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Action of Sodium Bisulfite on Lignin Model Compounds

by Biswanath Sen

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ACTION OF SODIUM BISULFITE ON LIGNIN MODEL COMPOUNDS

A thesis submitted by

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INTRODUCTION

Although a vast amount of research has been carried out on the chemistry of lignin in the past hundred years, the structure of lignin is still an open question. Part of the difficulty lies in the fact that lignin is an amorphous high polymer whose composition varies in accordance with the method of isolation and from different sources. Some of these isolated lignins are quite different from the original or protolignin present in wood. Only Brauns' isolated native lignin (1) seems to be present as such in wood. However, the yield of native lignin is small in comparison with the total lignin content of the wood.

As the result of extensive research, lignin chemists have, by now, come to accept several pertinent facts about the constitution of lignin. The presence of aromatic residues in lignin has been proved by various workers. Vanillin has been isolated by alkaline hydrolysis of lignosulfonic acid and by alkaline oxidation of isolated lignin, or even protolignin in the presence of nitrobenzene (2). Recently Lange (3) has used a physical method to show the presence of aromatic rings in protolignin.

Harris and co-workers (4,5) isolated propylcyclohexane derivatives in a fairly large yield by the hydrogenolysis of methanol maple lignin and thereby showed the presence of a three-carbon side chain attached to an aromatic ring. This was anticipated by Klason (6), who assumed that lignin is formed by polymerization of coniferyl aldehyde. Freudenberg and co-workers (7) have recently obtained a ligninlike material by the dehydropolymerization of coniferyl alcohol.

Although it is generally accepted that gymnosperm lignin is built mainly of guaiacyl units with a three-carbon side chain in the meta position to the methoxyl group, the constitution of the side chain and the manner of linking of the lignin building stones is still uncertain. Free alcoholic groups are present in lignin, and the presence of carbonyl group is also indicated (8). The presence of aliphatic double bonds in lignin is controversial, although more and more evidences are accumulating to indicate that such double bonds do exist in lignin (9). The presence of oxygen-containing heterocyclic rings in lignin has been postulated from time to time, though no direct proof has been obtained. However, Glading (10) and Aulin-Erdtman (11) postulated that ultraviolet spectroscopic measurements indicate the presence of such heterocyclic rings in lignin.

The individual lignin building stones may be linked by an ether linkage between the phenolic hydroxyl group of one building stone and a carbon atom in the side chain of another building stone. But there is also evidence for carbon-carbon linkage between lignin building stones. Freudenberg and co-workers (12,13) obtained isohemipinic acid by a mild alkali treatment of methylated lignin, followed by methylation and oxidation. The production of isohemipinic acid indicates that some of the benzene rings in lignin have an additional carbon side chain at the 5-position from the usual side chain. Evidently, if both types of linkage, e.g., a phenolic ether linkage, and a carbon-carbon linkage, are present between the same two lignin building stones, a heterocyclic ring would be formed.

Among the reactions used for the study of lignin, sulfonation has been widely employed. Unlike oxidation or hydrogenation, sulfonation does not break down lignin into simple monomolecular compounds. The study of the product of sulfonation, or the mechanism of sulfonation should therefore contribute important information about the structure of lignin. Many theories have been advanced for the mechanism of sulfonation, and on the basis of these, the presence of certain chemical groups in lignin have been inferred. However, none of these theories are entirely satisfactory.

In recent years, the sulfonation reaction has been used to evolve a new approach to lignin chemistry. This is the approach of model reactions. If an organic model compound can be sulfonated in the same way as lignin, then such a grouping as is present in the model compound may also be present in lignin, and may be responsible for the sulfonation of lignin. However, this is an oversimplified picture, because many organic compounds containing different chemical groups undergo sulfonation. In order to make good use of the model reactions in the field of lignin chemistry, we must (a) start with a good model compound, i.e., one which has groups similar to those present in lignin; (b) compare the process of sulfonation with that of lignin, e.g., rate, conditions, and changes in the physical properties during sulfonation; (c) show what chemical change has taken place in the model compound as the result of sulfonation; and (d) compare the product of sulfonation with lignosulfonic acid. The advantage of model reactions is that we deal with low-molecular weight compounds of known structure, and that we can easily trace the change effected by sulfonation.

HISTORICAL REVIEW

As was mentioned earlier, many theories have been proposed from time to time for the mechanism of sulfonation of lignin. It is generally recognized that the sulfonation of wood proceeds in two stages (14). First, a solid lignosulfonic acid with a low sulfur content is formed. Further treatment with bisulfite results in hydrolysis, solubilization, and further sulfonation of the solid lignosulfonic acid. Freudenberg and co-workers (15) showed that sulfonation takes place entirely in the side chain. Experiments by Hibbert and Tomlinson (16) seem to indicate further that in lignosulfonic acid the sulfonic acid is probably attached to the carbon in the side chain next to the benzene ring.

The sulfonation of lignin may occur in one or more of the following ways:

1. Replacement of an alcoholic hydroxyl group by the sulfonic acid group
2. Addition of sodium bisulfite to an activated double bond
3. Splitting of an ether linkage and subsequent sulfonation of the liberated alcoholic hydroxyl group
4. Opening of a pyran or furan ring with subsequent sulfonation of the liberated alcoholic hydroxyl group.

Model experiments have been carried out to show that alcoholic hydroxyl groups can be replaced by sulfonic acid groups by the action of bisulfite. Holmberg and Hedén (17) obtained the corresponding sulfonic acids from α-phenylethanol, β-phenylethanol, and benzhydrol by cooking with bisulfite. However, Kratzl and Däubner (18) contend that the yields of these sulfonic acids are low compared to that of lignosulfonic acid. Lindgren (19) showed that substituted benzyl alcohols, such as vanillyl alcohol, veratryl alcohol, 3-methoxy-4-hydroxyphenylmethylcarbinol, and 3,4-dimethoxyphenylmethylcarbinol were more suitable lignin models in giving good yields of the corresponding sulfonic acids.

However, the presence of other groups in the side chain beside hydroxyl has a decided effect on sulfonation. Kratzl, Däubner, and Siegens (20) showed that α-hydroxyketones like phenylacetylcarbinol, benzoin, and α-hydroxypropiovanillone gave little or no sulfonic acid. However, β-hydroxyketones were very readily sulfonated. These workers also showed that the sulfonic acids from the β-hydroxyketones were easily hydrolyzed (reversed aldol reaction) by alkali in the following way:

$$RCH(SO_3H)CH_2COR' \longrightarrow RCH(OH)CH_2COR' \longrightarrow RCHO + CH_3COR'.$$

Wacek and Kratzl (21) believe that the "hydrolyzable" vanillin is formed from lignosulfonic acid according to the above mechanism.

It was Klason (22) who first observed that in lignosulfonic acid a part of the sulfur is loosely combined and is split off by dilute acid or alkali. He explained this by assuming that the firmly bound sulfur in lignosulfonic acid is formed by the addition of sulfurous acid to a double bond, whereas the loosely bound sulfur is obtained by the addition of sulfurous acid to a carbonyl group. This led Hägglund (23) to use cinnamic aldehyde as a model substance; this reacted with bisulfite to give hydrocinnamic aldehyde β-sulfonic acid.

The above findings have been generalized by Kratzl and co-workers (18,20), who showed that although isolated double bonds have little or no tendency to react with bisulfite, a double bond in an α,β-unsaturated aldehyde or ketone readily adds sulfurous acid. They obtained good yields of sulfonic acids from a number of chalcones, e.g., 2'-hydroxychalcone, 4'-hydroxy-3'-methoxychalcone, and 4'-hydroxy-3,4,3'-trimethoxychalcone. This they explained by pointing out that the double bond in such compounds is activated by the carbonyl group in the β-position, and that β-hydroxyketones and α,β-unsaturated ketones are readily interconvertible.

$$RCH(OH)CH_2COR' \underset{+H_2O}{\overset{-H_2O}{\rightleftharpoons}} RCH{=}CHCOR'$$

Ethers have also been used as lignin models. After finding a suitable model compound in α-phenylethanol, Holmberg (24) considered that the lignin molecule may be bound in the wood by benzyl ether linkages to carbohydrate molecules or to other lignin molecules. Freudenberg and co-workers (25,26) also considered the possibility that ether groups may be responsible for the sulfonation of lignin.

Working with phenolic ethers as model substances, Richtzenhain (27) showed that benzyl guaiacyl ether was split into benzyl alcohol and guaiacol by the action of sulfurous acid. However, p-methoxybenzyl guaiacyl ether was converted by the action of sodium sulfite into guaiacol and sodium *p*-methoxybenzyl sulfonate. The ether of an aromatic secondary alcohol was found to be a better model compound than that of an aromatic primary alcohol. In contrast with benzyl guaiacyl ether, guaiacyl methylphenylcarbinyl ether was not only split, but the alcohol was partly converted into the sulfonic acid.

Lindgren (28) showed that both diveratryl ether and veratryl ethyl ether can be sulfonated to 3,4-dimethoxytoluene-ω-sulfonic acid.

Freudenberg (13) proved that sulfonation can occur by the opening of an oxygen ring by showing that Erdtman's acid is sulfonated by bisulfite as a result of the opening of a furan ring and subsequent sulfonation of the liberated alcoholic hydroxyl group. Richtzenhain (27) studied the behavior of a number of oxygen-containing heterocyclic compounds toward bisulfite. He found that whereas flavanone gives sodium β-(2-hydroxybenzoyl)phenylethyl-α-sulfonate by ring opening, flavan, 8-methoxyflavan, α-methylcoumaran, α-methyl-β-phenylcoumaran, and 2-phenylcoumarone did not react with bisulfite. All types of pyran or furan rings are therefore not opened by cooking with bisulfite.

PRESENTATION OF THE PROBLEM

The problem deals with a search for further model compounds which are more suitable for the study of sulfonation of lignin than those hitherto used. As described in the previous section, various model compounds have already been studied as to their behavior toward bisulfite, giving some information about the possible mechanism of the sulfonation of lignin. However, further work with model substances more closely related to the structure of lignin, as suggested in recent years, has to be done in order to gain a more complete knowledge of the sulfonation of lignin. The present problem deals with the synthesis of a series of such model compounds and with a study of the behavior of these compounds toward bisulfite under the conditions used in the bisulfite sulfonation of lignin.

Because there is ample indication that in lignin the benzene rings of the building stones are joined by a three-carbon side chain, compounds having a C_6-C_3-C_6 group are of particular interest. The work of Kratzl and co-workers on the sulfonation of chalcones and other α,β-unsaturated ketones show that these compounds are readily sulfonated. However, it is not yet known whether pyran or furan ring formation, or easy susceptibility to ring formation of the three-carbon side chain would change the reactivity of the α,β-unsaturated ketonic grouping toward bisulfite. The problem also includes a study of this aspect of sulfonation and would therefore involve model compounds with α,β-unsaturated ketonic groups linking two aromatic nuclei; the three-carbon side chain is open in some, but forms part of a heterocyclic ring in others.

The presence of differently substituted benzopyran groups has been postulated in lignin from time to time, but no definite proof has been obtained. However, sulfonation of some model compounds which contain substituted benzopyran groups has been studied in the past in order to see whether such groups can be sulfonated. But in order to draw definite conclusions, more extensive work has to be done in this direction, especially on the use of model compounds which contain groups known to be present in lignin. As a beginning in this direction, several substituted benzopyran compounds, not previously studied, are to be investigated in this work as to their behavior towards bisulfite.

The present work specifically involves syntheses of several suitable substituted benzofuran and benzopyran lignin model compounds and a study of the action of bisulfite cooking liquor on these and other model compounds. The model compounds to be studied are (1) 2-vanilloylbenzofuran, (2) 2-vanillylidene-3-coumaranone, (3) 3-methoxy-2',4-dihydroxychalcone, (4) 3'-methoxy-4'-hydroxyflavone, (5) 3'-methoxy-2,4'-dihydroxychalcone, (6) 3'-methoxy-4'-hydroxyflavylium chloride, (7) 3'-methoxy-4'-hydroxyflavanone, (8) 3'-methoxy-4,4'-dihydroxyflavan, (9) quercetin, and (10) 2,3-dihydroquercetin. Of these, compounds 7, 9, and 10 have been previously reported in the literature.

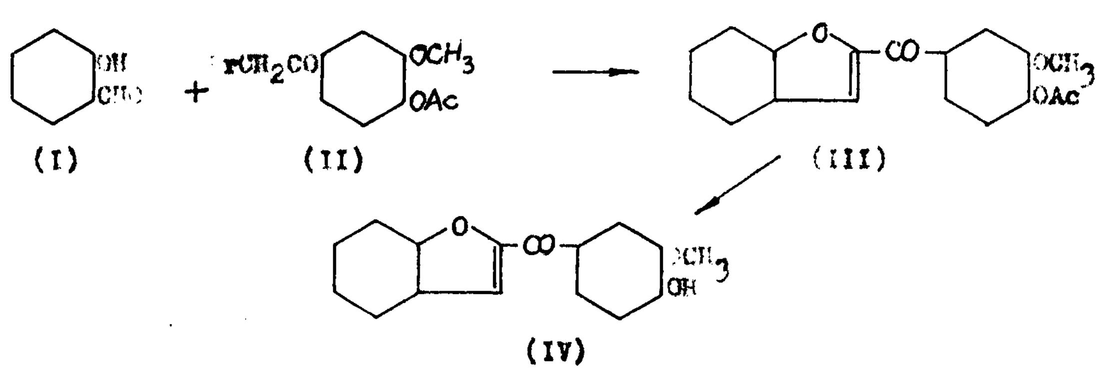
SYNTHESES OF MODEL COMPOUNDS

The lignin model compounds were synthesized following conventional organic reactions. The details of synthesis, as well as discussions on each model compound synthesized, are presented in this section.

2-VANILLOYLBENZOFURAN (IV)

Two procedures employing a common synthetic method for the preparation of benzofuran derivatives were used for synthesizing 2-vanilloylbenzofuran (IV). This method involves a combined hydrobromic acid elimination and aldol condensation between properly substituted aromatic aldehydes and ketones under the influence of anhydrous potassium carbonate (29).

ω-Bromoacetovanillone acetate (II) is a known compound and can be prepared fairly easily from acetovanillone (30,31). In the first procedure, therefore, this was used as a starting material. Salicylaldehyde (I) and ω-bromoacetovanillone (II) were condensed in absolute ethanol solution in the presence of anhydrous potassium carbonate to give 2-vanilloylbenzofuran acetate (III). This was hydrolyzed to give 2-vanilloylbenzofuran (IV).



The yield of 2-vanilloylbenzofuran acetate (III) in the condensation step was very poor; an appreciable amount of a resinous material was formed. This can be explained by the instability of the ester group under the alkaline condition of the reaction. If the acetyl group of ω-bromoacetovanillone acetate (II) was hydrolyzed during the condensation, then ω-bromoacetovanillone thus formed would react with itself with the conceivable formation of a long-chain resin. The final step of hydrolysis of 2-vanilloylbenzofuran acetate (III) proceeded smoothly.

2-VANILLOYLBENZOFURAN ACETATE (III)

Acetovanillone was acetylated by acetic anhydride and sodium acetate according to the method given by Neitzel (30). The resulting acetovanillone acetate was brominated in anhydrous chloroform solution according to the method given by Brickman, Hawkins, and Hibbert (31).

Freshly distilled salicylaldehyde (12.2 grams) and dry ω-bromoacetovanillone acetate (27.6 grams) as obtained above were dissolved in absolute ethanol (100 ml.) in a 250-ml. round-bottom flask. Anhydrous potassium carbonate (14 grams) were added, and the mixture was boiled for one hour on the steam bath. The mixture was added to excess water, and the precipitated oil was extracted with ether. The ether solution was dried over anhydrous sodium sulfate and the ether was evaporated. A thick viscous residue resulted which did not crystallize on cooling in the refrigerator for several days. Vacuum distillation of the viscous product resulted in appreciable decomposition, but the product which distilled over at 250° and 7-10 mm. crystallized on cooling and scratching the sides of the container with a glass rod. Recrystallized repeatedly from dilute ethanol, the yield of the pure product melting at 126-127° was only 0.2 grams.

Analysis.[1] Calculated for 2-vanilloylbenzofuran acetate, $C_{18}H_{14}O_5$: C, 69.7%; H, 4.5%; MeO 10.0%. Found: C, 69.7%; H, 4.6%; MeO, 10.6%.

2-VANILLOYLBENZOFURAN (IV)

2-Vanilloylbenzofuran acetate (2 grams) was dissolved in 50 ml. of ethanol, and 5 ml. of concentrated hydrochloric acid were added. After refluxing for 12 hours on a steam bath, the solution was evaporated to half its volume. On dilution with water, the solution yielded a crystalline product. The product was dissolved in dilute ethanol, decolorized with Darco, and recrystallized. About one gram of pure 2-vanilloylbenzofuran melting at 137.5-138.5° was obtained.

Analysis. Calculated for 2-vanilloylbenzofuran, $C_{16}H_{12}O_4$: C, 71.6%; H, 4.5%; MeO, 11.6%. Found: C, 71.7%; H, 4.6%; MeO, 11.6%.

Because the over-all yield in the first procedure was very poor, an alternative procedure was developed for the synthesis of 2-vanilloylbenzofuran. ω-Bromoacetovanillone benzyl ether (VII) was not known, but was fairly easily prepared by first benzylating acetovanillone (V) in the usual way and then brominating the product. While

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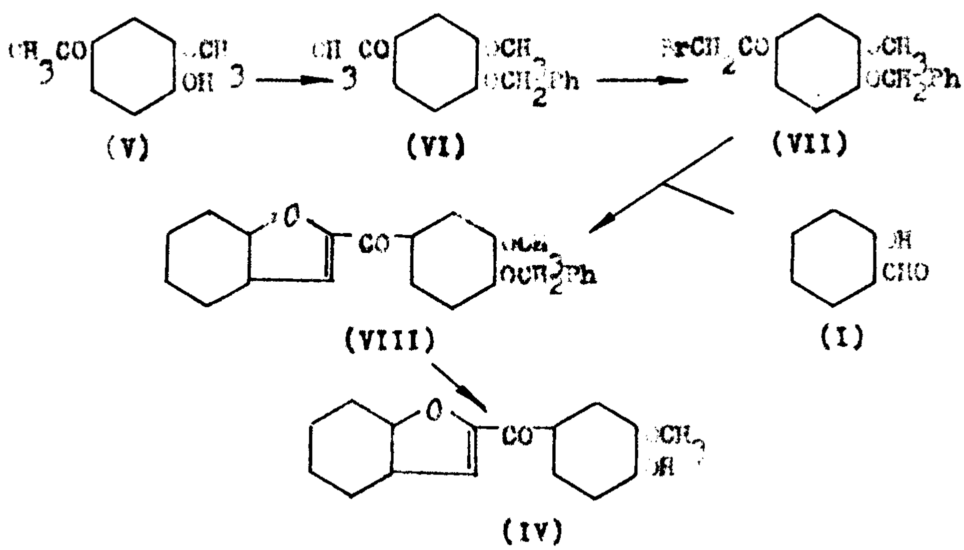
[1] All analyses were carried out by Mr. Donald Macdonnell.

this work was in progress, Leopold (32) reported the preparation of ω-bromoacetovanillone benzyl ether (VII) in a very similar way. This compound was condensed with salicylaldehyde (I) in ethanol solution in the presence of anhydrous potassium carbonate to form 2-vanilloylbenzofuran benzyl ether (VIII) in good yields. This benzyl ether was very rapidly debenzylated in ethyl acetate solution by molecular hydrogen at room temperature and at a pressure of 50 pounds under the catalytic influence of 10% palladium on activated charcoal. 2-Vanilloylbenzofuran (IV) was obtained in a good yield.



The general method of preparation, the fact that the same compound was obtained by two alternative procedures, and the elementary analysis data leave no doubt as to the structure of the synthesized compound.

[illegible] (VI)

Acetovanillone (45 grams) and benzyl chloride (20 grams) were dissolved in 100 ml. of acetone in a 250-ml. round-bottom flask.

Anhydrous potassium carbonate (35 grams) was added, and the mixture was refluxed for eight hours on the steam bath. The reaction mixture was poured into an excess of ice water and extracted with ether. The ether solution was washed with dilute sodium hydroxide solution and water, and finally dried over anhydrous sodium sulfate. The ether was distilled off, and the residual solid was recrystallized from petroleum ether (b.p. 60-110°). The yield of the pure compound, melting at 86-86.5°, was 50 grams.

<u>Analysis</u>. Calculated for benzylacetovanillone, $C_{16}H_{16}O_3$: C, 75.0%; H, 6.3%; MeO, 12.1%. Found: C, 75.0%; H, 6.4%; MeO, 12.4%.

ω-BROMOBENZYLACETOVANILLONE (VII)

Benzylacetovanillone (5 grams) was dissolved in 50 ml. of dry chloroform in a round-bottom flask fitted with a dropping funnel and a stirrer. Then 3.2 grams of bromine in 25 ml. of dry chloroform were added dropwise with stirring over a period of 45 minutes at room temperature. Stirring was continued for an additional 30 minutes. The product was washed with water and bicarbonate solution. The chloroform solution was dried with anhydrous sodium sulfate and evaporated under reduced pressure. The residue crystallized when allowed to stand and the sides of the container were scratched with a glass rod. The crystals were dissolved in ethanol, decolorized with charcoal, and recrystallized from dilute ethanol. The yield of pure ω-bromobenzylacetovanillone, melting at 102-103° was 4 grams.

<u>Analysis</u>. Calculated for ω-bromobenzylacetovanillone, $C_{16}H_{15}O_3Br$: MeO, 9.3%. Found: MeO, 9.5%.

2-VANILLOYLBENZOFURAN BENZYL ETHER (VIII)

Dry ω-bromobenzylacetovanillone (4 grams) and freshly distilled salicylaldehyde (3 grams) were dissolved in 50 ml. of absolute ethanol in a 250-ml. round-bottom flask. Anhydrous potassium carbonate (1.5 grams) was added, and the reaction mixture was refluxed for two hours on the steam bath. The reaction mixture was poured into excess water and extracted with ether. The ether extract was washed with dilute sodium hydroxide solution and water, and dried over anhydrous sodium sulfate. The ether was distilled and the residue crystallized from dilute ethanol. Recrystallization from 95% ethanol gave 3.8 grams of the pure benzyl ether, m.p. 119-120°.

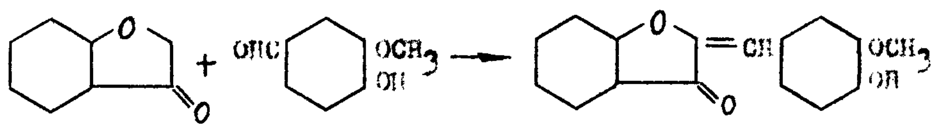
<u>Analysis</u>. Calculated for 2-vanilloylbenzofuran benzyl ether, $C_{23}H_{18}O_4$: C, 77.1%; H, 5.0%; MeO, 8.7%. Found: C, 77.1%; H, 5.2%; MeO, 9.0%.

2-VANILLOYLBENZOFURAN (IV)

2-Vanilloylbenzofuran benzyl ether (1.4 grams) was dissolved in ethyl acetate (100 ml.), and 1 gram of 10% palladium on activated charcoal was added. The mixture was shaken under hydrogen at 26° and 50 pounds pressure for 15 minutes. The catalyst was filtered, and the ethyl acetate solution was extracted with dilute sodium hydroxide solution. Crushed ice was added to the alkaline extract, which was then acidified with hydrochloric acid with vigorous stirring. A semisolid precipitate was formed which solidified completely on continued stirring. The precipitate was filtered and recrystallized twice from dilute ethanol. The yield of the pure compound, melting at 137.5-138.5°, was 0.7 gram. The mixed melting point with a sample of 2-vanilloylbenzofuran obtained by the previous method showed no depression.

2-VANILLYLIDENE-3-COUMARANONE (XI)

The active methylene group in the 2-position of 3-coumaranone is known to condense readily with aldehydes in the presence of hydrochloric acid (23,24). This fact was utilized in synthesizing 2-vanillylidene-3-coumaranone (XI), which was formed in good yield when vanillin (X) and 3-coumaranone (IX) were condensed in ethanol solution in the presence of hydrochloric acid.



(IX) (X) (XI)

By using the same general method, the condensation product from 3-coumaranone and veratraldehyde was prepared by Freudenberg and co-workers (33), and its melting point was reported to be 150°. This same compound was made by methylating 2-vanillylidene-3-coumaranone (XI) with dimethyl sulfate and alkali, but its melting point was found to be 158-160°. A sample of 2-veratrylidene-3-coumaranone was therefore prepared according to the method given by Freudenberg and co-workers. Its melting point and mixed melting point with methylated 2-vanillylidene-3-coumaranone was found to be 158-160°. The general method of preparation, elementary analysis data, and the formation of the known methyl derivative establishes the structure of the compound (XI) synthesized.

2-VANILLYLIDENE-3-COUMARANONE (XI)

3-Coumaranone was prepared according to the following method given by Fries and Pfaffendorf (35). Phenol chloroacetate was formed from phenol and chloroacetyl chloride. Fries' rearrangement of phenol chloroacetate by heating with anhydrous aluminum chloride yielded o-hydroxychloroacetophenone. The latter compound was heated with sodium acetate in ethanol solution to effect ring closure, thereby producing 3-coumaranone.

3-Coumaranone (14.2 grams) and vanillin (15.8 grams) were dissolved in the minimum amount of absolute ethanol at 50-60°. Ten milliliters of concentrated hydrochloric acid were added dropwise at this temperature. An orange-yellow crystalline product was precipitated. The precipitated solid was filtered, washed with dilute alcohol, and dried. Recrystallization from a chloroform-methanol mixture and then from dioxane yielded 15.5 grams of the desired product, orange-yellow needles, melting at 206-207°.

Analysis. Calculated for 2-vanillylidene-3-coumaranone, $C_{16}H_{12}O_4$: C, 71.6%; H, 4.5%; MeO, 11.6%. Found: C, 71.6%; H, 4.6%; MeO, 11.5%.

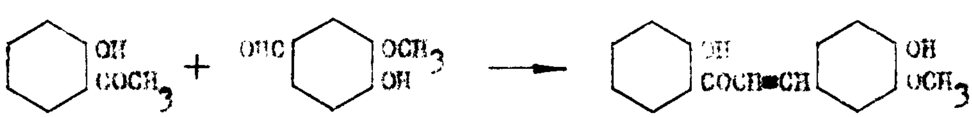
2-VERATRYLIDENE-3-COUMARANONE

A small amount of 2-vanillylidene-3-coumaranone was methylated in the usual way by dimethyl sulfate and alkali. The product was washed thoroughly with dilute alkali solution and water, and then dried. Repeated recrystallizations from methanol yielded a compound melting at

158-160°. The melting point was not depressed when mixed with a sample of a 2-veratrylidene-3-coumaranone (m.p. 158-160°) prepared by condensing veratraldehyde and 3-coumaranone under conditions as described above.

3-METHOXY-2',4-DIHYDROXYCHALCONE (XIII)

Russell and Todd (36) prepared 3-methoxy-2',4-dihydroxychalcone (XIII) in the following way. Vanillin (X) and *o*-hydroxyacetophenone (XII) were benzoylated and the products were condensed by passing dry hydrogen chloride in anhydrous ethyl acetate solution. The benzoylated chalcone thus formed was debenzoylated to give 3-methoxy-2',4-dihydroxychalcone (XIII). The over-all yield of this procedure was not reported by Russell and Todd. An attempt to make the chalcone by the above procedure did not result in a satisfactory yield. Therefore a modified procedure (37) was used to synthesize the chalcone. The procedure consisted of condensing vanillin (X) and *o*-hydroxyacetophenone (XII) directly in the presence of alkali, and gave a satisfactory yield of 3-methoxy-2',4-dihydroxychalcone (XIII).



(XII) (X) (XIII)

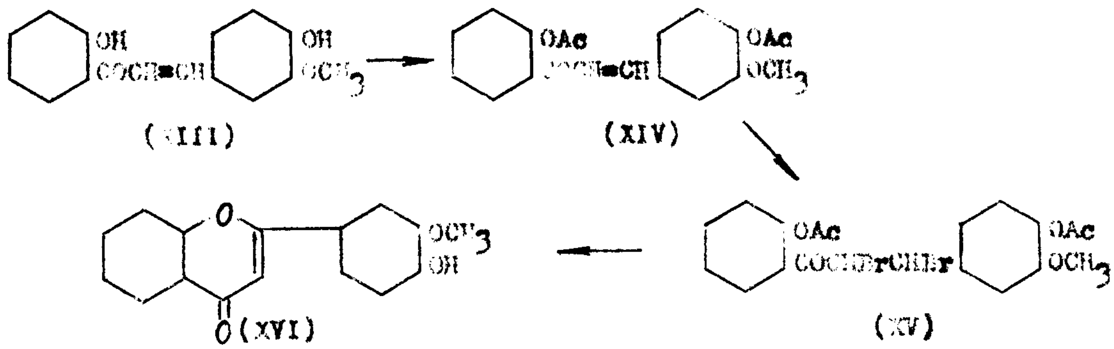
3-METHOXY-2',4-DIHYDROXYCHALCONE (XIII)

Vanillin (15.2 grams) and *o*-hydroxyacetophenone (13.6 grams) were dissolved in 50 ml. of ethanol and cooled to 0°. A 75-ml. portion of a 60% potassium hydroxide solution was cooled to 0° and added to the above alcoholic solution with stirring. The flask was well stoppered and kept for seven days at room temperature with occasional shaking. The color of the solution and the suspended solids gradually turned to bright orange. After seven days, crushed ice was added to the mixture, which was then acidified (congo red) with 6 *N* hydrochloric acid. The semisolid oily precipitate was extracted with ether, washed with a saturated solution of sodium bisulfite, and finally subjected to steam distillation. The residue could then be crystallized from dilute ethanol to yield 12 grams of 3-methoxy-2',4-dihydroxychalcone. The melting point and the mixed melting point with an authentic sample prepared by Russell and Todd's method was 129°.

3'-METHOXY-4'-HYDROXYFLAVONE (XVI)

3-Methoxy-2',4-dihydroxychalcone (XIII) was used as the starting material for the synthesis of 3'-methoxy-4'-hydroxyflavone (XVI). The general method of preparing flavones from chalcones is to treat the latter with bromine to give the chalcone dibromide, which yields the flavone on treatment with alcoholic potash (38).

Instead of using 3-methoxy-2',4-dihydroxychalcone (XIII) itself, the diacetate (XIV) was used for bromination, because the presence of hydroxyl groups in the ring sometimes causes simultaneous substitution and addition on treatment with bromine (39), whereas with acetates of chalcones the reaction is generally limited to addition (40). 3-Methoxy-2',4-diacetoxychalcone dibromide (XV) thus obtained was treated with hot aqueous alcoholic potassium hydroxide to give 3'-methoxy-4'-hydroxyflavone (XVI).



The structure of the flavone is established by the general method of preparation and the elementary analysis data.

3-METHOXY-2',4-DIACETOXYCHALCONE (XIV)

3-Methoxy-2',4-dihydroxychalcone (2 grams) was dissolved in 15 ml. anhydrous pyridine in a 50-ml. Erlenmeyer flask, and 8 ml. of acetic anhydride were gradually added. The flask was stoppered and kept overnight at room temperature. The reaction mixture was poured into an excess of ice water. The precipitated solid was filtered and washed first with very dilute hydrochloric acid solution and then with water. The product was recrystallized from 95% ethanol to give pale yellow crystals melting at 133°. The yield of the pure product was 2.35 grams.

<u>Analysis.</u> Calculated for 3-methoxy-2',4-diacetoxychalcone, $C_{20}H_{18}O_6$: C, 67.8%; H, 5.1%; MeO, 8.8%. Found: C, 67.9%, H, 5.1%, MeO, 9.0%.

3-METHOXY-2',4-DIACETOXYCHALCONE DIBROMIDE (XV)

3-Methoxy-2',4-diacetoxychalcone (2.35 grams) was dissolved in dry chloroform (20 ml.) and a solution of bromine (1.1 grams) in dry chloroform (10 ml.) was added dropwise to the above solution at room temperature. The bromine was consumed as rapidly as it was added. The solvent was completely evaporated on the steam bath, leaving a solid residue. This was recrystallized several times from dilute acetone, yielding white crystals melting at 170-171°.

Analysis. Calculated for 3-methoxy-2',4-diacetoxychalcone dibromide, $C_{20}H_{18}O_6Br_2$: C, 46.7%; H, 3.5%; Br, 31.1%. Found: C, 46.9%; H, 3.7%; Br, 31.0%.

3'-METHOXY-4'-HYDROXYFLAVONE (XVI)

3-Methoxy-2',4-diacetoxychalcone dibromide (1 gram) was dissolved in ethanol (20 ml.), and the solution was heated on the steam bath. A 60% potassium hydroxide solution (10 ml.) was gradually added to the above solution, causing the solution to turn red. The solution was heated on the steam bath for 15 minutes. It was then cooled, acidified with dilute hydrochloric acid, and kept in the refrigerator, causing the separation of yellow crystals, which were filtered and recrystallized twice from dilute ethanol. The product, melting at 194-195°, gave a negative sodium-fusion test for halogen.

Analysis. Calculated for 3'-methoxy-4'-hydroxyflavone, $C_{16}H_{12}O_4$: C, 71.6%; H, 4.5%; MeO, 11.6%. Found: C, 71.5%; H, 4.6%; MeO, 11.6%.

3'-METHOXY-2,4'-DIHYDROXYCHALCONE (XVII)

3'-Methoxy-2,4'-dihydroxychalcone (XVII) was prepared by the same general method used for making the other chalcone (XIII).

Salicylaldehyde (I) and acetovanillone (V) were condensed directly in the presence of alkali.

(I) (V) (XVII)

The condensation product, however, could not be obtained in a sufficient state of purity. This was because of the fact that the compound was slowly converted to a red product when it was heated in solution of a solvent, e.g., during recrystallization. It was therefore converted into its benzoyl derivative (XVIII), prepared according to Schotten-Baumann (41,42) starting with a fairly pure sample of the product. The elementary analysis of the purified benzoyl derivative proved that the compound formed during the alkaline condensation was 3'-methoxy-2,4'-dihydroxychalcone (XVII). Further proof to this effect was obtained by the fact that this chalcone could be very easily converted to 3'-methoxy-4'-hydroxyflavylium chloride by passing hydrogen chloride in a glacial acetic acid solution. Such conversions to flavylium salts are characteristic of 2-hydroxychalcones (43).

3'-METHOXY-2,4'-DIHYDROXYCHALCONE (XVII)

In a 250-ml. Erlenmeyer flask, salicylaldehyde (12.2 grams) and acetovanillone (16.6 grams) were dissolved in 100 ml. of ethanol and the solution was cooled to 0°. A 75-ml. portion of a 60% potassium hydroxide solution was also cooled to 0° and then added to the above solution with stirring. The container was well stoppered, and the reaction mixture was kept at room temperature for five days. It was then added to an excess of ice water and acidified with 6 N hydrochloric acid, whereby a sticky semisolid precipitate was obtained. This was extracted with ether, and the ether solution was washed with sodium bisulfite solution and with water. The ether solution was dried over anhydrous sodium sulfate and the ether distilled. The sirupy residue was triturated with a little ethanol, whereby brownish crystals separated out. These were filtered and recrystallized repeatedly from dilute ethanol.

The product did not have a sharp melting point and on heating slowly in the melting point bath, it gradually intensified in color and melted to a deep blue liquid at 152-157°. The compound could not be purified further by repeated recrystallization.

3'-METHOXY-2,4'-DIBENZOXYCHALCONE (XVIII)

A small amount of 3'-methoxy-2,4'-dihydroxychalcone was dissolved in 10% sodium hydroxide solution, and an excess of benzoyl chloride was added in portions with shaking. The mixture was shaken vigorously until the odor of benzoyl chloride no longer persisted. The solid was filtered, washed thoroughly with water, and recrystallized from ethanol. White crystals melting at 146.5-147.5° were obtained.

Analysis. Calculated for 3'-methoxy-2,4'-dibenzoxychalcone, $C_{30}H_{22}O_6$: C, 75.3%; H, 4.7%. Found: C, 75.4%; H, 4.8%.

3'-METHOXY-4'-HYDROXYFLAVYLIUM CHLORIDE (XIX)

3'-Methoxy-4'-hydroxyflavylium chloride was prepared by treating the above chalcone in glacial acetic acid solution with anhydrous hydrogen chloride. This is a very general method of synthesizing flavylium chlorides (42).

(XVIII) (XIX)

The structure of the final product was indicated by the general method of synthesis, intense red color of the product, (flavylium salts are deeply colored), the formation of a double salt with ferric chloride, and the analysis of the purified salt.

3'-METHOXY-4'-HYDROXYFLAVYLIUM CHLORIDE (XIX)

A fairly pure sample of 3'-methoxy-2,4'-dihydroxychalcone (XVIII) (1.5 grams) was dissolved in 15 ml. of glacial acetic acid. The mixture was cooled in an ice bath, and dry hydrogen chloride was passed through the solution for 10 minutes. An intense red-colored solid separated out. The precipitate was filtered by suction. The residue was recrystallized from 70% ethanol saturated with hydrogen chloride. The resulting deep red crystals were filtered and dried.

3'-METHOXY-4'-HYDROXYFLAVYLIUM FERRICHLORIDE

A small amount of 3'-methoxy-4'-hydroxyflavylium chloride was dissolved in glacial acetic acid, and a glacial acetic acid solution of ferric chloride (0.75 gram in 10 ml. of glacial acetic acid) was added. Glistening crystals with a metallic luster separated out. These were filtered by suction and recrystallized from glacial acetic acid.

Analysis. Calculated for 3'-methoxy-4'-hydroxyflavylium ferrichloride, $C_{16}H_{13}O_3Cl.FeCl_3$: Fe, 12.4%. Found: Fe, 14.6%.

3'-METHOXY-4'-HYDROXYFLAVANONE (XX)

Starting with 3-methoxy-2',4-dihydroxychalcone (XIII), 3'-methoxy-4'-hydroxyflavanone (XX) was obtained by the usual isomerization procedure (44).

(XIII) (XX)

A mixture of the flavanone and the chalcone resulted from heating the chalcone with alcoholic hydrochloric acid for 24 hours. The flavanone had to be purified by repeated recrystallizations from ethanol and finally from dry ethyl acetate. The structure of the final compound is proved by the general method of preparation and the elementary analysis data.

3'-METHOXY-4'-HYDROXYFLAVANONE (XX)

3-Methoxy-2',4-dihydroxychalcone (5 grams) was dissolved in hot ethanol (150 ml.) on the steam bath, and a 3% hydrochloric acid solution was added until a permanent turbidity appeared. This turbidity disappeared as the reaction mixture was heated on the steam bath. The solution was refluxed for 24 hours. On cooling the solution, crystals separated. These were filtered, decolorized with charcoal, and recrystallized several times from absolute ethanol. Final recrystallization from dry ethyl acetate yielded white crystals melting at 171-172°.

Analysis. Calculated for 3'-methoxy-4'-hydroxyflavanone, $C_{16}H_{14}O_4$: C, 71.1%; H, 5.2%; MeO, 11.5%. Found: C, 71.2%; H, 5.3%; MeO, 11.9%.

3'-METHOXY-4,4'-DIHYDROXYFLAVAN (XXI)

An attempt to prepare 3'-methoxy-4,4'-dihydroxyflavan (XXI) from 3'-methoxy-4'-hydroxyflavanone (XX), by catalytic reduction with molecular hydrogen in the presence of platinum catalyst was unsuccessful. However, this reduction was made possible by aluminum isopropoxide according to the method of Meerwein and Ponndorf (45,46).

(XX) (XXI)

The structure of the compound was proved by the method of formation, the formation of a diacetyl derivative, and the combustion analytical results. Crystallized from dilute ethanol, the compound formed a monohydrate which loses its water of crystallization at 100° in a vacuum.

3'-METHOXY-4,4'-DIHYDROXYFLAVAN (XXI)

3'-Methoxy-4'-hydroxyflavanone (2 grams) and aluminum isopropoxide (5 grams) were covered with 150 ml. of absolute isopropanol in a round-bottom flask fitted with a short upright condenser (25 cm.), the top of which was attached to another small condenser set for distillation. A boiling chip was added, and the solution was refluxed on the steam bath with no water passing through the upright condenser. The rate of distillation was five drops per minute. A positive test for acetone in the distillate given by 2,4-dinitrophenylhydrazine reagent showed that the reduction of the carbonyl group to the secondary alcoholic group was taking place. After the acetone test became negative, water was passed through the upright condenser for five minutes. The water was then removed from the upright condenser, and the first five drops of the distillate were tested for acetone. A positive test was obtained, and the distillation was continued. This procedure of intermittent refluxing and distillation was continued until no further acetone was being produced. The reduction was completed in 30 hours, and additional absolute isopropanol had to be added to the reaction mixture during this period. Most of the isopropanol was then removed under reduced pressure. The cooled residue was then hydrolysed with cold dilute hydrochloric acid solution. The yellow crystalline product which separated out was filtered and dried. Hot petroleum ether (b.p. 60-110°) leaching left the major portion of the product as a white residue. Recrystallized three times from dilute ethanol, the white crystals melted at 160-161°, when the temperature of the melting point bath was raised very gradually. However, the compound melted at a much lower temperature (the exact temperature could not be determined) if the rate of heating of the melting point bath was rapid.

<u>Analysis</u>. Calculated for 3'-methoxy-4,4'-dihydroxyflavan monohydrate, $C_{16}H_{16}O_4 \cdot H_2O$: C, 66.2%; H, 6.2%; MeO, 10.7%. Found after drying the compound over phosphorus pentoxide for three hours at 60° and 2 mm. pressure: C, 67.1%; H, 6.3%; MeO, 11.1%.

Calculated for 3'-methoxy-4,4'-dihydroxyflavan, $C_{16}H_{16}O_4$: C, 70.6%; H, 5.9%. Found after drying the compound for eight hours over phosphorus pentoxide at 100° and 1 mm. pressure: C, 70.6%; H, 6.0%.

3'-METHOXY-4,4'-DIACETOXYFLAVAN

3'-Methoxy-4,4'-dihydroxyflavan (0.1 gram) was dissolved in anhydrous pyridine (5 ml.), and 2 ml. of acetic anhydride were added. The solution was kept overnight at room temperature in a stoppered Erlenmeyer flask. It was then diluted with 50 ml. of ice water with stirring, whereby crystals separated. These were filtered, washed with dilute hydrochloric acid and water, and dried. Recrystallization from ethanol gave white crystals, m.p. 121.5-122°.

Analysis. Calculated for 3'-methoxy-4,4'-diacetoxyflavan $C_{20}H_{20}O_6$: MeO, 8.9%. Found: MeO, 8.7%.

ACTION OF SODIUM BISULFITE ON MODEL COMPOUNDS

In general the model compounds were cooked either with a sodium bisulfite solution or a soda-base acid cooking liquor, containing 3.9% free sulfur dioxide and 5.4% total sulfur dioxide, as analyzed by the Palmrose method (47). The cooking was done in a sealed glass tube at 135° in a glycerin bath with occasional shaking. Because the rate of solution and the treatment after cooking differed considerably with different compounds, each experiment will be discussed individually. Evidence of reaction is based upon the solution of the compound in the sulfite liquor and the preparation of the benzylisothiuronium salt.

3-METHOXY-2',4-DIHYDROXYCHALCONE

3-Methoxy-2',4-dihydroxychalcone (1 gram) was heated with a solution of sodium bisulfite (1 gram) in distilled water (25 ml.) in a sealed tube at 135° with occasional shaking. The compound went completely into solution within two hours to form a colorless solution. On standing at room temperature, a white crystalline precipitate separated. The tube was opened, and the white crystals were filtered, washed with a small amount of 50% ethanol, dried, and weighed. The yield was 1.3 grams. The theoretical yield of sodium sulfonate is 1.34 grams.

The white crystals were dissolved in a little distilled water. The solution was faintly acidified with hydrochloric acid, and a solution of 5 grams of benzylisothiourea hydrochloride in the minimum amount of distilled water was added to it. White crystals appeared almost immediately, and the precipitation was complete on standing. These crystals were filtered, washed with a little water, and recrystallized from dilute ethanol. The pure crystals thus obtained melted at 212°.

Analysis. Calculated for the benzylisothiuronium salt of the 3-methoxy-2',4-dihydroxychalcone sulfonic acid, $C_{24}H_{25}O_7N_2S_2$: S, 12.35%. Found: S, 12.2%.

3-Methoxy-2',4-dihydroxychalcone (1 gram) was heated with 20 ml. of a 5% neutral sodium sulfite solution at 135°. The compound was dissolved completely within five minutes.

2-VANILLOYLBENZOFURAN

2-Vanilloylbenzofuran (0.2 gram) was heated with a solution of sodium bisulfite (0.2 gram) in distilled water (10 ml.) in a sealed tube at 135° with occasional shaking. The compound did not go into solution even after heating for seven hours. The residue was filtered, washed thoroughly with water, dried, and weighed. The weight was 0.2 gram. Recrystallized from dilute ethanol, the product melted at 137.5-138.5°. Therefore the starting material was not changed under the conditions used.

The above experiment was repeated with acid cooking liquor. 2-Vanilloylbenzofuran (0.2 gram) was heated with cooking liquor (25 ml.) in a sealed tube at 135°. The compound was left undissolved even after heating for 24 hours. The original weight of the starting material was recovered in the same way as above.

2-VANILLYLIDENE-3-COUMARANONE

2-Vanillylidene-3-coumaranone (2 grams) was heated with a solution of sodium bisulfite (2 grams) in distilled water (40 ml.) in a sealed tube at 135° with occasional shaking. The compound was dissolved quite slowly, and a residue was left even after five hours of heating. After cooling, the solid residue was filtered, washed thoroughly with water, dried, and weighed. The weight of the residue was 0.75 gram. Recrystallized from ethanol, the orange crystals melted at 206-207°. The mixed melting point with a sample of the starting material was the same.

The filtrate from the solid residue was washed with ether, and the free sulfur dioxide was driven off from the solution in a current of nitrogen. An excess of the cation-exchange resin Duolite-C3 as supplied was added to the solution, which was then filtered, and the liberated sulfur dioxide again driven off by a current of nitrogen. The solution was neutralized with sodium hydroxide solution, faintly acidified with hydrochloric acid, and cooled. To this cold solution, a cooled solution of 5 grams of benzylisothiourea hydrochloride in the minimum amount of water was added with stirring. An oil separated which could not be further purified.

The above experiment was repeated with acid cooking liquor. 2-Vanillylidene-3-coumaranone (2 grams) and cooking liquor (40 ml.) were heated in a sealed tube at 135°. The rate of solution was similar to the above cook. A little residue was left after heating for five hours. Heating was continued, and the compound went completely into solution in 24 hours. The solution after the cook was yellow. On cooling and extraction with ether, the yellow color was removed by the ether, and the colorless aqueous solution was treated in the same way as above. Here also the benzylisothiuronium salt was obtained as an oil.

An attempt was therefore made to prepare the barium salt of the sulfonic acid. After completion of the cook, the solution was freed from all cations and sulfur dioxide by treatment with Duolite-C3 resin in the same way as above. The resulting solution was treated with barium hydroxide solution until the mixture attained a faint pink color. Carbon dioxide was passed through this solution for 10 minutes, whereby the red color vanished completely. The mixture was heated on the water bath for five minutes, and the precipitate of barium carbonate was filtered by suction. The yellowish filtrate was decolorized by carbon and filtered again.

A portion of the above solution was evaporated to a very small volume under vacuum and then poured into an excess of ethanol. Nothing but a silky haze was formed. The same result was obtained when a concentrated solution of the barium sulfonate was poured into an excess of dioxane.

A part of the aqueous solution of the barium sulfonate was evaporated to dryness at 50° under reduced pressure. The resulting solid was yellow in color and did not completely dissolve in water. After washing thoroughly with water, the yellow residue was recrystallized from dilute ethanol. The melting point and the mixed melting point with 2-vanillylidene-3-coumaranone was 206°. The barium sulfonate is therefore quite unstable and easily decomposes into 2-vanillylidene-3-coumaranone.

3'-METHOXY-4'-HYDROXYFLAVONE

3'-Methoxy-4'-hydroxyflavone (0.1 gram) and a solution of sodium bisulfite (0.1 gram) in distilled water (5 ml.) were heated in a sealed tube at 135° with occasional shaking. The compound remained undissolved even after heating for seven hours. After cooling, the residue was filtered, washed with water, and dried. The dried residue weighed 0.1 gram. Recrystallization from dilute ethanol gave crystals, whose melting point and mixed melting point with a sample of the starting material was 194-195°.

The above experiment was repeated with acid cooking liquor. 3'-Methoxy-4'-hydroxyflavone (0.1 gram) was heated with cooking liquor (10 ml.) in a sealed tube at 135°. The compound was left undissolved even after heating for 24 hours. The original weight of the starting material was recovered following the above procedure.

3'-METHOXY-4'-HYDROXYFLAVANONE

A suspension of 3'-methoxy-4'-hydroxyflavanone (0.5 gram) in acid cooking liquor (25 ml.) was heated in a sealed tube at 135° with occasional shaking. The compound went completely into solution within two hours to give colorless solution. On standing at room temperature a white crystalline precipitate separated. This was filtered, washed with a small amount of cooled distilled water, and dried.

The white crystals were dissolved in a little distilled water, and the solution was cooled. This solution was faintly acidified with hydrochloric acid and then added to a cooled solution of 5 grams of benzylisothiourea hydrochloride in the minimum amount of distilled water. The resulting white crystalline precipitate was filtered, washed with a little water, and recrystallized from dilute ethanol. The pure crystals melted at 212°. There was no depression in a mixed melting point determination with the benzylisothiuronium salt of the product from sulfite cooking of 3-methoxy-2',4-dihydroxychalcone.

3'-Methoxy-4'-hydroxyflavanone (0.5 gram) was also cooked with a solution of pure sodium bisulfite (0.5 gram) in distilled water (10 ml.) under similar conditions as described above. The compound went completely into solution within two hours. The product was identical with that from the above cook.

3'-Methoxy-4'-hydroxyflavanone (1 gram) was heated with 20 ml. of a 5% neutral sodium sulfite solution at 135°. The compound was dissolved completely in about one hour.

3'-METHOXY-4'-HYDROXYFLAVYLIUM CHLORIDE

SULFITE COOK

3'-Methoxy-4'-hydroxyflavylium chloride (0.5 gram) was dissolved in acid cooking liquor (25 ml.) to give a colorless solution. The solution was then heated in a sealed tube at 135° for four hours. No apparent change took place during cooking, and no solid material separated from the solution on standing at room temperature for several days. The solution was extracted with ether, but on evaporation to dryness, the ether extract left no residue. Nitrogen gas was passed through the aqueous solution to drive off free sulfur dioxide. The solution was then treated with an excess of Duolite-C3 resin and filtered. The liberated sulfur dioxide was again driven off in a current of nitrogen. The solution was decolorized with carbon, filtered, and evaporated to dryness in a vacuum desiccator. On evaporation, the colorless solution yielded a bright red solid residue, similar to the starting material. The dry weight of the residue was approximately 0.5 gram.

A small amount of the red residue was dissolved in glacial acetic acid, and a few drops of concentrated hydrochloric acid were added. To this solution was added a glacial acetic acid solution of ferric chloride. Shining crystals of the ferrichloride derivative were obtained.

Sodium fusion test on the red residue indicated the absence of halogens but presence of sulfur.

REACTION WITH SULFUR DIOXIDE

3'-Methoxy-4'-hydroxyflavylium chloride in aqueous solution was decolorized by passing sulfur dioxide gas through the solution or by adding to it a solution of sodium bisulfite or neutral sodium sulfite.

3'-Methoxy-4'-hydroxyflavylium chloride (0.5 gram) was dissolved in distilled water (25 ml.) and gaseous sulfur dioxide was passed through the solution. Free sulfur dioxide was removed from the decolorized solution by passing nitrogen through it. A portion of the solution was evaporated to dryness at ordinary temperature to give a bright red solid residue. A solution of the residue in glacial acetic acid was treated with a few drops of concentrated hydrochloric acid, and a glacial acetic acid solution of ferric chloride was added. Shining ferrichloride crystals were formed.

When the sulfur dioxide decolorized solution was extracted with ether and the ether extract was evaporated to dryness, no residue was left. The decolorized solution was passed through an anion-exchange column containing Duolite-A2 resin, and the resulting solution was evaporated to dryness. No residue was left. However, on cation exchange by Duolite-C3, the resulting solution on evaporation to dryness yielded a red solid residue. This residue gave a ferrichloride following the procedure mentioned above. The red residue was dissolved in water, and the resulting colored solution could again be decolorized by passing sulfur dioxide through it.

A portion of the sulfur dioxide decolorized solution was evaporated to dryness under reduced pressure at room temperature, and the residue was dissolved in distilled water. A part of the solution was treated with barium chloride solution, but very little precipitate was formed. Another part was passed through a column containing Duolite-C3 resin, and the resulting colorless solution was tested for sulfite. A positive test for sulfite was obtained.

The red color could also be regenerated by the addition of concentrated hydrochloric acid to a decolorized solution, whereby the red color appeared slowly. After the addition of concentrated hydrochloric acid to a decolorized solution, a barium chloride solution was added. There was no precipitation of barium sulfate.

A portion of the sulfur dioxide-decolorized solution was evaporated very slowly at room temperature and pressure. In this way, a quantity of well-formed red crystalline material was obtained. This was filtered from the mother liquor, washed with water, and dried. This compound was not further purified, as an attempt at recrystallization by heating the compound in water resulted in decomposition.

__Analysis.__ Calculated for 3'-methoxy-4'-hydroxyflavylium sulfite, $C_{32}H_{26}O_9S$: S, 5.45%. Calculated for 3'-methoxy-4'-hydroxyflavylium sulfate, $C_{32}H_{26}O_{10}S$: S, 5.3%. Found: S, 5.3%.

Another portion of the sulfur dioxide decolorized solution was cooled and added to a cooled solution of benzylisothiourea hydrochloride in the minimum amount of water. The mixture was kept in the refrigerator overnight. The solid was filtered under suction and recrystallized from a small amount of ethanol. The product melted at 175-177°.

__Analysis.__ Calculated for benzylisothiuronium salt of the compound obtained by isomerization of 3'-methoxy-4'-hydroxyflavylium sulfite, $C_{39}H_{26}O_9S$, $C_7H_{10}SN_2$: S, 5.65%. Found: S, 5.2%.

3'-METHOXY-2,4'-DIHYDROXYCHALCONE

A fairly pure sample of 3'-methoxy-2,4'-dihydroxychalcone (0.5 gram) was heated with a solution of sodium bisulfite (0.5 gram) in distilled water (25 ml.) in a sealed tube at 135° with occasional shaking. The compound went completely into solution within one hour to form a colorless solution. The cooking was continued for another hour. The resulting solution was cooled, and to a portion of this solution, concentrated hydrochloric acid was added. A red color appeared gradually.

Nitrogen was passed through the remaining portion of the solution to drive off the free sulfur dioxide. An excess of Duolite-C3 resin was added, and the solution filtered. Nitrogen was again passed through the solution to remove the liberated sulfur dioxide. A part of this solution was evaporated to dryness at ordinary temperature and pressure. A bright red residue was left. This was dissolved in glacial acetic acid and treated with a few drops of concentrated hydrochloric acid. On the addition of a glacial acetic acid solution of ferric chloride to the above solution, shining crystals of a ferrichloride were formed.

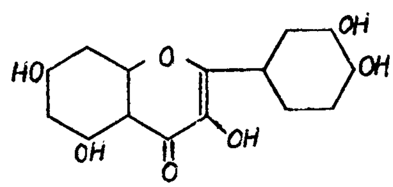
In all its other reactions, the cooked solution behaved exactly like a solution of 3'-methoxy-4'-hydroxyflavylium chloride decolorized with sodium bisulfite.

3'-METHOXY-4,4'-DIHYDROXYFLAVAN

3'-Methoxy-4,4'-dihydroxyflavan (1 gram) was heated with 25 ml. of acid cooking liquor in a sealed glass tube at 135° with occasional shaking. A dark oily residue was left undissolved even after 12 hours of heating. The product was extracted with ether, and the ether solution was evaporated to dryness to give 0.1 gram of residue. The aqueous solution after ether extraction was freed from dissolved sulfur dioxide by passing a current of nitrogen through it. The solution was then treated with an excess of Duolite-C3 resin, the solution filtered, and the liberated sulfur dioxide again removed by passing nitrogen through it. The aqueous solution was then almost neutralized with sodium hydroxide solution and cooled.

A cooled solution of 2 grams of benzylisothiourea hydrochloride in the minimum amount of water was added with stirring. An oily precipitate resulted which could not be further purified.

QUERCETIN

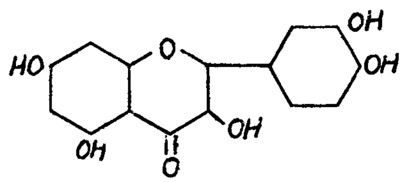


Quercetin (1 gram) was heated with acid cooking liquor (50 ml.) in a sealed glass tube at 135° with occasional shaking. After five hours of heating, a part of the starting material went into solution, but was reprecipitated on cooling. The residue was filtered, washed thoroughly with water, and recrystallized from dilute ethanol. The melting point of the product was above 300°.

A portion of the product (0.5 gram) was heated for one hour with acetic anhydride (20 ml.) and sodium acetate (2 grams) on a steam bath. The product was cooled and diluted with ice water and, after decomposition of the acetic anhydride, the residual solid was filtered, washed with water, and recrystallized from dilute ethanol. The pure product melted at 198.5-200°.

Analysis. Calculated for quercetin pentaacetate, $C_{25}H_{20}O_{12}$: C, 58.6%; H, 3.9%. Found: C, 58.6%; H, 4.1%.

2,3-DIHYDROQUERCETIN[1]



2,3-Dihydroquercetin (0.6 gram) was heated with acid cooking liquor (90 ml.) in a sealed glass tube at 135° with occasional shaking. The compound was dissolved completely by the time the temperature of the bath reached 100°. After one hour at 135°, yellow crystals began to separate from the solution. The heating was continued for a total of five hours. The yellow crystals were filtered, washed thoroughly with water, and dried. The weight of the product was 0.55 gram. This was recrystallized from dilute ethanol. The product melted above 300°.

A small amount of the product was acetylated by heating with acetic anhydride and sodium acetate on a steam bath for one hour. The product was diluted with ice water to precipitate the acetate. The solid

[1]This compound was prepared by Mr. J. C. Pew of the U. S. Forest Products Laboratory, Madison, Wisconsin.

was filtered, washed with water, and recrystallized from dilute ethanol. The product melted at 198.5-200°. The melting point was not depressed by mixture with authentic quercetin pentaacetate.

In another experiment, 2,3-dihydroquercetin (0.5 gram) was heated with acid cooking liquor (25 ml.) in a sealed tube at 100° for half an hour. A clear solution was thereby obtained, but when the solution was cooled, a solid separated out. This was filtered, washed with water, and recrystallized from hot water. The product melted at 130-135° with decomposition. The mixed melting point with a sample of 2,3-dihydroquercetin was also the same.

2,3-Dihydroquercetin (0.5 gram) was also heated with distilled water (35 ml.) in a sealed glass tube at 135° for eight hours. The product went into solution and stayed in solution until the end of the cook. On cooling, a solid precipitated out of solution. This was filtered, and recrystallized from hot water. The melting point and the mixed melting point with 2,3-dihydroquercetin was 130-135°.

ULTRAVIOLET ABSORPTION SPECTRA OF THE MODEL COMPOUNDS AND THEIR SULFONATED DERIVATIVES[1]

The ultraviolet absorption spectra of the model compounds and some of their sulfonated derivatives were studied for comparative purposes, and for tracing the change effected by the sulfonation of model compounds.

Strong absorption in the ultraviolet is given by compounds capable of resonating between several configurations. The benzene ring, which is capable of such resonance gives a strong absorption band at 200-220 mμ. Substitution of the benzene ring, e.g., by hydroxyl or amino groups, increases the number of possible resonating structures, and therefore introduces other absorption bands. Some more or less self-contained resonating systems, e.g., (C=O), $-NO_2$, and -N=N-, produces characteristic absorption bands.

The ultraviolet absorption spectrum of a compound is the integrated result of the different overlapping bands, characteristic of the different groups. Some of these bands may be strong enough so that their maxima stand out prominently, whereas other less strong bands may be lost in the over-all curve as a result of overlapping. Therefore, although the over-all curve is a distinctive property of a particular compound, distinction of compounds within a complex family is hardly achieved by the study of the ultraviolet spectrum. The location and intensity of the maxima are the most significant features of an ultraviolet absorption curve.

[1]The ultraviolet spectra were determined by Mr. Donald Macdonnell.

The ultraviolet spectrum of lignin has been compared with those of a series of model compounds by Glading (10), Patterson and Hibbert (48), and Aulin-Erdtman (49). Although these authors agree that the ultraviolet spectrum of lignin is that of a substituted benzene ring, their interpretation of the over-all curve is quite different. This is to be expected in view of the above discussion about the limitations of the ultraviolet spectrum. In fact, the number of organic compounds whose ultraviolet spectra closely resemble that of lignin is continually growing. This should be kept in mind in comparing the ultraviolet spectra of the model compounds with that of lignin (Fig. 1b).

Ultraviolet absorption spectrum has also been used to trace the change effected by sulfonation of model compounds. Erdtman and co-workers (50) have shown that the replacement of a hydroxyl group in a model compound by a sulfonic acid group involves practically no change in the ultraviolet curve. However, a major change in the model compound, such as the opening up of a heterocyclic ring, would give an entirely different ultraviolet curve from that of the original model compound. Therefore, a study of the ultraviolet spectrum may be valuable in tracing the change effected by sulfonation.

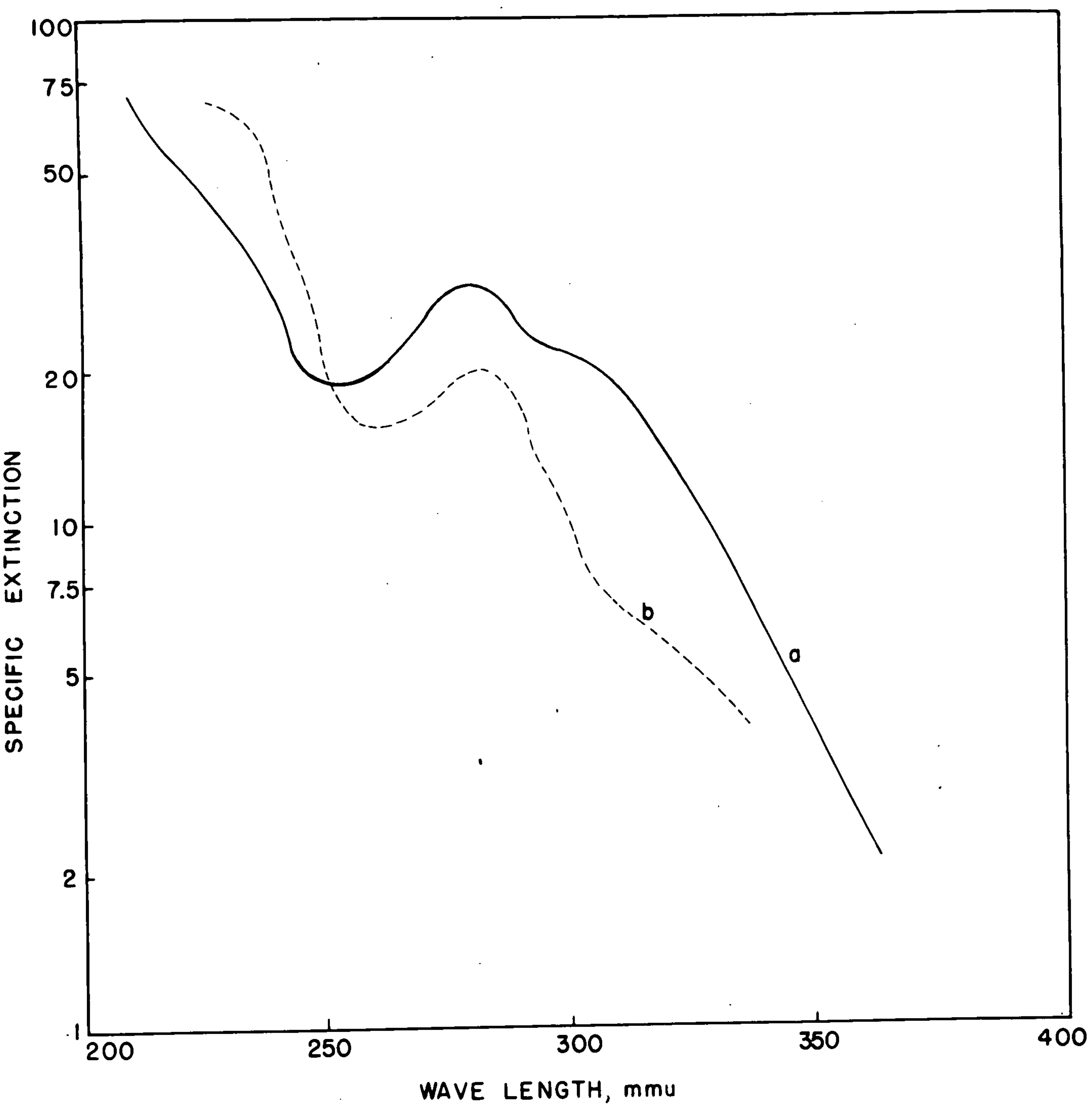
The ultraviolet spectra of the model compounds and of some of their sulfonic acids were determined in ethanol or aqueous solution. In case of those sulfonic acids which could not be isolated, aqueous solutions were obtained by cooking the model compounds with sulfur dioxide solution at 135°. After the model compound had dissolved, excess sulfur dioxide was removed from the solution by passing a current of purified nitrogen through it.

FIGURE 1

ULTRAVIOLET ABSORPTION SPECTRA

a - Sulfonic acid from 3'-methoxy-4'-hydroxyflavylium chloride

b - Native spruce lignin



100
75
50
20
10
7.5
5
2
1
SPECIFIC EXTINCTION
200
250
300
350
400
WAVE LENGTH, mmu
a
b

Figure 1

The ultraviolet spectra are presented in Figures 2-5. It is interesting to note that of all the compounds studied, only the sulfur dioxide-bleached 3'-methoxy-4'-hydroxyflavylium chloride gave a close spectrum (Fig. 1a) to native lignin or lignosulfonic acid. The structure of the compound obtained by the sulfur dioxide bleaching of 3'-methoxy-4'-hydroxyflavylium chloride has not been established. However, if it is a sulfonic acid, of which indications have been obtained, then the structure of this sulfonic acid may be similar to that of lignosulfonic acid.

The spectra of the model compounds having α,β-unsaturated ketone grouping connecting two aromatic nuclei have been compiled in Figure 2. All these compounds have prominent absorption bands in the longer wavelength region. From the striking dissimilarity of these spectra from that of native lignin, it is inferred that α,β-unsaturated ketone groups are not present in lignin, or the amount of such groups is insignificant. However, this does not preclude the possibility of the presence of masked aldol-type groups in lignin.

Figure 2 also shows that the maxima for 3-methoxy-2',4-dihydroxychalcone and 2-vanillylidene-3-coumaranone (a and b) are close together around 400 mmu, whereas those for 2-vanilloylbenzofuran and 3'-methoxy-4'-hydroxyflavone (c and d) appear at 300-350 mmu. It is interesting to note that the former two compounds react with bisulfite to give sulfonic acids, whereas the latter two do not react with bisulfite at all.

The spectra of the α,β-unsaturated ketonic model compounds, which could be sulfonated, and their sulfonated derivatives have been

FIGURE 2

ULTRAVIOLET ABSORPTION SPECTRA

a - 3-Methoxy-2',4-dihydroxychalcone

b - 2-Vanillylidene-3-coumaranone

c - 2-Vanilloylbenzofuran

d - 3'-Methoxy-4'-hydroxyflavone

FIGURE 3

ULTRAVIOLET ABSORPTION SPECTRA

a - 3-Methoxy-2',4-dihydroxychalcone

a'- Sulfonic acid from above

b - 2-Vanillylidene-3-coumaranone

b'- Sulfonic acid from above

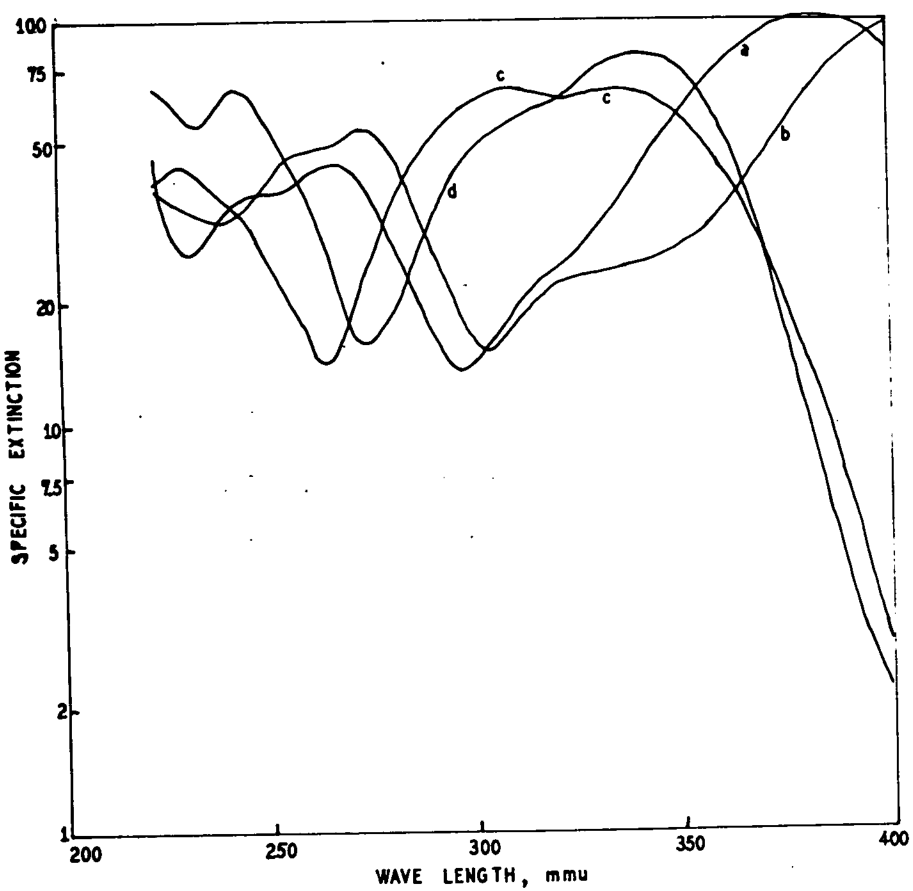


Figure 2

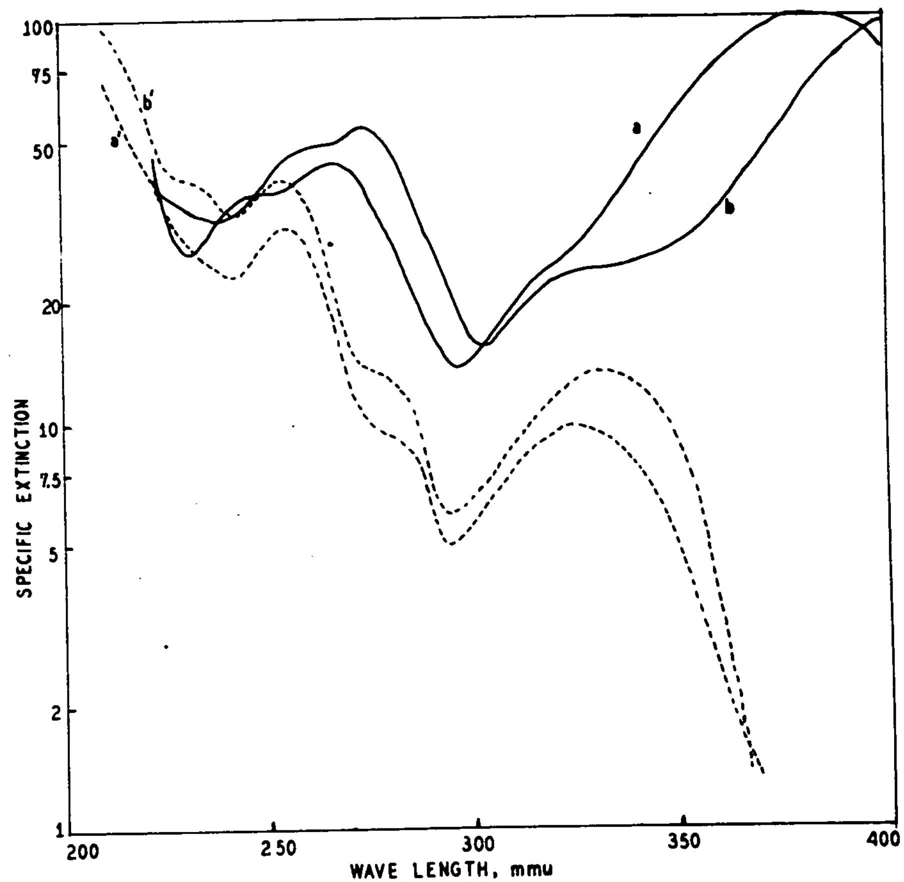


Figure 3

FIGURE 4

ULTRAVIOLET ABSORPTION SPECTRA

a - 3-Methoxy-2',4-dihydroxychalcone

b - 3'-Methoxy-4'-hydroxyflavanone

c - Sulfonic acid from bath

FIGURE 5

ULTRAVIOLET ABSORPTION SPECTRA

a - 3'-Methoxy-4'-hydroxyflavylium chloride

a'- Sulfonic acid from above

b - 3'-Methoxy-4,4'-dihydroxyflavan

b'- Sulfonic acid from above

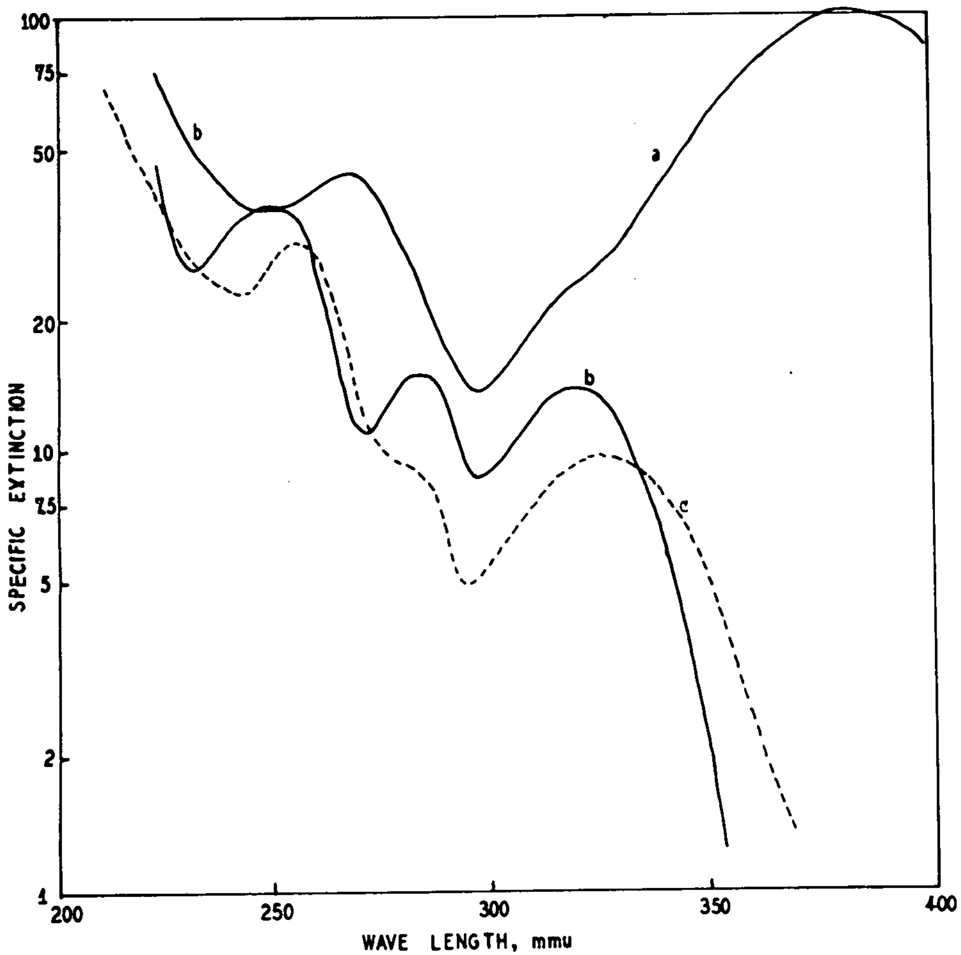


Figure 4

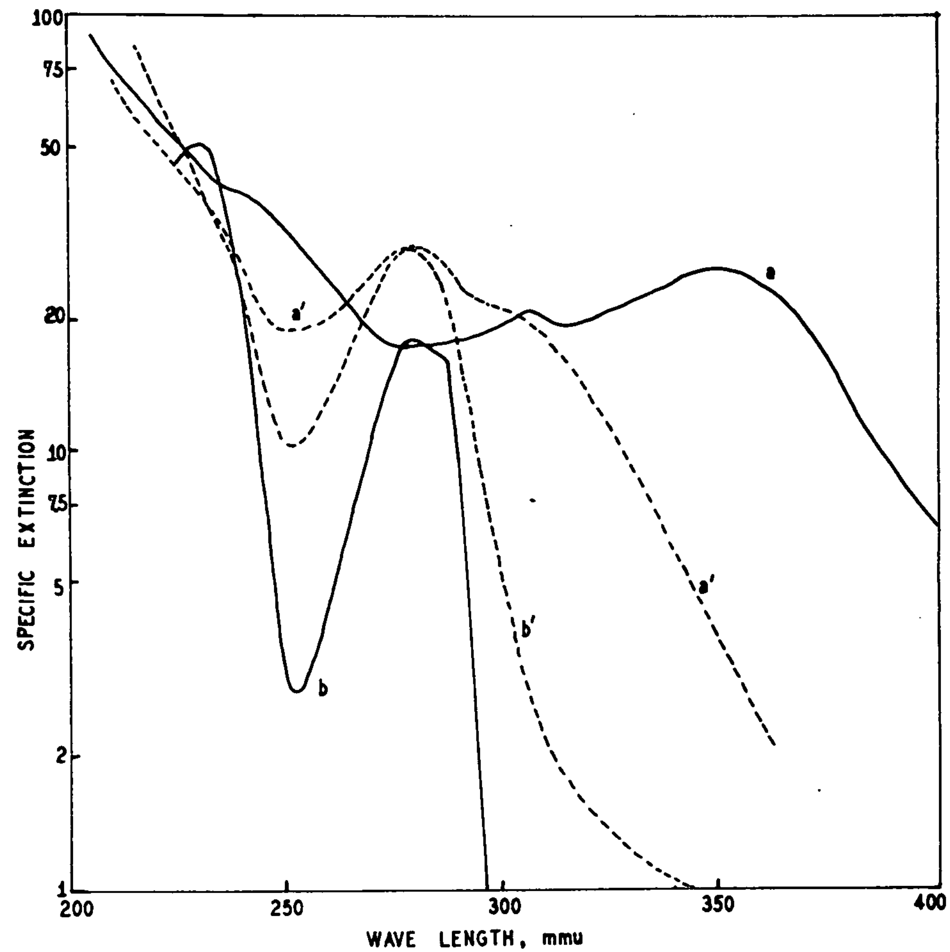


Figure 5

compiled in Figure 3. From chemical evidence, it is known that with the chalcone (a), sulfonation takes place by the addition of sulfurous acid to the double bond. This removes the conjugated system, and also the strong band in the longer wavelength region. The sulfonic acid from 2-vanillylidene-3-coumaranone was too unstable to be isolated for analysis, and therefore the structure could not be decided by chemical means. However, by comparing the ultraviolet spectra, we find that in the sulfonic acid from 2-vanillylidene-3-coumaranone, the strong absorption due to the α,β-unsaturated ketone group has disappeared. Further, the difference between the spectra of 3-methoxy-2',4-dihydroxychalcone (a) and its sulfonic acid (a') is closely duplicated by that between 2-vanillylidene-3-coumaranone (b) and its sulfonic acid (b'). The probable change effected by sulfonation of 2-vanillylidene-3-coumaranone is, therefore, addition of sulfurous acid to the double bond in the following way.

(XI) (XXIV)

Comparison between 3-methoxy-2',4-dihydroxychalcone (a), 3'-methoxy-4'-hydroxyflavanone (b), and the common sulfonic acid is made in Figure 4. It is seen that all three curves are different from each other. This is to be expected because sulfonation is accompanied by ring opening in one case and by the disappearance of a conjugated system in the other. However, the ring opening in 3'-methoxy-4'-hydroxyflavanone is not associated with as much change in the ultraviolet spectrum (compare b and c), as would be expected from the findings of Erdtman and co-workers (50).

The ultraviolet spectra of 3'-methoxy-4,4'-dihydroxyflavan, 3'-methoxy-4'-hydroxyflavylium chloride, and their sulfonated derivatives are compared in Figure 5. In the case of the flavylium chloride, it is seen that a drastic change in the structure takes place as a result of sulfonation (compare a and a'). Further, as already pointed out, the curve for the product of sulfonation (a') resembles that of native lignin, or lignosulfonic acid. The curves for 3'-methoxy-4,4'-dihydroxyflavan (b), and its sulfonic acid (b') were quite similar excepting at the lower wavelength region. Better comparisons could have been made if ultraviolet absorption at the lower wavelength region were more fully recorded. As it is, nothing definite can be inferred as to whether or not ring opening takes place during sulfonation.

INFRARED ABSORPTION SPECTRA OF THE MODEL COMPOUNDS[1]

The infrared absorption spectra of some of the model compounds were studied with a view to support the structural formulas assigned to them.

In recent years, infrared absorption spectra have been extensively used as a supplement to chemical methods in determining the structural formulas of organic compounds. The infrared absorption spectra of organic compounds are the result of the resonant vibration of atomic and molecular groups. The frequency of the resonant vibration of the characteristic groups determines the position of absorption bands in the infrared region, and herein lies the usefulness of the infrared spectrum in the field of organic chemistry.

During the past 20 years, the infrared spectra of a great number of compounds have been systematically accumulated, so that some correlations between chemical groups and their characteristic absorption bands could be established. Correlation tables such as those of Williams (51) and Colthup (52) are available in the literature for the purpose of interpreting infrared spectra of organic compounds. Browning (53) has also made correlation charts after studying the infrared spectra of a great number of organic compounds. All three sources were consulted freely in interpreting the infrared spectra of the model compounds.

The infrared absorption spectra of six of the model compounds, as determined by a Perkin-Elmer Model 12B infrared spectrophotometer,

[1]The infrared spectra were determined by Mr. Richard Messner and Mr. Robert J. Buxton.

is shown in Figure 6. All those compounds were obtained as films from suitable solvents. As seen in Figure 6, the infrared spectra of the model compounds are quite complicated and do not lend themselves to a complete analysis. A few conclusions can, however, be deduced from the curves.

All the compounds show the characteristic hydroxyl bands near 3500 cm.$^{-1}$. Phenolic and alcoholic hydroxyl groups are not usually distinguishable in infrared curves. In the present work, however, it was found that two bands were obtained in the hydroxyl region in the curve of compound D, which contains both phenolic and alcoholic hydroxyl groups. Vanillyl alcohol and coniferyl alcohol also have two bands in the hydroxyl region. In certain cases, therefore, it seems that different bands for alcoholic and phenolic hydroxyl groups may be obtained.

All the compounds have two bands at about 1500 and 1600 cm.$^{-1}$ as a result of the presence of aromatic rings. The aliphatic double bond is indicated by the bands at 1587 cm.$^{-1}$ in compound A, and 1642 cm.$^{-1}$ in compound E. The band at 1587 cm.$^{-1}$ in compound A probably contains overlapping bands for both the aromatic ring and the aliphatic double bond. Because of its ease of isomerization to the chalcone, the compound B also shows a band for a double bond at 1645 cm.$^{-1}$. In compounds C and F, the double bonds probably have lost their aliphatic character; they are in conjugation with a condensed aromatic ring. Accordingly, the bands for the aliphatic double bond are missing in these two compounds.

FIGURE 6

INFRARED ABSORPTION SPECTRA

A - 3-Methoxy-2',4-dihydroxychalcone

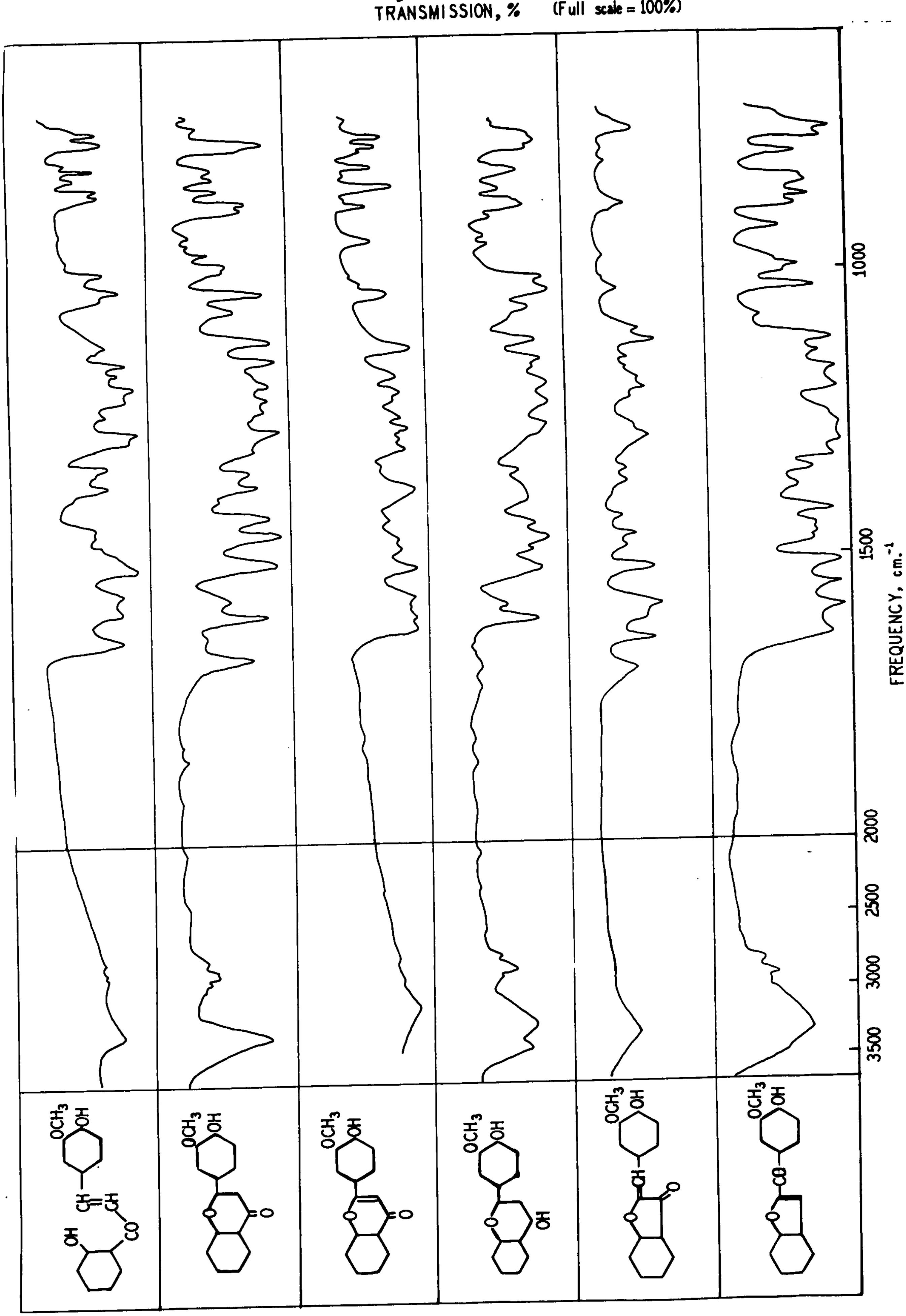
B - 3'-Methoxy-4'-hydroxyflavanone

C - 3'-Methoxy-4'-hydroxyflavone

D - 3'-Methoxy-4,4'-dihydroxyflavan

E - 2-Vanillylidene-3-coumaranone

F - 2-Vanilloylbenzofuran



TRANSMISSION, % (Full scale = 100%)
FREQUENCY, cm.⁻¹
1000
1500
2000
2500
3000
3500

The pyran or furan ring is usually associated with a band between the 1500 and 1600 cm.$^{-1}$ bands for the aromatic ring. All the compounds revealed such a band. The presence of a similar band in the curve of compound A indicated again that it might have resulted from the isomerization of the compound A to the flavanone.

The carbonyl group was represented by bands at 1636 cm.$^{-1}$ in compound A, 1694 cm.$^{-1}$ in compound B, 1618 cm.$^{-1}$ in compound C, 1691 cm.$^{-1}$ in compound E, and 1637 cm.$^{-1}$ in compound F. The carbonyl band was absent in the curve of compound D, as is to be expected.

Carbon-hydrogen linkages are represented by bands at 1450-1470 cm.$^{-1}$ in all the compounds. A band at 1350-1370 cm.$^{-1}$ was present in all the compounds and may be ascribed to the phenolic hydroxyl group. All the compounds also showed absorption in the vicinity of 1270 cm.$^{-1}$, which may be due to methoxyl groups. The 1120 cm.$^{-1}$ band, present in all the curves, is due to dioxane which was used as a solvent in order to make the films.

The trisubstituted benzene ring is represented by bands in the vicinity of 1030 and 850 cm.$^{-1}$ in all the compounds. All the compounds also showed infrared absorption near 760 cm.$^{-1}$, which is characteristic of the disubstituted benzene ring.

GENERAL DISCUSSIONS

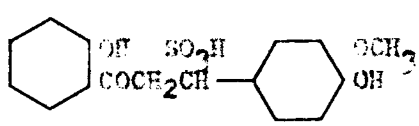
Two types of model compounds were studied in the bisulfite cooking experiments. The first type comprises compounds containing a $-\overset{|}{C}=\overset{|}{C}-CO-$ group connecting two aromatic nuclei, namely, 2-vanilloylbenzofuran, 2-vanillylidene-3-coumaranone, 3-methoxy-2',4-dihydroxychalcone, 3'-methoxy-4'-hydroxyflavone, and 3'-methoxy-2,4'-dihydroxychalcone. Compounds containing α,β-unsaturated ketone groups readily add sodium bisulfite to the double bond according to Kratzl and co-workers (18,20). These compounds were therefore heated with an aqueous solution of analytical-grade sodium bisulfite in a sealed tube. In the case of compounds which were insoluble or difficultly soluble in sodium bisulfite solution, another cook was carried out in an acid cooking liquor, containing 2.9% free sulfur dioxide and 5.4% total sulfur dioxide.

The second group studied comprises compounds containing the three-carbon side chain connecting two aromatic nuclei in the form of a pyran ring. These compounds were 3'-methoxy-4'-hydroxyflavylium chloride, 3'-methoxy-4'-hydroxyflavanone, 3'-methoxy-4,4'-dihydroxyflavan, quercetin, and 2,3-dihydroquercetin. All these compounds were cooked with a soda-base acid cooking liquor, which contained 2.9% free sulfur dioxide and 5.4% total sulfur dioxide.

Of the first group of compounds, 2-vanilloylbenzofuran and 3'-methoxy-4'-hydroxyflavone did not react with bisulfite. Although these compounds contain an α,β-unsaturated ketone grouping, evidently ring formation and conjugation with the benzene ring has conferred stability to sulfite pulping conditions. Quercetin which contains a similar α,β-unsaturated ketone group in addition to a free hydroxyl group in the pyran ring also shows stability towards bisulfite cooking.

Unlike 2-vanilloylbenzofuran, 2-vanillylidene-3-coumaranone was slowly sulfonated to form a soluble derivative. The benzylisothiuronium salt was oily and unstable, and a pure product was not obtained for analysis. The barium salt was also unstable and decomposed very readily to 2-vanillylidene-3-coumaranone. Therefore it could not be determined whether sulfonation takes place by addition at the double bond or by other ways, such as ring opening. The formation of a ketone hydroxysulfonate by the addition of sodium bisulfite at the carbonyl group is improbable in spite of the relative instability of the sulfonic acid. This is because of the fact that the carbonyl group in 2-vanillylidene-3-coumaranone is attached to a benzene nucleus and a tertiary carbon, and is unlikely to form a hydroxysulfonate with sodium bisulfite (54). In fact, attempts to make the oxime and the semicarbazone from 2-vanillylidene-3-coumaranone were unsuccessful, probably because of steric effects. Further, during the attempted isolation of the barium sulfonate, the sulfonic acid was not converted to 2-vanillylidene-3-coumaranone by merely making the product of the sulfite cook alkaline with excess barium hydroxide. The bisulfite addition products of carbonyl compounds are decomposed under similar conditions (55). Although the structure of the sulfonic acid could not be determined by chemical methods, ultraviolet spectroscopic measurements seem to indicate that the sulfonic acid is obtained from 2-vanillylidene-3-coumaranone by the addition of sulfurous acid across the double bond, in accordance with the views of Hoeck and Kratzl. Unlike 2-vanilloylbenzofuran, sulfonation of 2-vanillylidene-3-coumaranone is possible probably because the latter, does not contain a double bond in the furan ring in conjugation with the condensed benzene ring.

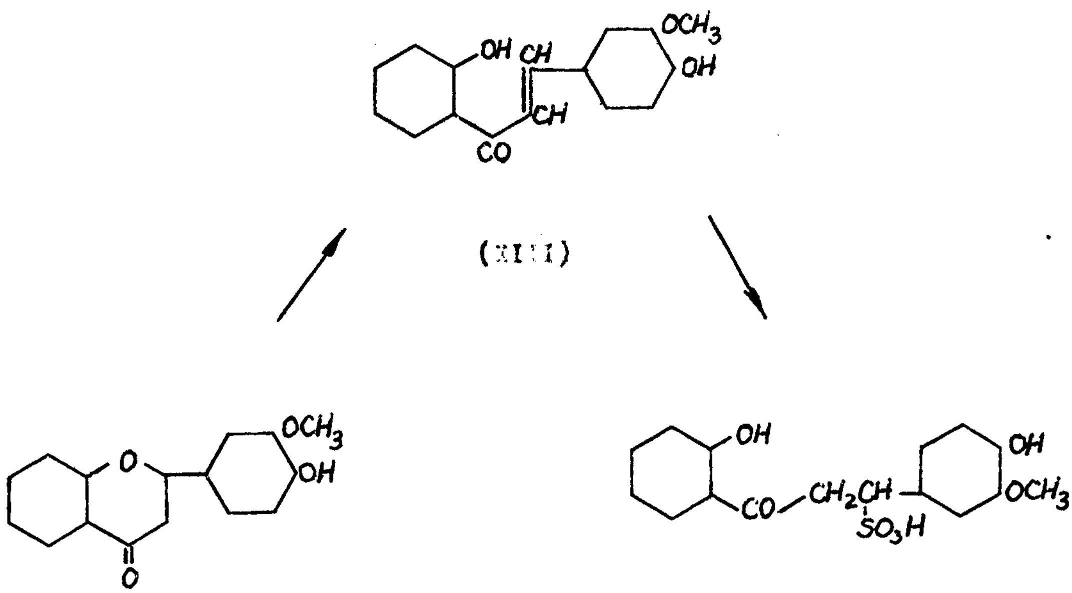
3-Methoxy-2',4-dihydroxychalcone was very readily sulfonated by heating with a solution of sodium bisulfite. The sulfonic acid was isolated as the slightly soluble sodium salt and also as the benzylisothiuronium compound. Analytical results showed that the sulfonic acid was formed by the addition of one molecule of sulfurous acid to the chalcone. Hägglund and Kratzl's work shows that sulfurous acid adds very readily to the double bond of different chalcones, in such a way that the sulfonic acid group is attached to the β-carbon atom from the carbonyl group; therefore the following structure is indicated for the sulfonic acid:



(XVII)

3'-Methoxy-4'-hydroxyflavanone dissolved readily on heating with acid cooking liquor or bisulfite solution. The sulfonic acid was isolated as the sodium salt and also as the benzylisothiuronium compound, and was found to be the same compound (XVII) as that obtained from 3-methoxy-2',4-dihydroxychalcone, as proved by the melting point and mixed melting point determinations of the benzylisothiuronium compounds. This was to be expected from the works of Richtzenhain (27) and Kratzl (18), who showed that the pyran ring in flavanone opened upon cooking with bisulfite solution, and the product was identical with that obtained from 2'-hydroxychalcone.

The rate of sulfonation of both the above chalcone and the corresponding flavanone was approximately the same with either bisulfite solution or acid cooking liquor. The sulfonation of the flavanone may proceed either by direct splitting of the ether linkage, or by intermediate formation of the chalcone. The acidic medium used for cooking the flavanone also facilitates flavanone-chalcone isomerization. Further, whereas α,β-unsaturated ketones have been known to give sulfonated derivatives, the only type of benzopyran compound that is known thus far to undergo sulfonation by ring opening is the flavanone. Therefore the idea that in the present case, the flavanone first isomerizes to the chalcone, and then the chalcone is sulfonated seems very plausible.



(XIII)

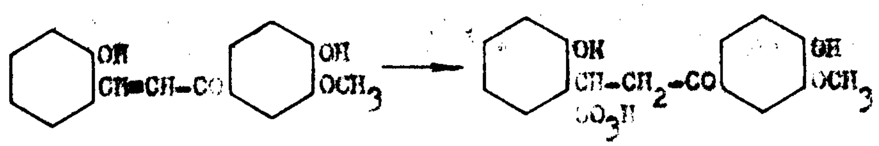
(XX)

(XXII)

When cooked in a neutral sodium sulfite solution at 135°, the chalcone dissolved within five minutes, whereas the flavanone took more than one hour to dissolve. The reaction rates of the sulfonation of the chalcone and the flavanone in neutral and acidic media can be explained according to the above hypothesis in the following way. In acidic media, the rate of sulfonation of the chalcone is slower than that of the formation of the chalcone from the flavanone. Therefore the over-all rate of sulfonation would be determined by the rate of sulfonation of the chalcone. Hence the rate of sulfonation of the chalcone and the flavanone would be the same in acidic media. However, in neutral media, the isomerization reaction should be slower than the sulfonation of the chalcone and should therefore determine the over-all rate for the sulfonation of the flavanone.

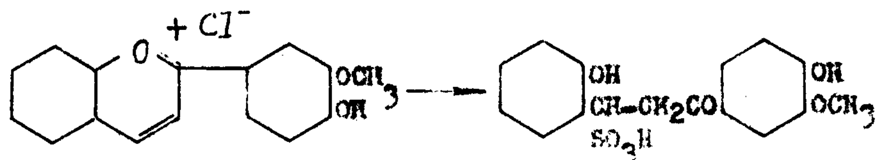
The above hypothesis is also supported by the fact that although ether linkages in some model compounds have been known to be hydrolyzed on cooking with bisulfite, the rate of such reaction is definitely slow under the usual sulfite pulping conditions, compared with the rate of sulfonation of 3'-methoxy-4'-hydroxyflavanone. However, the ready sulfonation of this flavanone may also be explained by the presence of proper substituents, which would make the ether linkage more susceptible to hydrolysis.

3'-Methoxy-2,4'-dihydroxychalcone, which was not obtained in a pure state, readily gave a water-soluble product when cooked with sodium bisulfite solution. Although Kratzl and co-workers (18) worked with several chalcones, none with a hydroxyl group in the 2-position had been subjected to bisulfite cooking. As already mentioned in an earlier section, the 2-hydroxychalcones have a great tendency to form flavylium compounds, especially in acidic media. The flavylium compounds have been known to be decolorized and therefore chemically changed by the action of sodium bisulfite. Therefore, if the above chalcone is heated with a solution of sodium bisulfite, it is very probable that it would be first converted to the 3'-methoxy-4'-hydroxyflavylium salt, which would then react with sodium bisulfite to form the colorless compound. In fact, the product of sulfonation of the above chalcone was found to be the same as that obtained by decolorizing a solution of 3'-methoxy-4'-hydroxyflavylium chloride with sodium bisulfite. However, according to the views of Kratzl and co-workers, the sulfonated product might have been obtained by the addition of sulfurous acid across the double bond of the chalcone.



(XVII) (XXIII)

If such is the case, the decolorization of 3'-methoxy-4'-hydroxyflavylium chloride by sodium bisulfite must be associated with ring opening.



(XIX) (XXIII)

However, we do not have any evidence whether the views of Kratzl and co-workers would hold in the case of 2-hydroxychalcones, which have a great tendency to form flavylium compounds. Further, as will be discussed later, although a sulfonic acid is probably formed by the decolorization of the flavylium chloride with bisulfite, the analysis of the benzylthiuronium compound did not agree with the above formula (XXIII).

The decolorization of 3'-methoxy-4'-hydroxyflavylium chloride is analogous to the sulfur dioxide or sulfite bleaching of the coloring matter of flowers, i.e., anthocyanins, as noted by Willstätter (56). This bleaching reaction has been usually regarded as a reduction effected by sulfur dioxide (57). However, the present work clearly shows that no reduction is involved in such decolorization. On evaporation to dryness, or on the addition of concentrated hydrochloric acid to the decolorized solution, the brilliant red color reappears. The red color also reappears when a solution of the flavylium chloride, decolorized with neutral sodium sulfite, is shaken with excess barium chloride. The red product obtained by evaporation of the decolorized solution gives a ferrichloride. Therefore we can conclude that the intermediate colorless product is not very stable and is readily reconverted into the flavylium compound.

The decolorized solution contained an organic anion, because ether extraction or a cation-exchange resin failed to remove the organic residue from the decolorized solution, whereas an anion-exchange resin adsorbed the organic portion completely. The organic anion contained sulfur, because if the sulfur dioxide decolorized solution was evaporated to dryness after the free sulfur dioxide had been driven off, the residue still contained sulfur, as shown by the sodium-fusion test. The red residue obtained by evaporation of the decolorized solution contains sulfur partly as sulfate and partly as sulfite. However, sulfites in solution are slowly oxidized to sulfates by dissolved oxygen, and therefore the sulfate in the red residue is probably the result of such oxidation. This is also supported by the fact that when a fresh sulfur dioxide-decolorized solution is treated with concentrated hydrochloric acid, and a barium chloride solution is then added, no precipitation of barium sulfate occurs.

The organic anion containing sulfur is most probably a sulfonic acid, which is obtained from 3'-methoxy-4'-hydroxyflavylium sulfite by some mechanism other than reduction. In support of this view, a benzylisothiuronium compound could be formed from the decolorized solution. It must be remembered, however, that some compounds other than sulfonic acids also give benzylisothiuronium salts (58,59). The analysis of the benzylisothiuronium salt is hard to explain, because only one sulfur atom was present per two flavylium units; the sulfonic acid would require one sulfur atom per flavylium unit. However, the benzylisothiuronium compound might not have been very pure, because it was highly soluble in water, from which it was recrystallized. No other suitable solvents were found for recrystallization.

The ultraviolet spectrographic studies show that decolorization of the flavylium chloride by sulfur dioxide is associated with a drastic structural rearrangement. As pointed out earlier, the possibility of ring opening to form a sulfonic acid (XXIII) is not precluded.

When 3'-methoxy-4'-hydroxyflavylium chloride was cooked with acid cooking liquor, the same bleaching action took place, and cooking at an elevated temperature was found to have no further effect.

3'-Methoxy-4,4'-dihydroxyflavan dissolved slowly in acid cooking liquor at 135°, but a residue was left at the end of the cook. The sulfonic acid gave an oily benzylisothiuronium compound, which could not be further purified. The hydroxyl group in the 4-position should be fairly easily sulfonated, because it is similar to the hydroxyl group in the α-phenylethanol, which was successfully sulfonated by Holmberg and Hedén (17). It could not be shown, however, whether the ring also opens up or not. The ultraviolet spectrographic study (see page 82) did not add any further information.

As already mentioned, quercetin was not sulfonated by cooking with acid cooking liquor. However, 2,3-dihydroquercetin was converted to quercetin under similar conditions. Pew (60) showed that 2,3-dihydroquercetin is oxidized to quercetin by heating with dilute sulfuric acid. Evidently bisulfite acid cooking liquor merely provides the acidity for the oxidation. During the process of cooking with acid liquor, 2,3-dihydroquercetin dissolves to form a colorless solution, from which quercetin ultimately separates, but no intermediate sulfonic acid is formed. This is proved by the fact that, once 2,3-dihydroquercetin is dissolved in acid cooking liquor, it can be reprecipitated as the original product by cooling. For identification purposes, quercetin was converted to the pentaacetate, which was easily purified to obtain well-formed crystals melting in the range of 175-177°.

SUMMARY AND CONCLUSIONS

A number of model compounds, in which two aromatic nuclei were joined by a three-carbon side chain, were synthesized. Because the substituents are known to have a definite influence on the sulfonation of model organic compounds, one of the aromatic nuclei was a guaiacyl group. This was done because such groups are known to be present in lignin.

In addition to providing several lignin model compounds for sulfonation, the present work also serves as a preliminary synthetic approach to the preparation of lignin models, in which both the aromatic nuclei would be properly substituted, as shown below. Both the structures are known to be present in lignin, as already discussed in the introduction.

The present work also opens up avenues of approach for synthesizing another type of better model compounds, in which the three-carbon side chains are properly substituted. Most of the side chains in lignin contain alcoholic hydroxyl groups, although the type and the position of such hydroxyl groups are not certain. One model compound containing a hydroxyl group in the side chain, namely, 3'-methoxy-4,4'-dihydroxyflavan, has been described in this investigation. However, other similar compounds may be synthesized by the proper reduction of some of the model compounds containing α,β-unsaturated ketone groups, which have been described in the present work.

In the bisulfite cooking experiments, model compounds containing α,β-unsaturated ketone groups showed a wide range of reactivity towards bisulfite cooking liquor. Both the chalcones were very readily sulfonated. The sulfonic acid was formed from 3-methoxy-2',4-dihydroxychalcone by the addition of sulfurous acid to the double bond. Although a sulfonated derivative was formed from 3'-methoxy-2,4'-dihydroxychalcone, its structure could not be established. Because of the hydroxyl group in the 2-position of the chalcone, the compound has a great tendency to form flavylium salts. In fact, the sulfonated derivative was identical with the product obtained by the bleaching of 3'-methoxy-4'-hydroxyflavylium chloride with sulfur dioxide. The sulfonation of 3'-methoxy-2,4'-dihydroxychalcone may proceed either through the intermediate formation of 3'-methoxy-4'-hydroxyflavylium salt or directly by the addition of sulfurous acid to the double bond.

In the model compounds, where the three-carbon side chain was involved in a five- or six-membered heterocyclic ring, the reactivity to bisulfite was strongly effected. 2-Vanilloylbenzofuran and 3'-methoxy-4'-hydroxyflavone, in which the double bonds are in the heterocyclic rings, and in conjugation with the condensed aromatic nuclei, did not react at all with bisulfite. However, 2-vanillylidene-3-coumaranone, which does not have as stable a configuration as the above compounds, reacts with bisulfite cooking liquor, although the reaction rate is quite slow. The sulfonic acid could not be isolated, but the structure was deduced from ultraviolet spectrographic studies. It appears that the sulfonic acid is formed by the addition of sulfurous acid to the double bond in the same way that the sulfonic acid is formed from 3-methoxy-2',4-dihydroxychalcone.

The ultraviolet spectra of the model compounds containing α,β-unsaturated ketone groups revealed some correlation with their chemical structures and their relative stabilities toward bisulfite. If the double bond was in the heterocyclic ring in conjugation with the condensed aromatic nucleus, then the maximum of the band for conjugation was at a lower wavelength than if the double bond was outside the ring. It was observed that the two model compounds whose maxima were in the wavelength region of 400 mmu could be sulfonated. However, the compounds whose maxima were in the region of 300-350 mmu did not react with bisulfite.

In the case of model compounds with a pyran ring, only 3'-methoxy-4'-hydroxyflavanone, 3'-methoxy-4,4'-dihydroxyflavan, and 3'-methoxy-4'-hydroxyflavylium chloride reacted with bisulfite cooking liquor to give sulfonated products. Richtzenhain's (27) work has shown that neither flavan nor 8-methoxyflavan react with bisulfite. The present work shows that 3'-methoxy-4'-hydroxyflavone, and quercetin also are stable toward bisulfite cooking liquor. However, 2,3-dihydroquercetin is oxidized to quercetin when heated with cooking liquor.

In the case of 3'-methoxy-4'-hydroxyflavanone, sulfonation takes place by the opening of the pyran ring. Experiments have been carried out to indicate that the sulfonation proceeds through the intermediate formation of the chalcone. The sulfonic acid obtained was identical with that obtained from 3-methoxy-2',4-dihydroxychalcone. The structures of the sulfonic acids from 3'-methoxy-4,4'-dihydroxyflavan and 3'-methoxy-4'-hydroxyflavylium chloride were not established.

As to the probable presence in lignin of configurations similar to those in the model compounds, it can be said that the α,β-unsaturated ketone groups are not present in lignin, at least not to any significant extent. The ultraviolet studies lend support to this view. However, a masked α,β-unsaturated group may be present in lignin. During sulfonation such a group would be initially converted into an α,β-unsaturated ketone group, which would subsequently react with bisulfite. The present work, therefore, constitutes partly a study of the second phase of sulfonation of a masked α,β-unsaturated ketone group provided, of course, that such a group does exist in lignin.

The present investigation does not give any information as to whether lignin contains oxygen-containing heterocyclic rings. However, the flavone and benzofuran configurations, even if they were present in lignin, which is doubtful because they are stable α,β-unsaturated ketones, definitely are not responsible for the sulfonation of lignin. The same is true for a flavanol or 2,3-dihydroflavonol structure. From the viewpoint of a model sulfonation reaction, flavanone, 4-hydroxyflavan, or a masked benzylidene-3-coumaranone configuration may be present in lignin. Although the deep-colored flavylium-ion configuration may not be present in lignin, the structure corresponding to the sulfonated derivative of 3'-methoxy-4'-hydroxyflavylium chloride may be similar to that of lignosulfonic acid, as shown by ultraviolet spectrographic studies.

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