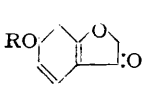
Survey of Anthoxanthins. Part IX. Isolation and Constitution of Palasitrin.*

By (Miss) B. Puri and T. R. Seshadri.

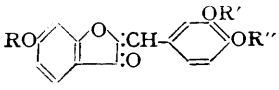
[Reprint Order No. 6020.]

A new glycoside, palasitrin, has been isolated from the flowers of *Butea frondosa*. It yields on hydrolysis 2 mols. of glucose and 2-(3 : 4-dihydroxybenzylidene)-6-hydroxycoumaran-3-one. The glucose units are in the 6- and the 3′-position since, on complete methylation and hydrolysis, the glycoside yields the 4′-monomethyl ether of the aglycone. For comparison all the monomethyl ethers of the aglycone have been synthesised. Butein and *iso*butrin have been converted into the aglycone and palasitrin by acetylation, addition of bromine, and treatment with alcoholic alkali.

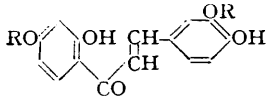
In Part III (Puri and Seshadri, *J. Sci. Ind. Res. India*, 1953, **12**, *B*, 462) were described the isolation and study of *iso*butrin, a new butein glycoside from the dried flowers of *Butea frondosa*. The existence of butrin (butin diglucoside) along with small amounts of the aglycones, butin and butein, had been recorded in earlier publications. Extraction of the fresh flowers with cold alcohol and chromatography showed the presence of *iso*butrin as almost the sole glycosidic component, butrin and the aglycones being absent. There was very little carotene in the flowers, but the deep orange-red colour suggested the presence of another pigment. After the removal of butrin (formed during the concentration) and *iso*butrin from the alcoholic extract by fractional crystallisation, the mother-liquors yield a brownish-red, crystalline pigment, $C_{27}H_{30}O_{15}$, which is named palasitrin, derived from the Indian name of the tree, Palas. On hydrolysis with acid this gives two mols. of glucose and one of an aglycone identified as 2-(3 : 4-dihydroxybenzylidene)-6-hydroxycoumaran-3-one (II; R = R′ = R″ = H) by comparison with a sample prepared by a modification of the method of Bruhl and Friedlander (*Ber.*, 1897, **30**, 297) and von Auwers and Pohl (*Annalen*, 1914, **405**, 243). Further, 6-hydroxycoumaran-3-one (I; R = H) was condensed with vanillin, and the 3′-methyl ether (II; R = R″ = H, R′ = Me) completely methylated, yielding the trimethyl ether of the aglycone.



(I)



(II)



(III; R = $C_6H_{11}O_5$)

When completely methylated and then hydrolysed, the glycoside gave a monomethyl ether, m. p. 241—243°, showing that the two glucose units were in different positions. It differed from the 3′-methyl ether already mentioned, and from the 6-methyl ether (m. p. 223—225°) obtained by the condensation of 6-methoxycoumaran-3-one (I; R = Me) with protocatechuic aldehyde, but resembled the 4′-methyl ether (II; R = R′ = H, R″ = Me) (m. p. 241—243°) prepared from 6-hydroxycoumaran-3-one and *iso*vanillin. Palasitrin is therefore the 6 : 3′-diglucoside. The fact that the positions occupied by the sugar units in this glycoside and in *iso*butrin (III) are similar is of significance : it appears that *iso*butrin undergoes oxidation to palasitrin or that they have a common origin. In the laboratory the conversion of a chalkone into a benzylidenecoumaranone is fairly easy : butein tetra-acetate yields its dibromide which gives the aglycone (II; R = R′ = R″ = H) on treatment with alcoholic potassium hydroxide. By the same procedure *iso*butrin was converted into palasitrin.

By chromatography it was shown that flowers, and particularly buds, collected at the beginning of the season contain only *iso*butrin whereas later a considerable quantity of palasitrin was present in the mature flowers. The occurrence of benzylidenecoumaranones along with chalkones or flavanones has been noted, *e.g.*, leptosin in *Coreopsis grandiflora*

* Part VIII, *J. Sci. Ind. Res. India*, 1955, **14**, *B*, 93.

(Geissman and Heaton, *J. Amer. Chem. Soc.*, 1943, **65**, 677), aureusin in *Antirrheinum majus* (Seikel and Geissman, *ibid.*, 1950, **72**, 5725), 6 : 3′ : 4′-trihydroxyaurone in yellow *Dahlia* (Nordstrom and Swain, *Chem. and Ind.*, 1953, 823), and sulphurein in *Cosmos sulphureus* (Shimokariyama and Hattori, *J. Amer. Chem. Soc.*, 1953, **75**, 1900).

Experimental

For circular paper chromatography Whatman No. 1 paper and water saturated with phenol at 34—36° were used.

Extraction of Fresh Flowers.—(a) *Chromatography of the extracts.* The orange-red fully grown flowers were extracted with cold alcohol for 2 days. The process was repeated twice with fresh alcohol. In circular paper chromatography it gave a marked orange-yellow ring without spraying, R_F 0·72, which corresponds to *iso*butrin. Another yellow ring was present which when sprayed with ammonia became purple (R_F 0·65). No rings corresponding to butein, butrin, or butin appeared. Similar extractions were carried out with the following materials: (1) Fresh buds collected in the beginning of the season gave only *iso*butrin. (2) Flowers air-dried indoors gave the same results as fresh flowers. (3) Sun-dried flowers gave two yellow rings, as with fresh flowers before spraying, but a third yellow ring (R_F 0·87) appeared after spraying, which corresponded to butrin. Thus sun-drying caused some conversion of *iso*butrin into butrin. (4) Extracts of fresh flowers concentrated under ordinary pressure gave results similar to those last noted, showing the effect of evaporation at atmospheric pressure.

(b) *Butrin.* The alcoholic extract of the flowers was concentrated at atmospheric pressure to a small volume and set aside. An orange-yellow crystalline solid separated. After two crystallisations from methyl alcohol and one from alcohol it formed colourless long needles, m. p. 194—195° (decomp.). It gave no colour with alcoholic ferric chloride and a deep pink colour with magnesium and hydrochloric acid. In paper chromatography no ring was visible before spraying and only after spraying with ammonia a yellow ring, R_F 0·87, appeared. This fraction was therefore butrin (Puri and Seshadri, *loc. cit.*).

(c) iso*Butrin.* The mother-liquor after removal of butrin was further concentrated and cooled. Bright yellow crystals were obtained which crystallised from methyl alcohol as small narrow prisms and needles, m. p. 190—191° (decomp.) after sintering at 185°. In paper chromatography the substance gave a marked yellow ring before spraying with ammonia (R_F 0·72). It gave an olive-brown colour with alcoholic ferric chloride but no colour with magnesium powder and hydrochloric acid. These properties agreed with those of *iso*butrin (Puri and Seshadri, *loc. cit.*).

A rough estimate of *iso*butrin was made by exhaustion of fresh flowers (150 g.) with alcohol, concentration of the extract, hydrolysis with aqueous sulphuric acid (7%), and ether-extraction of the aglycone mixture (butin and butein mostly). From the yield of the dry aglycone mixture, *iso*butrin was found to constitute 5% of the fresh flowers and 25% of the air-dried flowers.

(d) *Palasitrin.* The mother-liquor from *iso*butrin was extracted repeatedly with ether, to remove any free aglycones. The aqueous layer was then saturated with ammonium sulphate and extracted with butyl alcohol. The extract was diluted with light petroleum and then extracted with small portions of aqueous 5% potassium carbonate. The carbonate extract was acidified in the cold and re-extracted with butyl alcohol. This alcohol extract was then evaporated, and the residue dissolved in methanol (50 c.c.) and passed down a column of magnesol (30 × 1·5 cm.) under suction to remove the last traces of *iso*butrin. *iso*Butrin formed the lower yellow zone and palasitrin an upper orange-red zone. The zones were then washed down with methyl alcohol, the extract of the new glycoside concentrated, and the residue recrystallised from butyl alcohol; it formed long prisms, m. p. 199—200° (decomp.) after sintering at 125° (Found: C, 53·3; H, 5·6; loss on drying at 110°, 2·9. $C_{27}H_{30}O_{15},H_2O$ requires C, 52·9; H, 5·2; H_2O, 3·0%). It gave a purple solution with aqueous sodium hydroxide, a deep-red solution with concentrated sulphuric acid, an olive-brown colour with alcoholic ferric chloride, and no colour with magnesium powder and hydrochloric acid. In paper chromatography it gave a yellow ring which became purple when sprayed with ammonia (circular R_F 0·66). The yield of palasitrin was approx. 0·2% of the fresh flowers.

Hydrolysis of Palasitrin.—The glycoside was boiled with 7% aqueous-alcoholic sulphuric acid for 2 hr. When the solution was cooled an orange-yellow solid separated which in paper chromatography gave a yellow ring becoming purple-red when sprayed with ammonia (R_F 0·45). The *aglucone* crystallised from alcohol as deep orange-yellow prisms, m. p. 310—312° (decomp.), gave

an olive-brown colour with alcoholic ferric chloride, no colour with magnesium and hydrochloric acid, an orange-red colour with concentrated sulphuric acid, and a purple-red colour with aqueous sodium hydroxide (Found: C, 67·0; H, 4·0. $C_{15}H_{10}O_5$ requires C, 66·7; H, 3·7%). The mixed m. p. with a synthetic sample was undepressed.

The aqueous solution was finally extracted with ether to remove the last traces of the aglucone and neutralised with barium carbonate. The solution was filtered off, concentrated and tested for sugar {circular R_F 0·60; 34—36°; phenol–water (9 : 1); indicates glucose [Found: glucose, 58·5; aglucone, 44·4. $C_{27}H_{30}O_{15},H_2O$ requires glucose (2 mols.), 58·8; aglucone, 44·1]}.

The aglucone trimethyl ether, prepared by methyl sulphate and potassium carbonate in boiling acetone (20 hr.), crystallised from dilute alcohol as colourless rectangular prisms and plates, m. p. 184—186°, alone or mixed with a synthetic sample.

Synthesis of 2-(3 : 4-*Dimethoxybenzylidene*)-6-*hydroxycoumaran*-3-*one*.—(a) 6-*Hydroxy*-2-(4-*hydroxy*-3-*methoxybenzylidene*)*coumaran*-3-*one*. A solution of 6-hydroxycoumaran-3-one (Balakrishna, Rao, and Seshadri, *Proc. Indian Acad. Sci.*, 1949, **29**, *A*, 399) (2 g.) and vanillin (4 g.) in alcohol (20 c.c.) was treated with 30 c.c. of aqueous potassium hydroxide (50 g. in 35 c.c.) and kept at room temperature for 3 days with occasional shaking, then diluted with water, and acidified with hydrochloric acid. The *monomethyl ether* crystallised from dilute alcohol as yellow rectangular prisms, m. p. 263—264° (Found, in the sample dried at 120° *in vacuo*: C, 67·5; H, 4·0. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·2%).

(b) *Methylation*. The monomethyl ether was heated in acetone with an excess of methyl sulphate and potassium carbonate for 12 hr. The *trimethyl ether*, crystallised twice from dilute alcohol, yielded colourless rectangular prisms and plates, m. p. 184—186° (Found: C, 69·2; H, 5·5. $C_{18}H_{16}O_5$ requires C, 69·2; H, 5·1%).

Complete Methylation and Hydrolysis of Palasitrin.—Palasitrin (0·5 g.) was heated in dry acetone (100 c.c.) with methyl sulphate (1·5 c.c.) and potassium carbonate (3 g.) for 30 hr. Acetone was then distilled off, and water added to dissolve the potassium salts and enough sulphuric acid to neutralise the carbonate and to make the acid strength 7%. The mixture was boiled for 2 hr., cooled, and extracted with ether. Evaporation yielded a yellow solid which crystallised from alcohol as orange-yellow prismatic needles and rods, m. p. 241—242° alone or mixed with synthetic 6-*hydroxy*-2-(3-*hydroxy*-4-*methoxybenzylidene*)*coumaran*-3-*one* (Found: C, 60·5; H, 4·5; loss at 120°, 10·5. $C_{16}H_{12}O_5,2H_2O$ requires C, 60·0; H, 5·0; H_2O, 11·2%. Found in the sample dried at 120°: OMe, 10·7. $C_{16}H_{12}O_5$ requires OMe, 10·9%).

Synthesis. Condensation of 6-hydroxycoumaran-3-one (1 g.) with *iso*vanillin (2 g.) was effected as with vanillin. The 4′-methyl ether crystallised from alcohol as orange-yellow prismatic needles and rods, m. p. 241—243° (Found, in the sample dried at 120°: OMe, 10·8. $C_{16}H_{12}O_5$ requires for 1OMe, 10·9%).

Synthesis of 2-(3 : 4-*Dihydroxybenzylidene*)-6-*hydroxycoumaran*-3-*one*.—The following modified procedure has been found convenient. A solution of 6-hydroxycoumaran-3-one (1·50 g.) and protocatechualdehyde (1·38 g.) in alcohol (20 c.c.) was saturated with hydrogen chloride (ice-cooling) in 2 hr., then diluted with water and extracted with ether. The solvent was evaporated off and the solid twice crystallised from alcohol, yielding orange-yellow prisms, m. p. 310—312° (decomp.).

2-(3 : 4-*Dihydroxybenzylidene*)-6-*methoxycoumaran*-3-*one*.—6-Methoxycoumaran-3-one (Balakrishna, Rao, and Seshadri, *loc. cit.*) (1·64 g.) was condensed with protocatechuic aldehyde (1·38 g.) as in the foregoing experiment. After two crystallisations from dilute alcohol the *product* formed red prisms, m. p. 223—225°. The solution in alcohol was yellow but the crystalline solid deep red (Found: C, 68·3; H, 4·7. $C_{16}H_{12}O_5$ requires C, 67·6; H, 4·2%).

Conversion of Butein into 2-(3 : 4-*Dihydroxybenzylidene*)-6-*hydroxycoumaran*-3-*one*.—(*a*) Butein tetra-acetate (Perkin and Everest, "Natural Organic Colouring Matters," Longmans, London, 1918, p. 169) (0·5 g.) in glacial acetic acid (5 c.c.) was treated dropwise with bromine in acetic acid. The mixture was cooled in ice and the orange-yellow solid *dibromide* which separated was filtered off and washed with water. It crystallised from acetone–light petroleum as pale red prisms, m. p. 86—88° (Found: Br, 25·6. $C_{23}H_{20}O_9Br_2$ requires Br, 26·7%).

(*b*) The crude dibromide was boiled with aqueous 10% potassium hydroxide (50 c.c.) until the whole was a clear solution (10 min.), cooled, acidified with hydrochloric acid, and extracted with ether. The solvent was evaporated and the residue tested chromatographically. It gave a marked yellow ring (R_F 0·45) which became purple when sprayed with ammonia. Another ring which appeared pale yellow only after spraying with ammonia (R_F 0·56) indicated a minor by-product, probably a flavone. The solid was repeatedly recrystallised from ethyl acetate till

a pure sample was obtained having m. p. 310—312° (decomp.) alone or mixed with the sample obtained by the hydrolysis of palasitrin.

Conversion of iso*Butrin into Palasitrin.*—*iso*Butrin (1 g.) was heated with acetic anhydride (15 c.c.) and sodium acetate (3 g.) for 3 hr., and then poured into ice-water. The product, crystallised repeatedly from alcohol, yielded iso*butrin acetate* as pale yellow rectangular prisms, m. p. 108—110° (Found: C, 56·0; H, 5·6. $C_{47}H_{52}O_{25}$ requires C, 55·5; H, 5·1%).

To the acetate (0·5 g.) in glacial acetic acid (5 c.c.) bromine in glacial acetic acid was added dropwise. Water was added and the resulting orange-yellow sticky solid filtered off and boiled with aqueous 10% potassium hydroxide (50 c.c.) until completely dissolved. The solution was cooled, acidified with hydrochloric acid, and extracted with butyl alcohol, and the extract concentrated. The residue gave in chromatography a yellow ring (R_F 0·64), becoming purple when sprayed with ammonia. It was purified by column chromatography as for natural palasitrin. The pure product crystallised from butyl alcohol as long prisms, m. p. 199—200° (decomp.) with sintering at 125°, alone or mixed with the natural sample. There was agreement in all colour reactions.

UNIVERSITY OF DELHI, DELHI-8.

[*Received, January 8th,* 1955.]