A NEW EFFECT OF HYDROGEN BOND FORMATION

Chelation and Stability of Flavanones

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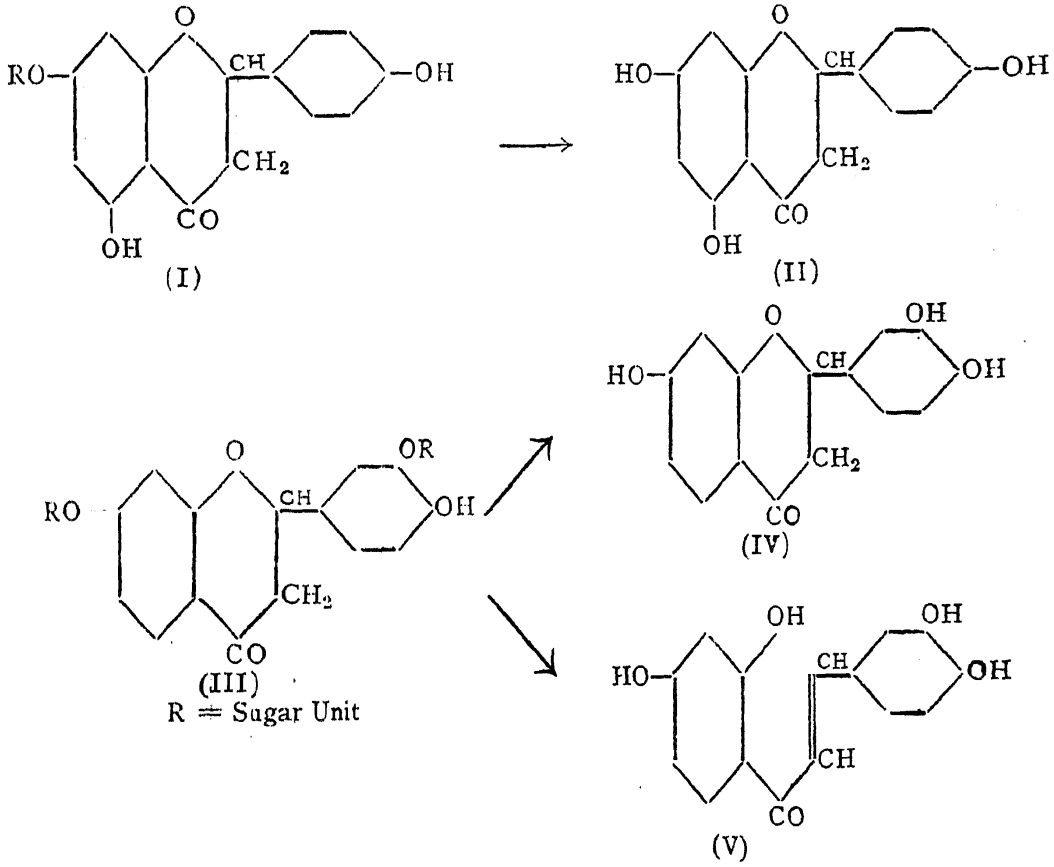
Received December 10, 1947

In the course of the study of the flavanone glycosides, naringin[1] (I) and butrin[2] (III), a remarkable difference was noticed in their behaviour when subjected to acid hydrolysis; whereas the former yields a single aglucone, naringenin (II) which gives all the reactions for a flavanone, the latter under the same conditions forms a mixture of aglucones which could be separated by employing their difference in solubility in water. It consists of the flavanone, butin (IV) and the chalkone, butein (V).

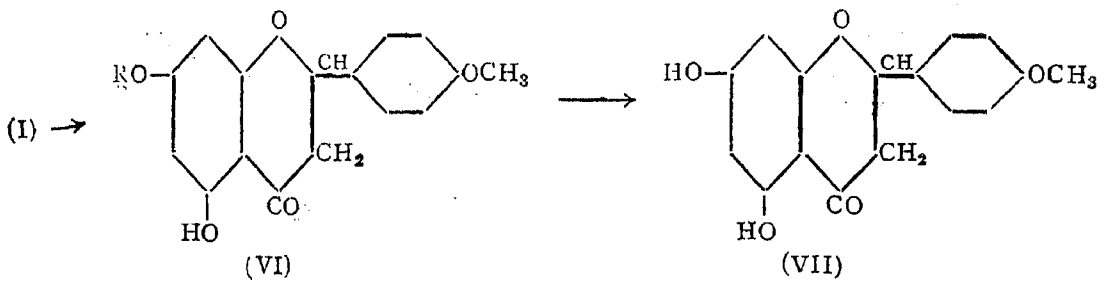


In order to make sure of the individual nature of each product and its freedom from admixture, the following characteristic colour tests have been employed besides the usual criteria of purity based on crystal structure and melting point. The colour reaction (bright red and allied colours) with magnesium and hydrochloric acid in an alcoholic solution is positive for

flavanones and is not given by the isomeric chalkones. On the other hand the chalkones give a positive boric-citric[3] reaction which is negative for the flavanones. Using a combination of these the aglucone obtained by the hydrolysis of naringin is found to be an unmixed flavanone (naringenin) and butrin yields a mixture as already stated.

A similar difference in behaviour is exhibited by the two isomeric flavanones, butin and naringenin when heated with mineral acids or dissolved in alkali and reprecipitated by acids; the former undergoes partial change into the chalkone, butein whereas the latter is unaffected. This phenomenon, *i.e.*, the difference in the stability of the flavanone ring as found in naringin and naringenin on the one hand and butrin and butin on the other should be capable of explanation based on the structures of these compounds. The apparent difference that could be directly noticed is the presence of a 5-hydroxyl group in naringin and naringenin and its absence in butrin and butin and this could be considered to be mainly responsible. This surmise is supported by the following experiments in the partial methylation of naringin and naringenin using dimethyl sulphate.

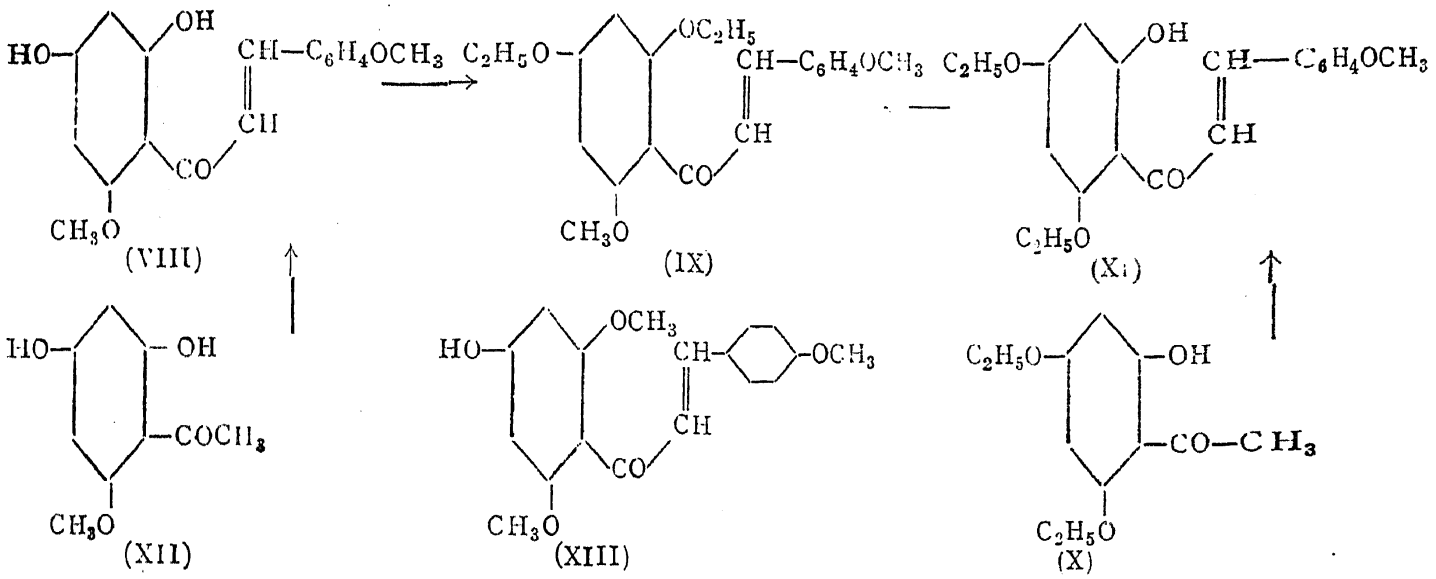
That naringin is a 7-glycoside of naringenin has already been mentioned. Methylation of it using diazomethane was originally investigated by Asahina and Inubuse.[4] He noticed that only one methyl group entered the molecule and when the product was hydrolysed it yielded isosakuranetin (VII) which is the 4′-methyl ether of naringenin. The same result has now been obtained by using just one molecular proportion of dimethyl sulphate for the methylation in dry acetone solution in the presence of anhydrous potassium carbonate as the condensing agent. The final product of hydrolysis (isosakuranetin) gives all the reactions of a flavanone and is free from chalkone. Again it is stable to cold alkali since it is recovered unchanged on acidifying the alkaline solution.



(VI) (VII)

But on methylation using two mols. of dimethyl sulphate, the hydroxyl in the 5-position is also methylated and when the glycoside dimethyl ether is hydrolysed the product is found to consist of 2:4-dihydroxy-6:4′-dimethoxy chalkone (VIII). The constitution of this chalkone has now been established in two ways. (1) It is ethylated and the diethyl ether shown to

be identical with a synthetic sample of 2:4-diethoxy-6:4′-dimethoxy-chalkone (IX). The latter has now been synthesised from phloracetophenone-diethyl ether (X) by first condensing it with anisic aldehyde and finally methylating the chalkone (XI). (2) The dihydroxy chalkone (VIII) has itself been synthesised in the following manner and direct comparison effected. The mono-methyl ether of phloracetophenone (XII) is prepared for this purpose by the partial demethylation of the trimethyl ether according to the method of Gulati and Venkatraman[5] and condensed with anisaldehyde.

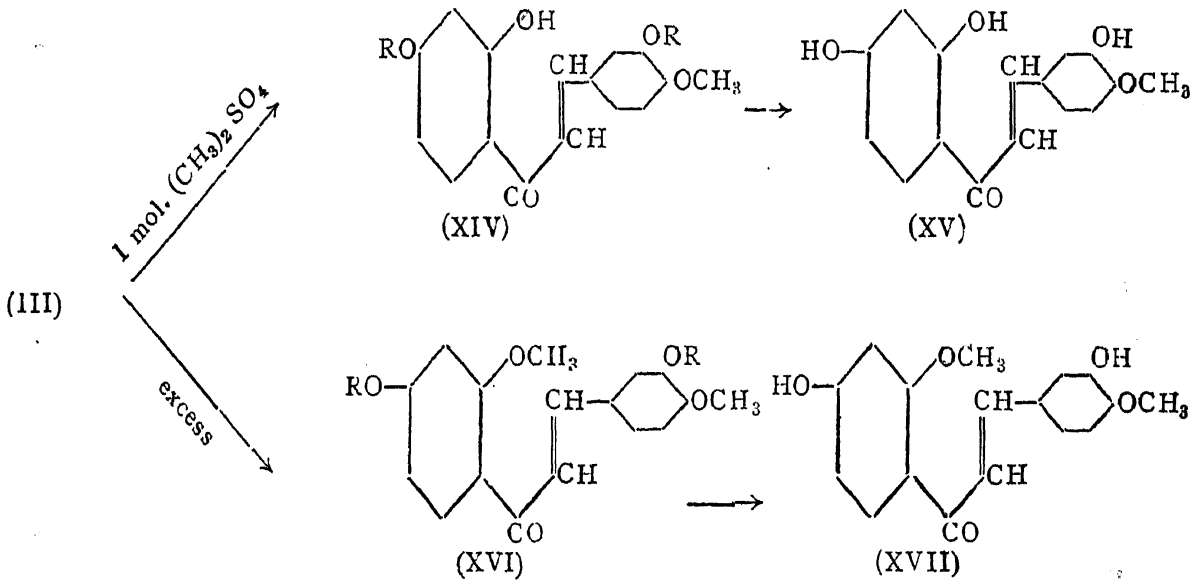


In this connection could be mentioned the work of Sonn and Bülow[6] who condensed acetonitrile with the monomethyl ether of phloroglucinol. The ketone thus obtained was provisionally given the structure 6-methoxy-2:4-dihydroxyacetophenone. This underwent smooth condensation with anisaldehyde to a chalkone melting at 169° which was given provisionally the constitution of 4′:6-dimethoxy-2:4-dihydroxychalkone. The properties of this compound agreed closely with the dimethyl ether (VIII) described above and thus the tentative constitution given by Sonn and Bülow for their product is shown to be correct. Using methoxy-acetonitrile and phloroglucinol-monomethyl ether, more recently Kuhn *et al.*[7] obtained ω:6-dimethyl-ether of phloracetophenone. This further supports Sonn and Bülow's constitution for their acetophenone derivative and chalkone. Obviously in these reactions the monomethyl ether of phloroglucinol behaves like resorcinol.

Methylation of naringin using three mols. of dimethyl sulphate and subsequent hydrolysis leads to the formation of 2:6:4′-trimethoxy-4-hydroxy-chalkone (XIII). In this case the third methyl group has entered after the flavanone ring opened out. The chalkone is found to be identical with a synthetic sample prepared by Rangaswami and Seshadri.[1] From

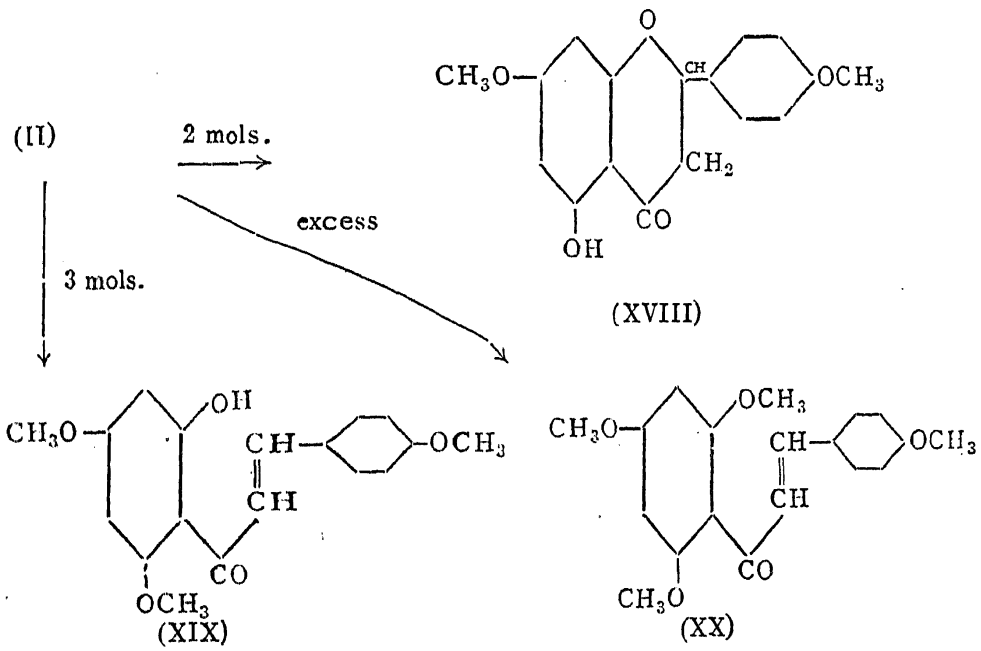
the experiments described above it will be clear that the methylation of the 5-hydroxyl affects the stability of the flavanone ring markedly and the corresponding chalkone is obtained as the product. It is possible that mixtures of chalkones and flavanones are produced in these reactions but the presence of the latter could not be detected by qualitative tests.

The methylation of butrin was effected earlier by Rao and Seshadri[2] using diazomethane. Under the mild conditions employed the free hydroxyl alone was methylated without any further change and the monomethyl ether was obtained fully in the flavanone form. It was colourless and it melted at 226–28°. Reichel *et al.*[8] later reported its synthesis and the melting point of the synthetic product given by them agrees with that noted earlier for methyl-butrin. In order to get information about the behaviour of butrin under the conditions of methylation now adopted for naringin, fresh experiments have now been conducted. Though butrin is only sparingly soluble in acetone a fine suspension of it in this solvent reacts with dimethyl sulphate and potassium carbonate. With one mol. of dimethyl sulphate a monomethyl ether is formed; but it is different from the one already described. It melts at 190° and has the properties of 2-hydroxy-4: 3′-diglucosidoxy-4′-methoxy chalkone (XIV) prepared synthetically by Reichel *et al.*[8] Thus the flavanone ring gets opened out during this methylation. On hydrolysis the new methyl ether yields a monomethyl butein (XV) which is found to be identical with a synthetic sample of 4′-methyl butein. With two mols. or excess of dimethyl sulphate butrin forms a dimethyl ether (XVI). This on hydrolysis produces a dimethyl butein (XVII) found to be identical with a synthetic sample of 2: 4′-dimethoxy-4: 3′-dihydroxy chalkone.



The special importance of the 5-hydroxyl group for the stability of the flavanone structure is again confirmed by methylation experiments using

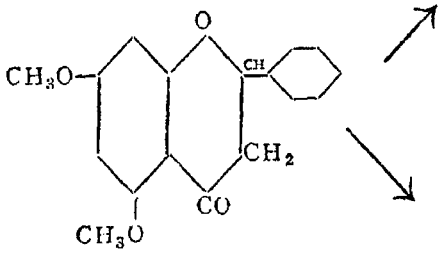
naringenin (II) itself. Shinoda and Sato[9] reported that they could obtain sakuranetin (7-methyl ether of naringenin) by using one molecular proportion of diazomethane. This result could not be obtained now by using one mol. of dimethyl sulphate. A mixture results and though it is found to consist only of flavanones, it could not be separated. However when two mols. of dimethyl sulphate are employed a definite compound, 7:4′-dimethoxy-5-hydroxy-flavanone (XVIII) (sakuranetin-methyl ether) is formed. This was obtained earlier by a number of workers, (1) by the methylation of sakuranetin with diazomethane (Asahina and Inubuse[4]), (2) by the methylation of isosakuranetin (Hattori[10]) and (3) by the methylation of naringenin itself using excess of diazomethane (Shinoda and Sato[9]). Geissman and Clinton[11] prepared the same compound by methylating naringenin with dimethyl sulphate and potash in methyl alcoholic solution. They could separate a small quantity of naringenin-4′-methyl ether (isosakuranetin) as a by-product. The dimethyl ether gives all the properties of a flavanone, can be recovered from an alkaline solution by acidification and is stable to boiling dilute acid. On the other hand with 3 mols. or excess of the methylating agent the chalkone tri-(XIX) and tetramethyl (XX) ethers are formed. These were identified by comparison with authentic samples synthetically obtained by the condensation of anisaldehyde with phloracetophenone di- and trimethyl ethers.[12]



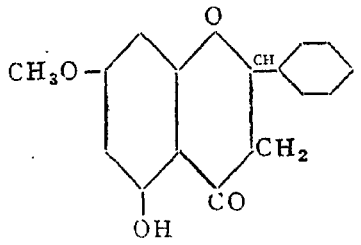
Methylation of butin was carried out earlier by Perkin and Hummel[17] using aqueous alkali and dimethyl sulphate. They obtained a mixture of butin and butein trimethyl ethers. This reaction has now been examined using potassium carbonate and dimethyl sulphate. It has not been found possible to effect partial methylation using one and two mols. of the reagent and isolate definite products. With three mols., butein 4:3′:4′-trimethyl

ether is readily obtained and with four mols. and excess butein tetramethyl ether. These have been characterised by comparison with synthetic samples.

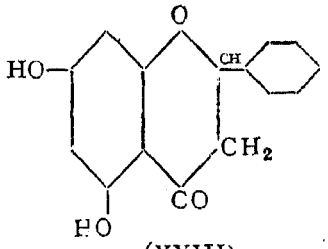
A few simpler compounds have also been tested for their stability to acid and alkali. 7-Methoxy-5-hydroxy-flavanone[13] (XXII), 5 : 7-dihydroxy-flavanone (XXIII) and 5-hydroxy-flavanone (XXIV) belong to one category. The first two have been obtained by the partial and complete demethylation of 5 : 7-dimethoxy flavanone (XXI) using aluminium chloride and the last prepared for the first time by a similar demethylation of 5-methoxy-flavanone.[19]



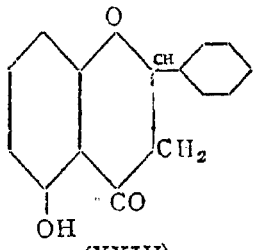
(XXI)



(XXII)



(XXIII)



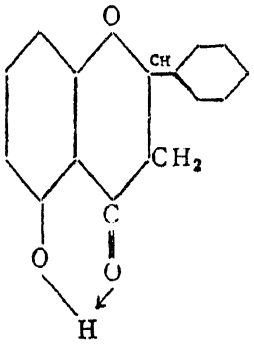
(XXIV)

All the three are found to be stable. On the other hand the methyl ethers, 5-methoxy-flavanone and 5 : 7-dimethoxy-flavanone (XXI) are unstable.

An interesting observation made in the past which is relevant to the present theme relates to the synthesis of polyhydroxy chalkones by the condensation of cinnamoyl chloride and its derivatives with polyhydroxy phenols in nitrobenzene solution using aluminium chloride as the condensing agent. It was observed by Shinoda and Sato[14] that when resorcinol was used the main products were chalkone derivatives whereas with phloroglucinol mainly flavanone derivatives were obtained. In the latter case the chalkones are unstable and by heating with glacial acetic acid or by heating

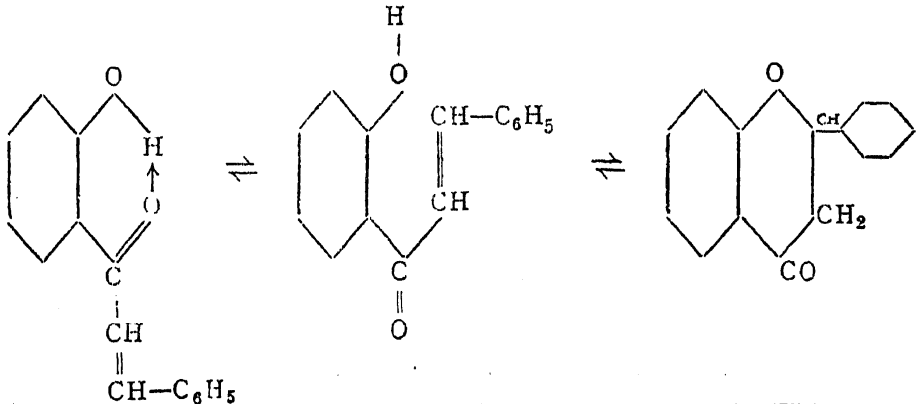
above their melting points they change over to flavanones. Employing the ethyl carbonate of *p*-hydroxy-cinnamic acid the above authors[14] and also Rosenmund[15] synthetically obtained the flavanone, naringenin by the above method. It may further be mentioned here that whereas with resorcinol derivatives like butrin the corresponding chalkone (butein) has also been found to occur in nature,[16] in the case of phloroglucinol derivatives only flavanones are found and the corresponding chalkones do not appear to have been isolated from natural products so far. The presence of a free 5-hydroxyl group may account for this feature and the flavanones concerned should have greater stability.

The above-mentioned significant influence of the 5-hydroxyl group is capable of being explained on the basis of the existence of a hydrogen bond (chelation) involving this hydroxyl and the neighbouring carbonyl group which contributes to the stability of the flavanone structure. That chelation exists in 5-hydroxy-flavanones (XXV) is established by the characteristic colour reactions and the resistance offered to the methylation of this hydroxyl group by mild methylating agents.



(XXV)

In order to explain clearly the part played by the chelation it is necessary to understand the flavanone-chalkone relationship and interconversion in detail. In simple cases it is known that chalkones and the corresponding flavanones exist in equilibrium in an acid or alkaline medium and hence there is certain amount of stability associated with each structure. The following may represent the relationship.



(XXVI) (XXVII) (XXVIII)

That chalkones are largely chelated is amply borne out by their properties and thus structure (XXVI) will correctly represent them. The existence of the non-chelate form (XXVII) though in small amounts in ortho-hydroxy-carbonyl compounds is revealed by the Raman Effect.[18] This was originally explained by giving the trans-structure to the OH group alone. It seems to be more correct to consider that the moving apart of the CO group is also involved as shown in (XXVII).

The presence of a 5-hydroxyl adds more stability to the flavanone structure by the formation of an additional chelate ring as shown in (XXV). Even if the flavanone ring opens the concerned groups are not able to move far enough to yield a stable chalkone. On the other hand whatever alternative constitution the chalkone takes in this case it is favourable for quick conversion into the flavanone. As soon as the 5-hydroxyl is methylated this influence vanishes and the normal conversion into a stable chalkone becomes possible.

Experimental

Hydrolysis of naringin

Naringin was boiled with dilute sulphuric acid (7%) It readily went into solution and after half an hour the aglucone separated out. After heating for 2 hours the solid was filtered off and crystallised from alcohol. It was quite colourless and melted at 243–44°. An alcoholic solution gave a bright red colour with magnesium and hydrochloric acid. No yellow colour was produced with boric and citric acids in dry acetone solution and thus the test for chalkone was negative.

Methylation of naringin

(*a*) *Using one mol. of dimethyl sulphate.*—A solution of naringin (2 g.) in dry acetone (30 c.c.) was refluxed with dimethyl sulphate (0·35 c.c., 1 mol.) and anhydrous potassium carbonate (3 g.) for 20 hours. The potassium salts were then separated by filtration and washed with warm acetone. The acetone filtrate was evaporated when an oily residue was obtained. It did not solidify even after 24 hours. It was, therefore, refluxed with 7% sulphuric acid for two hours. The liquid was cooled and extracted with ether. The ethereal extract was dried over calcium chloride and evaporated. A colourless crystalline solid was obtained. It crystallised from alcohol in the form of colourless tiny prisms, melting at 187–89°.

The potassium salts were dissolved in water, acidified with sulphuric acid and refluxed for two hours. When the resulting liquid was extracted with ether and the ether extract evaporated a small quantity of the above compound was obtained. Total yield, 0·5 g.

It gave reddish brown colour with alcoholic ferric chloride and blue colour with concentrated sulphuric acid.[10] When reduced with magnesium and hydrochloric acid the alcoholic solution was scarlet red. No colour was developed in the boric-citric acid test. It was found to be identical with isosakuranetin (VII).[4]

(*b*) *Using two mols. of dimethyl sulphate.*—The above experiment was repeated with naringin (2 g.) and two molecular proportions of dimethyl sulphate (0·7 c.c.). When the acetone solution was evaporated, an oily residue was obtained. It was hydrolysed by refluxing with 7% sulphuric acid for two hours. The liquid was ether-extracted and the ether removed by evaporation. When the yellow solid residue was crystallised from ethyl acetate, it was obtained in the form of yellow prisms and needles melting at 165–67°. A small quantity of the same compound was also recovered from the potassium salts after boiling with aqueous acid. Total yield, 0·5 g.

It gave a brown colour with ferric chloride in alcoholic solution but developed no colour with magnesium and hydrochloric acid. It was soluble in aqueous sodium hydroxide yielding an yellow solution and dissolved in sulphuric acid with red colour. A mixed melting point determination with a synthetic sample of 2:4-dihydroxy-6:4′-dimethoxy chalkone (VIII) described below showed no depression.

Ethyl derivative.—The above product (0·2 g.) was dissolved in dry acetone (20 c.c.) and ethyl iodide (1 c.c.) and anhydrous potassium carbonate (3 g.) were added. The mixture was refluxed for 20 hours. The solvent was then removed by evaporation and water added to the residue when a yellow crystalline solid separated out. It was filtered and crystallised from a mixture of ethyl acetate and alcohol when it came out as pale yellow broad rectangular plates melting at 120–22°. A mixed melting point determination with a synthetic sample of 6:4′-dimethoxy-2:4-diethoxy-chalkone (IX) described later on showed no depression.

(*c*) *Using excess of dimethyl sulphate.*—Naringin (2 g.) was methylated as before with excess of dimethyl sulphate (2 c.c.). The residue obtained after evaporating the acetone solution was hydrolysed by refluxing with 7% sulphuric acid for 2 hours. On cooling, a yellow crystalline solid separated. It was collected and crystallised from alcohol when it came out in the form of pale yellow needles and micaceous plates melting at 202–4°. A small quantity of the same compound could also be recovered from the potassium salts. It developed no colour with ferric chloride in alcoholic solution and dissolved in aqueous sodium hydroxide yielding an yellow solution. Its melting point was not depressed when mixed with a synthetic sample of 2:6:4′-trimethoxy-4-hydroxy chalkone[1] (XIII).

Synthesis of 2:4-*dihydroxy*-6:4'-*dimethoxy-chalkone* (*VIII*).—To a solution of 2:4-dihydroxy-6-methoxy acetophenone (0·5 g.) and anisaldehyde (2 c.c.) in alcohol (20 c.c.) was added potassium hydroxide (2 g. in 2 c.c. of water) little by little with shaking. More alcohol was added to obtain a clear solution and the container was corked air-tight and left aside for 60 hours. The solution was then diluted with a large amount of water and extracted with ether to remove any unreacted aldehyde. The remaining aqueous solution on acidification deposited a bright yellow oil which was taken up in ether. The ethereal extract was washed with dilute sodium bicarbonate solution to remove any free anisic acid. On evaporating the ether, a yellow crystalline solid was obtained which was again crystallised from alcohol. The chalkone was thus obtained as yellow prisms and needles melting at 165–7°. Yield, 0·2 g. (Found: C, 67·8; H, 5·6; $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·3%.)

2-*Hydroxy*-4'-*methoxy*-4:6-*diethoxy chalkone* (*XI*)

4:6-Diethoxy-2-hydroxy-acetophenone (X) obtained by the partial ethylation of phloroacetophenone (1 g.) and anisaldehyde (5 c.c.) were dissolved in alcohol and potassium hydroxide (8 g. in 8 c.c. of water) added to it little by little with shaking. The condensation was carried out as given above. The crude product separated as a yellow solid; it was filtered and washed with sodium bicarbonate solution to remove any anisic acid and then crystallised from alcohol. The chalkone was thus obtained in the form of bright yellow rectangular plates melting at 110–12°. Yield, 1 g. (Found: C, 70·6; H. 6·6; $C_{20}H_{22}O_5$ requires C, 70·2 and H, 6·4%.)

2:4'-*Dimethoxy*-4:6-*diethoxy-chalkone* (*IX*)

The above chalkone (0·5 g.) was methylated in anhydrous acetone medium with dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (2 g.). After refluxing the mixture for 15 hours, the solvent was removed by distillation and water added to the residue. The yellow solid that separated out was filtered, washed and crystallised from a mixture of ethyl acetate and alcohol. It was obtained as lemon yellow broad rectangular plates melting at 120–22°. Yield, 0·4 g. The chalkone was insoluble in alkali and sparingly soluble in alcohol. It gave no colour with ferric chloride in alcoholic solution. (Found: C, 70·4; H, 7·0; $C_{21}H_{24}O_5$ requires C, 70·8; and H, 6·7%).

Methylation of butrin

(*a*) *With* 1 *mol. of dimethyl sulphate.*—Butrin (1 g.) was finely powdered and suspended in dry acetone (40 c.c.), dimethyl sulphate (0·2 c.c.) and

anhydrous potassium carbonate (0·5 g.) added and the mixture refluxed on a water-bath for 14 hours. It was then filtered and the residue washed with hot acetone. On evaporating acetone from the filtrate only a very small quantity of the methylated product (XIV) could be obtained. A little more of it could be secured by treating the residue on the filter with the minimum quantity of water and filtering off quickly. After one crystallisation from rectified spirits it melted at about 190° (decomp.); Reichel *et al.*[8] recorded m.p. 191–99° for their synthetic product. It gave no colour with magnesium and hydrochloric acid in alcoholic solution, but gave a deep brown colour with alcoholic ferric chloride. But most of the methylated product was in the aqueous carbonate solution. This was directly hydrolysed by acidifying with sulphuric acid and refluxing the solution for 2 hours. After cooling it was ether extracted and ether removed by evaporation when a yellow solid separated out. When recrystallised from alcohol it was obtained as yellow flat needles and rectangular plates melting at 206–8°. The mixed melting point with a synthetic sample of 4′-O-methyl-butein (XV)[2] was undepressed. It gave an olive brown colour with alcoholic ferric chloride. Yield, 0·2 g.

(*b*) *With excess dimethyl sulphate.*—Finely powdered butrin (1 g.) was suspended in dry acetone (40 c.c.), dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (3 g.) added and the mixture refluxed on a water-bath for 30 hours. It was then filtered and the residue washed with hot acetone. On evaporating acetone from the filtrate a small quantity of the methylated product (red solid) separated. More was obtained by treating the residue on the filter with a little water and filtering and washing it with small quantities of water. The aqueous solution (A) still contained considerable quantity of it and could be directly hydrolysed. Butrin dimethyl ether crystallised from alcohol as pale yellow glistening crystals melting at 210° (decomp.). Yield, 0·2 g. It gave no colour with alcoholic ferric chloride. It was rather hygroscopic and was also contaminated with mineral matter; satisfactory analytical results could not be obtained.

Hydrolysis.—The dimethyl glycoside obtained in the previous experiment was hydrolysed by refluxing with 7% aqueous sulphuric acid (20 c.c.) for 2 hours. The solution was cooled and extracted with ether. On removing the ether a pale yellow solid separated out. It was crystallised from rectified spirits when it was obtained as colourless tiny prisms melting at 95–97°. Further quantity of this compound could be obtained by acidifying the aqueous carbonate solution (A) with sulphuric acid and heating under reflux. The compound was soluble in hot sodium carbonate solution and gave no colour with ferric chloride in alcoholic solution. The mixed

melting point with a synthetic sample of 2:4′-dimethyl butein (see below) was undepressed. (Found: C, 68·2; H, 4·9; $C_{17}H_{16}O_5$ requires C, 68·0; and H, 5·3%.)

Synthesis of 2:4′-dimethyl butein.—Isopeonol (1 g.) and isovanillin (1 g.) were dissolved in alcohol and sodium hydroxide (5 g. in 4 c.c. of water) added to the solution. The mixture was corked air tight and allowed to stand for 48 hours at the room temperature. The solution was then diluted with water and acidified with hydrochloric acid. The chalkone that separated out was extracted with ether and the ether solution washed with aqueous sodium bicarbonate to remove any isovanillic acid formed during the reaction. The ether was removed by distillation when 2:4′-dimethyl butein separated as a pale yellow solid. It crystallised from rectified spirits as colourless tiny prisms melting at 95–97°. It gave no colour with alcoholic ferric chloride and was soluble in hot sodium carbonate solution. Yield (0·5 g.). It was identical with the product of hydrolysis of dimethyl butrin described above.

Methylation of Naringenin

(*a*) *Using two mols. of dimethyl sulphate.*—The methylation of naringenin (1 g.) was carried out as in other cases using dimethyl sulphate (0·8 c.c.) and anhydrous potassium carbonate (3 g.) in acetone medium. After refluxing for ten hours, the acetone solution was filtered and evaporated. The residue obtained thereby, was sparingly soluble in aqueous alkali. It was washed twice with cold alkali and then with water. It then crystallised from alcohol in the form of colourless long rectangular prisms melting at 120–22°. Yield, 0·5 g.

An alcoholic solution of the substance developed a scarlet red colour with magnesium and hydrochloric acid and with ferric chloride, a deep reddish brown colour. It was found to be identical with sakuranetin-monomethyl ether.[9]

(*b*) *Using three mols. of dimethyl sulphate*

Naringenin (1 g.) was methylated as in previous cases using three molecular proportions of dimethyl sulphate (1·2 c.c.) and anhydrous potassium carbonate (4 g.) in dry acetone medium. After refluxing for 10 hours, the acetone solution was filtered and evaporated. A yellow semi-solid was obtained which crystallised from alcohol as lemon yellow stout prisms, melting at 112–13°. Yield of the pure product 0·5 g.

It dissolved in aqueous alkali forming an yellow solution. Alcoholic ferric chloride developed an yellowish red colour. No red colour was

noticed on reduction with magnesium and hydrochloric acid in alcoholic solution. A bright yellow colour was obtained when the substance was treated with boric and citric acids in dry acetone solution. From a mixed melting point determination, it was found to be identical with an authentic sample of 4: 6: 4′-trimethoxy-2-hydroxy-chalkone.[12]

(*c*) *Using four mols. of dimethyl sulphate*

The above experiment was repeated with naringenin (1 g.) and four molecular proportions of dimethyl sulphate (2 c.c.). When the acetone solution was evaporated a yellow crystalline solid was obtained. It crystallised from alcohol in the form of yellow aggregates of plates melting at 120–21°. It was insoluble in alkali and gave no colour changes with ferric chloride in alcoholic solution and also with magnesium and hydrochloric acid. It was found to be identical with a synthetic sample of 2: 4: 6: 4′-tetramethoxy chalkone.[12]

Methylation of butin

(*a*) *With* 3 *mols. of dimethyl sulphate.*—To a solution of butin (0·5 g.) in dry acetone (40 c.c.), dimethyl sulphate (0·6 c.c.) and anhydrous potassium carbonate (2 g.) were added and the mixture refluxed on a water-bath for 12 hours. It was then filtered and the residue washed with hot acetone. On evaporating the acetone from the filtrate a yellow solid separated out. When crystallised from alcohol it was obtained as glistening yellow leaflets melting at 156°. It was identical with butein-4: 3′: 4′-trimethyl ether[17] and the mixed melting point with a synthetic sample was undepressed. It gave a brown colour with alcoholic ferric chloride.

(*b*) *With excess of dimethyl sulphate.*—Dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (3 g.) were added to a solution of butin (0·5 g.) in dry acetone (40 c.c.) and the mixture refluxed for 24 hours on a water-bath. It was then filtered and the residue washed with hot acetone. On evaporating the acetone solution the methylated product was obtained as a yellow solid. By crystallising from alcohol twice butein-tetramethyl ether was obtained as yellow irregular plates melting at 120–22°. It gave no colour with ferric chloride in alcoholic solution and was insoluble in alkali. The mixed melting point with a synthetic sample of tetramethyl butein described below was undepressed.

Synthesis of tetramethyl butein.—Resacetophenone dimethyl ether (0·5 g.) and veratraldehyde (1 g.) were dissolved in alcohol (30 c.c.) and potash (5 g. in 4 c.c. of water) added. More alcohol was added to get a clear solution and the mixture kept corked air tight for 48 hours at room temperature,

noticed on reduction with magnesium and hydrochloric acid in alcoholic solution. A bright yellow colour was obtained when the substance was treated with boric and citric acids in dry acetone solution. From a mixed melting point determination, it was found to be identical with an authentic sample of 4: 6: 4′-trimethoxy-2-hydroxy-chalkone.[12]

(*c*) *Using four mols. of dimethyl sulphate*

The above experiment was repeated with naringenin (1 g.) and four molecular proportions of dimethyl sulphate (2 c.c.). When the acetone solution was evaporated a yellow crystalline solid was obtained. It crystallised from alcohol in the form of yellow aggregates of plates melting at 120–21°. It was insoluble in alkali and gave no colour changes with ferric chloride in alcoholic solution and also with magnesium and hydrochloric acid. It was found to be identical with a synthetic sample of 2: 4: 6: 4′-tetramethoxy chalkone.[12]

Methylation of butin

(*a*) *With* 3 *mols. of dimethyl sulphate.*—To a solution of butin (0·5 g.) in dry acetone (40 c.c.), dimethyl sulphate (0·6 c.c.) and anhydrous potassium carbonate (2 g.) were added and the mixture refluxed on a water-bath for 12 hours. It was then filtered and the residue washed with hot acetone. On evaporating the acetone from the filtrate a yellow solid separated out. When crystallised from alcohol it was obtained as glistening yellow leaflets melting at 156°. It was identical with butein-4: 3′: 4′-trimethyl ether[17] and the mixed melting point with a synthetic sample was undepressed. It gave a brown colour with alcoholic ferric chloride.

(*b*) *With excess of dimethyl sulphate.*—Dimethyl sulphate (1 c.c.) and anhydrous potassium carbonate (3 g.) were added to a solution of butin (0·5 g.) in dry acetone (40 c.c.) and the mixture refluxed for 24 hours on a water-bath. It was then filtered and the residue washed with hot acetone. On evaporating the acetone solution the methylated product was obtained as a yellow solid. By crystallising from alcohol twice butein-tetramethyl ether was obtained as yellow irregular plates melting at 120–22°. It gave no colour with ferric chloride in alcoholic solution and was insoluble in alkali. The mixed melting point with a synthetic sample of tetramethyl butein described below was undepressed.

Synthesis of tetramethyl butein.—Resacetophenone dimethyl ether (0·5 g.) and veratraldehyde (1 g.) were dissolved in alcohol (30 c.c.) and potash (5 g. in 4 c.c. of water) added. More alcohol was added to get a clear solution and the mixture kept corked air tight for 48 hours at room temperature,

Then the solution was diluted and the chalkone that separated was filtered and washed with water. When crystallised from rectified spirits twice using animal charcoal it was obtained as yellow plates melting at 120–22°. It gave no colour with ferric chloride in alcoholic solution and was insoluble in aqueous alkali. It was sparingly soluble in ether but readily soluble in ethyl acetate and alcohol. (Found: C, 70·0; H, 6·3; $C_{19}H_{20}O_5$ requires C, 69·5; H, 6·1%.)

5: 7-*Dihydroxy-flavanone.*—This was originally made by Shinoda and Sato[14] by condensing phloroglucinol with cinnamoyl chloride in nitrobenzene solution using aluminium chloride as condensing agent. It has now been obtained by the demethylation of 5: 7-dimethoxy flavanone with anhydrous aluminium chloride in benzene solution.

5: 7-Dimethoxy-flavanone (0·5 g.) was dissolved in dry benzene (5 c.c.), anhydrous aluminium chloride (1 g.) added and the mixture refluxed on a water-bath for 1 hour. Benzene was then removed by evaporation. The aluminium chloride complex was decomposed by treating with ice-cold dilute hydrochloric acid when the dihydroxy-flavanone separated out. This was ether extracted and ether distilled off. On crystallising twice from rectified spirits using animal charcoal it was obtained as pale grey aggregates of broad rectangular plates melting at 201–3°. Yield (0·3 g.). It gave a pink colour with alcoholic ferric chloride and dissolved in aqueous sodium hydroxide forming a yellow solution (Shinoda and Sato reported the melting point as 203°). (Found: C, 69·8; H, 4·5; $C_{15}H_{12}O_4$ requires C, 70·3; H, 4·6%.)

5-*Hydroxy-flavanone.*—This was prepared by demethylating 5-methoxy-flavanone with anhydrous aluminium chloride.

5-Methoxy-flavanone[19] (0·5 g.) was dissolved in dry benzene (10 c.c.) and anhydrous aluminium chloride (1 g.) was added. After refluxing the mixture for one hour, the product was worked up as in the above preparation. The 5-hydroxy-flavanone crystallised from benzene (charcoal) as straw-yellow rectangular prisms melting at 152–54°. It gave a green colour with ferric chloride in alcoholic solution and dissolved in aqueous alkali on warming to form an yellow solution. (Found: C, 64·8; H, 6·0; $C_{15}H_{12}O_3$, $2H_2O$ requires C, 65·2; H, 5·8%.)

Action of alkali on some flavanones

1. *Naringenin.*—Naringenin dissolved readily in cold 10% aqueous sodium hydroxide forming a yellow solution. After half an hour it was acidified with dilute sulphuric acid when a colourless solid was precipitated,

This was filtered, washed and dried. It was identified as naringenin since it had all the required properties and reactions and the mixed melting point was not depressed.

2. *Isosakuranetin.*—It was also readily soluble in 10% cold aqueous sodium hydroxide and was recovered almost quantitatively from the yellow alkaline solution by acidification after half an hour. The melting point and colour reactions were the same as those of the original substance.

3. *Naringenin-7 : 4′-dimethyl ether.*—It was sparingly soluble in cold 10% aqueous sodium hydroxide. It however went into solution completely on warming. When acidified after keeping the alkaline solution for half-an-hour, the original compound was reprecipitated almost quantitatively.

4. 5 : 7-*Dihydroxy flavanone.*—It dissolved in cold aqueous sodium hydroxide (10%) forming a yellow solution. It could be completely recovered unchanged by acidifying the alkaline solution after half-an-hour.

5. 5-*Hydroxy-7-methoxy-flavanone.*[13]—This compound dissolved in aqueous sodium hydroxide (10%) on warming. It could be recovered unchanged by acidification of the alkaline solution after half-an-hour.

6. 5-*Hydroxy-flavanone.*—It went slowly into solution in 10% cold aqueous sodium hydroxide. The alkaline solution was yellow and deposited the original 5-hydroxy-flavanone when acidified with sulphuric acid.

7. 5 : 7 : 4′-*Trimethoxy-flavanone*[20].—This compound was synthesised by refluxing an alcoholic solution of 2-hydroxy-4 : 6 : 4′-trimethoxy chalkone[12] (m.p. 112°) containing 4% sulphuric acid for 24 hours. On evaporation of the alcohol, a mixture of the flavanone and chalkone was obtained. The components were separated by fractional crystallisation from ethyl acetate in which the flavanone was less soluble. It was finally crystallised from the same solvent when it was obtained as colourless prismatic crystals melting at 123–24°.

The trimethoxy-flavanone was insoluble in the cold aqueous sodium hydroxide (10%). But it gradually turned yellow and showed a tendency to go into solution slowly. This dissolution could be hastened by warming. The alkaline solution was yellow and when acidified with sulphuric acid a yellow semi-crystalline solid was obtained. It was taken up in ether. On the removal of ether by evaporation, a yellow oil was left behind which solidified on scratching with a glass rod. It crystallised from alcohol in the form of stout prisms melting at 112–13° and was identified to be 2-hydroxy-4 : 6 : 4′-trimethoxy-chalkone by direct comparison.

8. *5-Methoxy-flavanone*[19].—It was insoluble in cold 10% aqueous alkali, but it could be got into solution by slight warming. The alkaline solution was yellow and when acidified with sulphuric acid after half-an-hour, a yellow solid different from the 5-methoxy-flavanone, was obtained. It melted at 127° and gave a brown ferric chloride colour. It was identified to be the 2-hydroxy-6-methoxy-chalkone[19] by direct comparison.

9. *5:7-Dimethoxy-flavanone*[13].—This behaved similar to the above. When the alkaline solution was acidified after half-an-hour a pale yellow crystalline solid was obtained. It crystallised from aqueous alcohol and melted at 91–92° and in all its properties was identical with 2-hydroxy-4:6-dimethoxy-chalkone.[13]

Summary

A marked difference in the behaviour of naringin and of butrin towards hydrolysing agents has been noticed previously. Thus naringin yields the flavanone, naringenin, while butrin yields a mixture of the flavanone, butin, and the chalkone, butein. Similar difference in behaviour is also exhibited by the flavanones, naringenin and butin, when treated with mineral acids or alkalis.

Methylation has now been conducted of the glycosides, naringin and butrin and their aglucones naringenin and butin using varying molar proportions of dimethyl sulphate in the presence of anhydrous potassium carbonate and in acetone solution. From the study of the products and also of a number of simpler flavanone derivatives it is clear that the presence of a free 5-hydroxyl gives stability to the pyranone ring. This stability is not found when the 5-hydroxyl is non-existent as in butrin and butin or gets methylated as in some of the methyl ethers of naringin and naringenin and other flavanones; the products are chalkones in these cases.

The special influence of the 5-hydroxyl group in stabilising flavanone structure is attributed to the existence of chelation between this hydroxyl and the carbonyl group of the pyranone ring. The mechanism of the chalkone-flavanone conversion is discussed.

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