obtained as deep yellow microcrystalline powder melting at 206–08°. It was sparingly soluble in water but it dissolved freely in alcohol or acetic acid. When treated with a drop of ferric chloride, an aqueous alcoholic solution of the substance gave an olive brown colour. On reduction with magnesium and hydrochloric acid the substance did not develop any pink colour. It was found to be identical with a synthetic sample of 4′-O-methylbutein and admixture with it did not depress the melting point. (Found: OCH_3, 11·0; $C_{15}H_{11}O_4OCH_3$ requires OCH_3, 10·8%.)

(*b*) *Preparation of* 4′-O-*methylbutin*.—The hot aqueous filtrate left after the separation of the methyl butein deposited on cooling a pale yellow substance. It was filtered and recrystallised from dilute alcohol, when it came out as pale yellow glistening rectangular plates melting at 204–06°. In alcoholic solution it did not yield any colour with ferric chloride, but on reduction with magnesium and hydrochloric acid it gave a beautiful pink colour. It was identical with a synthetic sample of 4′-O-methylbutin, and the mixed melting point with it was undepressed. (Found: OCH_3, 11·1%; $C_{15}H_{11}O_4OCH_3$ requires OCH_3, 10·8%.)

This compound could be easily distinguished from the isomeric 4′-O-methylbutein, since it was only pale yellow in colour and crystallised as glistening rectangular plates, whereas the butein ether was a deep yellow microcrystalline powder. The melting point of a mixture of the two substances was 188–91°.

Synthesis of 4′-O-*methylbutein*—

The conditions for the preparation of chalkones according to the method of Kostanecki have recently been studied in these laboratories[5], and it has been found that the best yields could be obtained by (1) taking an excess of one of the components (aldehyde or ketone) the choice being dependent on the availability of the particular substance and (2) refluxing immediately after the addition of the alkali to the reactants for an hour. This procedure was adopted in the synthesis of 4′-O-methylbutein.

Isovanillin (1 g.; 1 molecular proportion) and resacetophenone (3 g.; 3 molecular proportions) were dissolved in 10 c.c. of alcohol and 25 c.c. of 50% potash were added to the solution at the room temperature. The mixture was refluxed for one hour on a water-bath and then kept out of contact with air for 24 hours. The product was then diluted and acidified with hydrochloric acid, cooling the flask under the tap. A yellow product separated out. It was filtered and recrystallised from alcohol. It came out as a microcrystalline powder melting at 206-08°. The yield of the pure product was 1 g. (Found: C, 65·2; H, 5·6; OCH_3, 10·6; loss on drying *in vacuo*

at 120-30°, 2·8; $C_{15}H_{11}O_4\,OCH_3,\frac{1}{2}H_2O$ requires C, 65·1; H, 5·4; OCH_3, 10·5; loss on drying (H_2O), 3·1%.)

Synthesis of 4′-O-methylbutin.—

The above methylbutein (1·5 g.) was dissolved in 50% alcohol (75 c.c.) and treated with concentrated sulphuric acid (3 c.c.). The solution was then boiled under reflux on a water-bath for 18 hours. It was then diluted with boiling water to 250 c.c. when a solid began to separate out. On shaking and slight cooling the solid coagulated. It was filtered and recrystallised from alcohol. It was a deep yellow substance melting at 205–07° and was found to be identical with the starting material. The filtrate deposited some more solid on cooling. It was only pale yellow in colour and crystallised from dilute alcohol as glistening rectangular plates. It produced the pink colour characteristic of flavanones, when reduced with magnesium and hydrochloric acid. It melted at 203–05°. Yield of the pure product was 0·5 g. (Found: C, 67·0; H, 4·9; OCH_3, 11·0; $C_{15}H_{11}O_4\,OCH_3$ requires C, 67·1; H, 5·1; OCH_3, 10·8%.)

Our thanks are due to Mr. G. V. L. Narasimha Murthy for some of the microanalyses.

Summary

On treatment with diazomethane, butrin gives rise to a monomethyl ether which yields on hydrolysis with acids a mixture of 4′-O-methylbutin and 4′-O-methylbutein, the latter being the major component. The constitutions of these two products have been established by alkaline oxidation, yielding isovanillic acid and comparison with synthetic samples. It is, therefore, concluded that butrin is 3′: 7-diglucoside of butin, and thus it is the first instance of a glycoside to contain the sugar residues in two different positions amongst the group of anthoxanthins and also the first instance to carry a sugar group in the side phenyl nucleus amongst both anthoxanthin and anthocyanin pigments.

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CONSTITUTION OF BUTRIN

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Received May 17, 1941

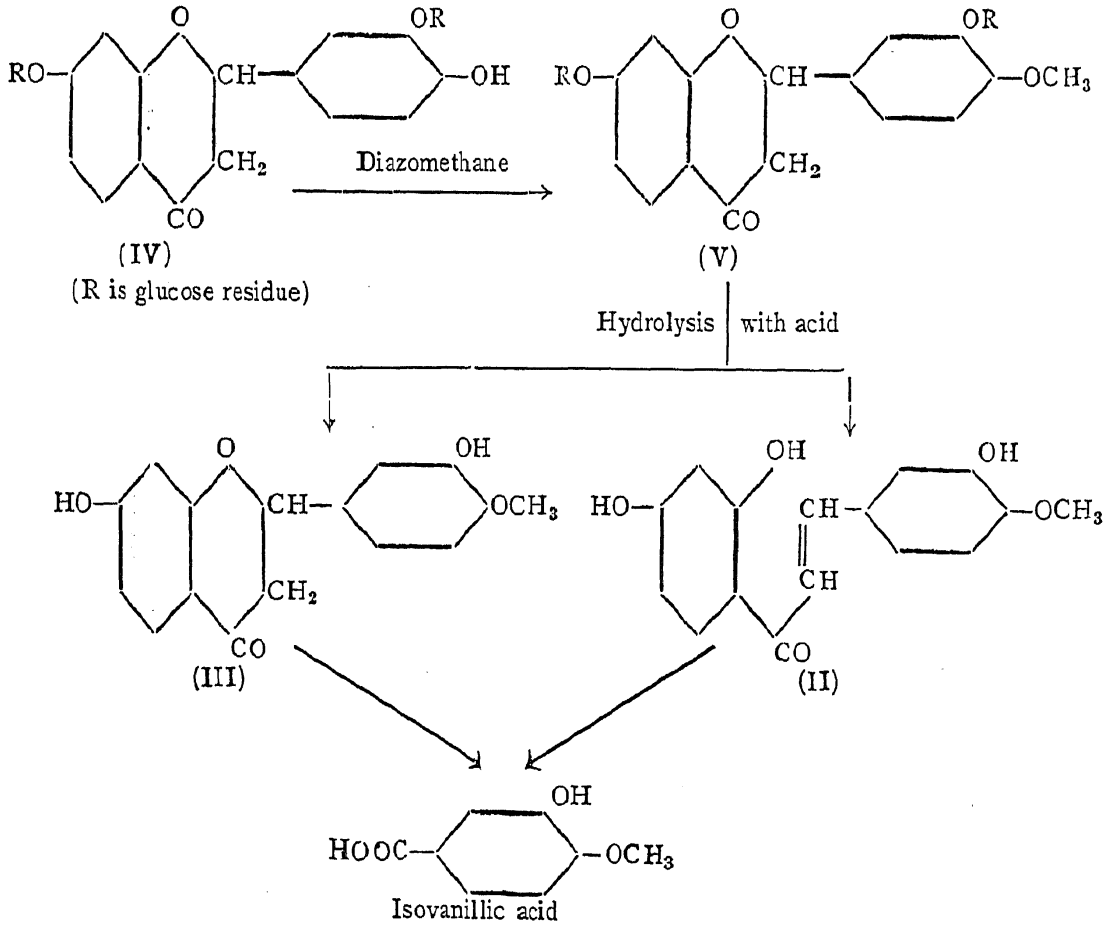
Butrin which was isolated by Lal and Dutt[1] from the flowers of *Butea frondosa* exhibits exceptional properties. Unlike the other flavanone glycosides, it contains no rhamnose unit and is remarkable for its sparing solubility in organic solvents and considerable solubility in water. In aqueous or alcoholic solution it gives no colour with ferric chloride. Its constitution is, therefore, of special interest in connection with the work on glycosides which is in progress in these laboratories.

The formula of butrin, $C_{27}H_{32}O_{15}$, indicates that it is a diglucoside and this has been confirmed recently by the estimation of the glucose and the aglucone obtained by acid hydrolysis.[2] In an attempt to establish the constitution of butrin, Lal[3] subjected the glucoside suspended in ethyl alcohol to ethylation with excess of ethyl iodide and potassium carbonate. He claimed to have isolated a colourless O-diethylbutrin and an isomeric bright yellow product, and therefore concluded that butrin was a bioside, the sugar group occupying only one hydroxyl group of butin.

For the present investigation diazomethane was chosen for the methylation of the glucoside since (*a*) it would not open up the pyranone ring because of its mild nature and (*b*) there is no hydroxyl group in position 5 to offer resistance to methylation by this reagent. The reaction was carried out in aqueous methyl alcoholic solution of the substance, and the product was found to be a monomethyl butrin. When reduced with magnesium and concentrated hydrochloric acid, it gave a bright pink colour, indicating the presence of the flavanone structure. Further it was not soluble in dilute alkali, thereby showing the absence of any free phenolic hydroxyl group. By the hydrolysis of the methylated glucoside with dilute acid in order to remove the sugar residues, an extraordinary result was obtained. The resulting methyl ether (I) gave no prominent colour when treated with magnesium and hydrochloric acid, and with ferric chloride produced a marked olive brown, characteristic of butein. It was, therefore, concluded that during hydrolysis complete rupture of the pyranone ring had taken place.[4] With a view to identify the monomethyl ether thus obtained, it was subjected to oxidative degradation with alkali and thereby isovanillic acid was produced. It should, therefore, be 4′-O-methylbutein.

In order to establish its identity further, a sample of 4′-O-methylbutein (II) was synthesised by condensing resacetophenone and isovanillin in the presence of alkali. Though the sample (I) obtained by the hydrolysis of the methylated butrin agreed closely in its properties with the synthetic 4′-O-methylbutein (II), it melted lower. This discrepancy which first offered great difficulties in the interpretation of the results became explicable, when it was realised later during a subsequent repetition of the experiment that (I) contained small quantities of a more easily soluble fraction (III) which differed from the major part in (*a*) giving no colour with ferric chloride and (*b*) producing a pink colour when treated with magnesium and hydrochloric acid. (III) was, therefore, a flavanone derivative. It was identified as 4′-O-methylbutin by taking the mixed melting point with an authentic sample obtained by heating the synthetic 4′-O-methylbutein (II) with dilute sulphuric acid for a number of hours. After the removal of this fraction, sample (I) melted correctly at 206–08° and the mixed melting point with (II) was undepressed.

From the above results the conclusion is inevitable that butrin is a dimonoside of butin, bearing the two glucose units in 3′ and 7 positions (IV). This explains the non-production of any colour by the glucoside with ferric chloride, the formation of only a monomethyl derivative (V) on methylating



the substance with diazomethane and the production of the monomethyl ethers of butin and butein and of isovanillic acid by degradation.

It may be interesting to note here that during the course of this methylation no methyl groups entered the sugar nuclei, as had been found to take place in some other cases. Further the hydrolysis of the methylated glucoside with acids has led to the formation of the butein compound in much larger proportion than has been found with butrin itself.[2]

The most important outcome of the present investigation is (1) that butrin is the first instance of an anthoxanthin containing two sugar groups in two different positions, though such cases are very common in anthocyanins and (2) that it is the first example for the occurrence of a glucose unit in the side phenyl nucleus in both anthoxanthins and anthocyanins. Another case wherein the side phenyl nucleus carries the sugar residue has also been recently discovered by us (unpublished work), thus showing that such type of glucosides is more common than originally imagined.

Experimental

The dried flowers of *Butea frondosa* were coarsely powdered and extracted in batches with methylated spirit. The total alcoholic extract was then distilled to recover the solvent and the concentrate was filtered, while still hot, through a fluted filter to remove the wax and the resin that separated out and then left for a week. At the end of this period, a crystalline solid along with some brown resin and wax was deposited. The contents were filtered and the residue washed with methylated spirit till it became lemon-yellow in colour. This crude product readily dissolved in boiling methyl alcohol, and when the solution was allowed to stand overnight, a pale yellow crystalline solid separated out. It was filtered and subsequently boiled with excess of methyl alcohol. This time, however, very little of the solid went into solution, as it was almost pure. It was once more boiled with excess of fresh methyl alcohol to completely remove the impurities and filtered. The substance was obtained by this procedure as almost colourless needles melting at 193–94° (decomp.). It was soluble in water and the aqueous solution did not develop any colour when treated with a drop of ferric chloride. A small part was dissolved in water, excess of concentrated hydrochloric acid added and then treated with magnesium powder, when a beautiful pink colour was produced.

Preparation of 4'-O-methylbutrin—

Butrin (3 g.) was dissolved in 80% methyl alcohol (200 c.c.) and treated with a large excess (10 g.) of diazomethane in ethereal solution in small

quantities. The contents were shaken vigorously after each addition. As soon as diazomethane was added, the solution became cherry red and the evolution of a small amount of gas also took place. Even during the course of the addition, a colourless crystalline solid began to separate out. The contents were left overnight to enable the reaction to be completed. The ether and the excess of diazomethane were then expelled from the reaction products and the colourless crystalline solid filtered. It was very sparingly soluble in methyl and ethyl alcohols, moderately soluble in glacial acetic acid and freely in boiling water or dilute acetic acid. From dilute acetic acid solution, it crystallised as colourless needles melting at 230–32°. It was not soluble in cold aqueous alkali and did not give any colour with ferric chloride. When reduced with magnesium powder and hydrochloric acid as already described, it developed the pink colour characteristic of flavanones. The yield of the pure product was 2 g. (Found in the sample dried *in vacuo* at 130–40°: C, 54·6; H, 5·3; OCH_3, 5·0; $C_{27}H_{31}O_{14}.OCH_3$ requires C, 55·1; H, 5·6; OCH_3 5·1%.)

Hydrolysis of Methylbutrin—

Experiment 1.—Methylbutrin (0·5 g.) was treated with 7% sulphuric acid (25 c.c.) and the mixture boiled under reflux on a wire-gauze. The solid went into complete solution within 5 minutes and after about 45 minutes, some yellow solid separated out giving rise to bumping. However, the heating was continued for 2 hours to complete the hydrolysis. The contents were then cooled and filtered. The residue was purified by crystallisation from dilute acetic acid. It was deep yellow in colour and produced an olive brown colour when treated with ferric chloride in dilute alcoholic solution. When reduced with magnesium and concentrated hydrochloric acid, it did not give the brilliant pink colour characteristic of flavanones. (Found: OCH_3, 11·2; $C_{15}H_{11}O_4OCH_3$ requires OCH_3, 10·8%.) The substance melted at 196–98°.

Oxidative Degradation of the Above Product: *Isolation of Isovanillic Acid*—

The substance (0·5 g.) was treated with 50% potash (20 c.c.) in a silver flask and heated under reflux for 6 hours. At the end of the period, the clear alkaline solution was acidified with hydrochloric acid and extracted with ether. From the ether solution an acid melting at 253–55° was obtained. It was found to be identical with isovanillic acid.

Experiment 2.—(*a*) *Preparation of* 4′-O-*methylbutein*: The hydrolysis of the methylated glucoside was carried out as already described. After the heating was over, the contents were immediately filtered hot and the filtrate allowed to cool. The residue was crystallised from dilute alcohol and was