be dehydrogenated by means of iodine yielding genkwanin (II). Earlier Chakravarti, *et al.*[2] reported that they could not carry out this transformation using selenium dioxide. The reaction using iodine is of a general nature and has been recently studied in detail with a number of examples.[8] Its application in the present case establishes elegantly the interrelationship between these two components obtained from the same bark (I *a* and II).

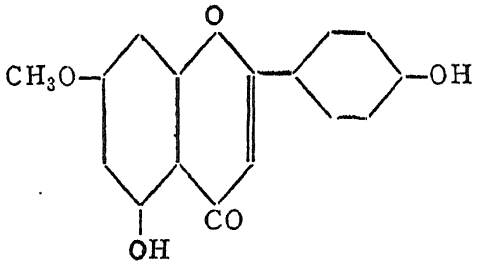
A careful examination of prunusetin has now shown that it is a slightly impure sample of prunetin (III). Chakravarti and Bhar[4] considered that it was different from prunetin and that it was a new substance because of the somewhat lower melting points they obtained for it and for its acetate and because of their failure to carry out partial methylation yielding the



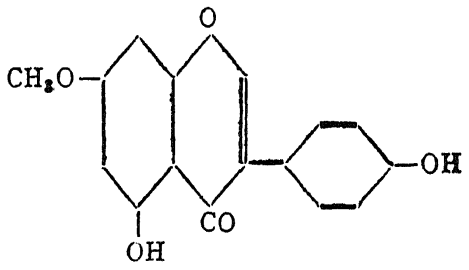
(I)

(*a*) R=H

(*b*) R=Glucose residue



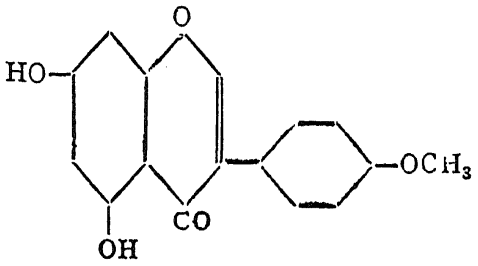
(II)



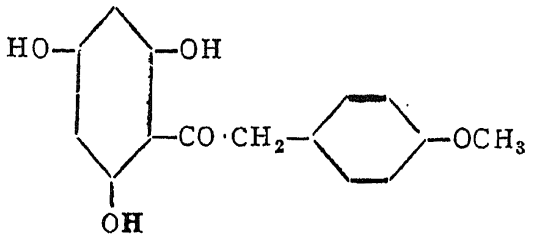
(III)

monomethyl ether. They could only get the dimethyl ether which was identical with genistein trimethyl ether. Of the three monomethyl ethers of genistein, prunetin is the 7-methyl ether earliest to be discovered in nature.[9] More recently the 4′-monomethyl ether (IV) has been obtained both synthetically[10] through the ketone (V) and from a natural source also as biochanin.[11] Chakravarti and Bhar came to the conclusion that prunusetin was the rather unusual 5-monomethyl ether of genistein; it would thus differ from the other two components of the *Prunus puddum* bark which are 7-methyl ethers. Their conclusion seemed to be erroneous even when their own observations were considered. For example, they recorded that prunusetin gives a brownish violet colour with ferric chloride which would suggest that in this compound the 5-hydroxy group is free; the presence of 7 and 4′-hydroxyls alone does not give rise to this colour. The insolubility of prunusetin in aqueous sodium carbonate would also indicate that the hydroxyl in the 7-position is not free. It is now found that careful purification of prunusetin by means of its acetate raises the melting point to 242° and its properties are identical with those of prunetin. Its partial methylation could be accomplished by using one mole of dimethyl sulphate and excess of anhydrous potassium carbonate in anhydrous acetone medium. The product, a dimethoxy compound, has the same melting point and properties as monomethyl prunetin (VI). Its acetate also has the required melting point. There seems to be no doubt therefore that prunusetin is essentially prunetin though slightly impure and the new name is not necessary. Not only does the bark the *Prunus puddum* contain representatives of the three major groups of anthoxanthins, a

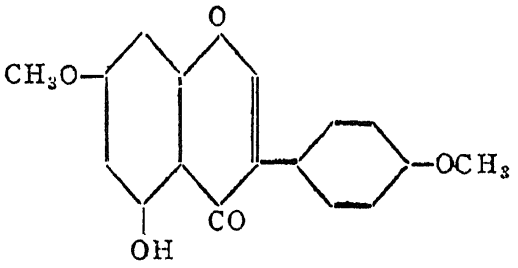
flavanone, flavone and isoflavone, but they all have the common feature that they are 7-methyl ethers of 5:7:4′-trihydroxy compounds.



(IV)



(V)



(VI)

Experimental

Extraction

The sun-dried bark of *Prunus puddum* (2 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 by distillation as much as possible and the concentrate (about 300 c.c.) was allowed to stand when after two days a deep red sticky solid separated out. This was filtered and extracted twice with hot benezene (100 c.c.) by refluxing on a water-bath for half an hour. The benzene extract (Fraction A) was set aside and worked up as described later. The residue on the filter was then boiled twice with water (100 c.c.) and filtered. The aqueous solution (Fraction B) contained the glucosidic portion. The solid left behind was then dissolved in excess of hot alcohol (Fraction C).

Fraction A (Sakuranetin) (I *a*)

The benzene solution when cooled slowly deposited a colourless crystalline solid which on recrystallisation from acetic acid was obtained as colourless prismatic needles melting at 151-52°. This product agreed in all its properties and colour reactions with sakuranetin. On evaporating the remaining benzene solution a pale yellow solid was obtained. This was recrystallised first from benzene and subsequently from acetic acid whereby a further quantity of sakuranetin was obtained. Total yield 20 g. (1% of the dry bark).

Acetylation

The sample of sakuranetin (0·5 g.) was dissolved in acetic anhydride (5 c.c.) and a few drops of pure concentrated sulphuric acid added. The solution was heated on a water-bath for 10 minutes and left at room temperature for 6 hours. It was then poured into ice-water with stirring. The semisolid that separated was extracted with ether. On evaporating the ether solution the product solidified slowly when kept in a vacuum desiccator. It crystallised from ethyl acetate as colourless tiny prisms melting at 98-99°. It gave no colour with ferric chloride in alcoholic solution.

Oxidation with iodine: Preparation of genkwanin (II)

Sakuranetin (0·5 g.) was dissolved in alcohol (10 c.c.), sodium acetate (1·5 g.) added and the solution boiled. To this a boiling solution of iodine (0·5 g.) in alcohol was added slowly. In the beginning there was rapid decolourisation and the completion of the reaction was indicated by a permanent colouration due to iodine. On cooling the solution a bright yellow solid separated. This was filtered and washed with sulphur dioxide water. It crystallised from alcohol in the form of yellow needles melting at 285-7°. A further quantity of the substance could be obtained by concentrating the alcoholic solution, diluting with water and crystallising the precipitated solid. Yield, 0·35 g. Its alcoholic solution gave a violet colour with ferric chloride and a scarlet red colour with magnesium and hydrochloric acid. It was identical in its properties with genkwanin and the mixed melting point with the natural sample described below was undepressed. (Found: C, 67·2; H, 4·1; $C_{16}H_{12}O_5$ requires C, 67·5; H, 4·2%.)

Fraction B (Sakuranin I b)

Since on cooling the aqueous solution and allowing to stand no product separated it was concentrated under reduced pressure on a water-bath (20 c.c.). The concentrated solution was shaken with excess of ether and allowed to stand. After a few days a colourless crystalline solid had slowly separated out. It crystallised from dilute alcohol as short needles melting at 210-12°. Its alcoholic solution gave a bright red colour with magnesium and hydrochloric acid but gave no colour with ferric chloride. It gave a deep green colour with concentrated nitric acid and dissolved in alkali giving a yellow solution. It agreed in all its properties with the description of sakuranin.[5] The aqueous mother-liquor when hydrolysed with 7% sulphuric acid yielded sakuranetin. Yield of sakuranetin 0·5 g.

Fraction C: Genkwanin and prunetin (II and III)

The alcoholic solution deposited on cooling a yellow crystalline solid which when recrystallised from alcohol melted at 285° and was identical

with genkwanin. The mother-liquor yielded on concentration a brownish yellow solid melting between 228° and 240°. This was boiled with small quantities of alcohol thrice and filtered. The residue yielded some more of pure genkwanin when recrystallised from ethyl acetate. The combined alcoholic extract deposited on cooling pale yellow needles melting at 236-8°. Recrystallisation from ethyl acetate raised the melting point to 238-39°. Yield, genkwanin 2 g., and prunusetin 6 g.

The prunusetin sample was acetylated by boiling with acetic anhydride and fused sodium acetate. The mixture was poured into ice-water, the colourless crystalline acetate was filtered, washed with water and dried. It crystallised from ethyl acetate as colourless rhombohedral prisms melting at 225-26° and agreed with the description of prunetin acetate. (Found: C, 64·9; H, 4·2; $C_{20}H_{16}O_7$ requires C, 65·2; H, 4·3%.) Deacetylation of the above acetate using alcoholic hydrochloric acid and crystallisation of the product from alcohol gave pure prunetin melting at 240-41°.

Prunetin monomethyl ether

Prunusetin sample (0·5 g.) was dissolved in dry acetone (20 c.c.), dimethyl sulphate (0·15 c.c.) and anhydrous potassium carbonate (1 g.) were added and the mixture refluxed for 6 hours. It was then filtered and the potassium salts washed with hot acetone. On distilling off acetone a pale yellow solid was obtained. It crystallised from ethyl acetate as thin rectangular plates melting at 139-40°. (Genistein dimethy ether, m.p. 140–41°.)[12] It gave a reddish brown colour with violet tinge with ferric chloride in alcoholic solution and it was sparingly soluble in aqueous sodium hydroxide (Found: C, 68·2; H, 5·0; $C_{17}H_{14}O_5$ requires C, 68·4; H, 4·7%.)

Its acetate prepared by using acetic anhydride and sodium acetate crystallised from ethyl acetate as colourless rectangular prisms melting at 202–3°. (Found: C, 66·8; H, 5·0; $C_{19}H_{16}O_6$ requires C, 67·1; H, 4·7%.) Deacetylation of the acetate and crystallisation of the product from ethyl acetate gave prunetin monomethyl ether, m.p. 140-41°.

Prunetin dimethyl ether

Prunusetin sample (0·5 g.) was methylated by boiling with excess of dimethyl sulphate (0·5 c.c.) and anhydrous potassium carbonate (1·5 g.) in dry acetone solution (20 c.c.) for 15 hours. Acetone was then distilled off and water added. The solid was filtered and washed with water. It crystallised from dilute alcohol as colourless needles melting at 161-62°; genistein trimethyl ether has m.p. 161-62°.[12] It was insoluble in aqueous alkali and gave no colour with ferric chloride in alcoholic solution.

Summary

The stem bark of Prunus puddum yields sakuranetin (I *a*), sakuranin (I *b*), genkwanin (II) and prunetin (III). The identification of sakuranetin is confirmed by the preparation of its diacetate and conversion to genkwanin. Prunusetin is shown to be only a slightly impure sample of prunetin. Though the components belong to different groups of anthoxanthins, they have the common feature that they are all 7-methyl ethers of 5: 7: 4′-trihydroxy compounds.

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