AN ABSTRACT OF THE THESIS OF

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Title Chemical Studies on Dihydroquercetin

Abstract approved  Redacted for privacy

Dihydroquercetin, 3,5,7,3',4'-pentahydroxyflavanone, obtained by the extraction of Douglas-fir bark, was treated with a number of reagents to determine the fundamental chemical properties of this compound.

Esterification of dihydroquercetin by acetic anhydride was the first reaction studied. Methyllations of dihydroquercetin were conducted using diazomethane and dimethyl sulfate. Nitration of pentamethyldihydroquercetin led to the formation of 6'-nitropentamethyldihydroquercetin. Bromination studies were performed using dihydroquercetin, pentamethyldihydroquercetin, and dihydroquercetin pentaacetate. Optically active dihydroquercetin was racemized by two different processes.

Dihydroquercetin and pentamethyldihydroquercetin failed to form ketonic derivatives with such reagents as hydroxylamine, phenylhydrazine, and 2,4-dinitrophenylhydrazine. Studies were also conducted using propylene oxide and chlorosulfonic acid. An attempt was made to replace the hetero oxygen atom of quercetin by a nitrogen atom. Several experiments were conducted involving the oxidation and dehydrogenation of dihydroquercetin. Pentamethyldihydroquercetin was also dehydrogenated to form pentamethylquercetin.

The ultraviolet spectra of dl-dihydroquercetin and of a number of related flavones and flavanones were determined for the first time. Using this data certain deductions were drawn regarding the structures of dihydroquercetin, quercetin, and pentamethyldihydroquercetin.
CHEMICAL STUDIES ON DIHYDROQUERCETIN

by

PETER COAD

A THESIS

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Date thesis is presented May 13, 1958
Typed by Raylene Coad
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DEDICATED

to

HERBERT L. HERGERT

for his inspiration, understanding, and friendship.
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CHEMICAL STUDIES ON DIHYDROQUERCETIN

I. Introduction

Dihydroquercetin is a compound which has been demonstrated by Kurth and his coworkers (22, p. 23) to be present in the cork fraction of Douglas-fir bark (*Pseudotsuga taxifolia* (Poir.) Britt.) to the extent of twenty per cent. Since the annual supply of Douglas-fir bark has been estimated to be in excess of two million tons and since the cork fraction in the bark runs from twenty-five per cent to over fifty per cent, the potential supply of dihydroquercetin is from ten thousand to twenty thousand short tons per year. Since this compound may well be of future commercial importance, a study of the chemistry of this flavanone was undertaken.

II. Historical

Dihydroquercetin (I) was first reported in the
literature in 1947 by Pew (33, p. 1), a research chemist working in Madison, Wisconsin. He isolated dihydroquercetin from Douglas-fir heartwood and proved the chemical structure. At the same time Graham and Kurth (12, p. 412), working in Corvallis, Oregon, independently of Pew, isolated the compound also from Douglas-fir heartwood and determined certain of its properties.

This compound belongs to a class of flavanones, the first example of which, designated alpinone (II),

![Chemical Structure of Alpinone (II)](image)

was discovered (18, p. 286) about sixteen years ago in a Japanese drug prepared from Alpinia japonica. Subsequently, five other 3-hydroxyflavanones were reported to occur naturally. Fustin (III) was isolated (31, p. 785) from the heartwood of Rhus succedanea; ampelopsin (IV) was isolated (21, p. 258) from Ampelopsis meliaefolia Kudo, a plant used as
a drug and condiment in China; and 3-hydroxynaringenin (V) was isolated by Pew (33, p.4) from both
a South American wood coigue (*Nothofagus dombeyi* Blume) and the heartwood of black cherry (*Prunus serotina* Ehrh.). In 1943 the 3-hydroxyflavanone, katuranin (VI) was isolated (36, p.467) from a Japanese wood (*Cercidiphyllum japonicum*). This work was published in the *Journal of the Agricultural Chemistry Society of Japan* and did not appear in *Chemical Abstracts* until 1951. Upon examination of the structural formula of katuranin, it was observed that katuranin is identical with 3-hydroxynaringenin. Thus, two names for the same compound appear in the current literature. Lindstedt (27, p.772) in Sweden reported and elucidated the structure of pinobanksin (VII) which is found in certain species of pine along with the 7-methyl ether of pinobanksin.

Pew (33, p.7) proved the structure of dihydroquercetin by oxidizing it to quercetin (VIII), by
reducing dihydroquercetin to eriodictyol (IX), and by

reducing quercetin to the racemic form of dihydroquercetin. An unknown product was also isolated in the latter reaction which has since been identified by Geissman (10, p. 3003) as 4, 6, 3', 4'-tetrahydroxy-2-benzyl-coumarone-3 (X).

III. Objectives of Investigation

The original goal proposed for this study was
the preparation of a series of compounds, some of which might have interesting commercial or medicinal properties. It soon became evident that before this could be done, more would have to be learned about the simple chemical reactions of the substance. Hence, the latter became the principal goal of this thesis. A second objective which developed was to determine the ultraviolet spectra of dihydroquercetin and of a number of related compounds.

In the present study optically active dihydroquercetin was prepared, as well as the racemic forms of this compound. Among the reactions that were conducted were acetylations, methylations, and brominations. The nitration, bromination, and sulfonation of pentamethyldihydroquercetin were performed. Dihydroquercetin was treated with typical ketone reagents
(e.g., hydroxylamine, phenylhydrazine, etc.), with propylene oxide, and with various oxidizing agents. An attempt was made to replace the hetero-oxygen of quercetin with a nitrogen atom.

The ultraviolet spectra of dihydroquercetin and of a number of related compounds were determined and reported for the first time. Several theoretical deductions were made regarding the structures of dihydroquercetin, quercetin, and pentamethyldihydroquercetin.

IV. Experimental

Preparation of Optically Active Dihydroquercetin

The dihydroquercetin for this investigation was obtained mainly by the extraction of "Silvacon 383", a Douglas-fir bark product of Weyerhauser Timber Company, Longview, Washington. Initial studies on the method of extraction were made using a Soxhlet extractor. The capacity of the cup of this extractor in which the bark was placed was approximately 250 ml.; a one liter flask was connected to the bottom of the extractor.

Twenty-nine g. of Douglas-fir bark was placed in the cup and was extracted with 600 ml. of benzene.
It was necessary to maintain a temperature of 60-70°C. in the cup in order to effect removal of the wax. The column of the extractor was wrapped with asbestos to prevent heat loss. The rate of extraction was adjusted so that the Soxhlet was refilled every ten minutes. Extraction was continued for six hours. Then the bark was removed from the apparatus and dried by spreading it out on a sheet of paper and allowing the benzene to evaporate at room temperature. It was found to be absolutely necessary to remove all traces of benzene at this stage. Otherwise, a dark brown colored impurity was obtained in the product, causing the crystallization of dihydroquercetin to be difficult, if not impossible.

The dried bark was returned to the Soxhlet and extracted with diethyl ether for nine hours (rate: 5 min. per extraction). The ether solution was collected and concentrated to about 50 ml. by distillation. To this residue 50 ml. of hot water was added slowly with stirring. The remaining ether was removed by distillation. A light tan solid was collected, 1.7 g. (6 per cent yield, based on weight of bark), m.p. 130-140°C.

In later work a larger Soxhlet extractor was employed that had a cup capacity of 1.5 liters and a
solvent flask with a capacity of 5 liters. Using this equipment 900-1050 g. of bark could be extracted at one time. In a typical run 900 g. of bark was extracted with 4 l. of benzene, dried, and then extracted with 4 l. of ether. Ninety-three g. of crude dihydroquercetin was obtained (10.3 per cent). This large-scale extraction process was repeated about twenty times in order to secure sufficient dihydroquercetin for the investigation which follows.

Dihydroquercetin was purified by repeated recrystallization from water. The purified compound consisted of fine white needles which melted on a melting point block at 236-238° C. The optical activity of the compound was obtained by dissolving 1 g. of dihydroquercetin in a mixture of 10 ml. of acetone and 10 ml. of water. It was found that

$$\left[\alpha\right]_{D}^{20^\circ} = \frac{a}{1 \times c} = \frac{430}{2 \times 0.05} = 43.0^\circ$$

The following data were obtained concerning the solubility of the dihydroquercetin produced in this laboratory.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.5 g./l. at 25°C. Soluble, boiling water</td>
</tr>
<tr>
<td>Solvent</td>
<td>Solubility</td>
</tr>
<tr>
<td>--------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Ether</td>
<td>Moderately soluble</td>
</tr>
<tr>
<td>Benzene</td>
<td>Insoluble, hot or cold</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Moderately soluble, cold</td>
</tr>
<tr>
<td></td>
<td>Soluble, hot</td>
</tr>
<tr>
<td>Ethyl alcohol</td>
<td>Soluble, cold</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>Very soluble</td>
</tr>
<tr>
<td>Dioxane</td>
<td>Very soluble</td>
</tr>
<tr>
<td>Acetone</td>
<td>Very soluble</td>
</tr>
</tbody>
</table>

Paper chromatography of dihydroquercetin using as solvent the alcohol rich layer of 40-10-50 n-butanol-acetic acid-water and developing with potassium hydroxide gave an $R_f$ of 0.475 with no separation of spots.

**Racemic Dihydroquercetins**

These compounds were prepared by reduction of quercetin (A) and by the action of hydrogen bromide on optically active dihydroquercetin (B).

(A) Ten g. of quercetin, prepared from active dihydroquercetin by the method to be described later in this thesis, was converted to dihydroquercetin by the procedure of Geissman (10, p. 3003). An intimate mixture of 10 g. of quercetin and 85 g. of
sodium carbonate was placed in a 2 l. round-bottom flask equipped with a stirrer and nitrogen inlet and outlet tubes. To the nitrogen-filled flask was added one liter of boiling water, and to the resulting dark-brown solution was added, with stirring and heating, 200 g. of sodium hydrosulfite (90% Na₂S₂O₄). In seven minutes a slow evolution of hydrogen sulfide began; in twelve minutes this had become vigorous. After twenty-five minutes the flask was cooled in ice and then 130 ml. of concentrated hydrochloric acid was added. After four hours at 0°C., the mixture was filtered, and 7.0 g. of unreacted quercetin was recovered. The filtrate was extracted continuously with ethyl acetate for forty-eight hours, and the ethyl acetate was removed by distillation. One hundred ml. of water was added to the residue; the mixture was heated to boiling and filtered. The insoluble material was 4,6,3',4'-tetrahydroxy-2-benzylcoumarone-3.

The filtrate was allowed to cool. A precipitate formed and was removed by filtration. The solid was avocado green with dark spots. Upon several recrystallizations from hot water, 1.5 g. of dihydroquercetin was obtained, m.p. 228-230° (dec.), \([\alpha]_D^{25} = 0.00°\) in a 50:50
mixture of acetone and water at 20°C. The pentaacetate was formed by treatment with acetic anhydride-pyridine according to method B to be described in the section dealing with acetylations. Upon recrystallization from ninety-five per cent ethyl alcohol the acetate was obtained in pure form, m.p. 144-146°C.

(B) Ten g. of optically active dihydroquercetin was dissolved in 100 ml. of glacial acetic acid and placed in a round-bottom flask equipped with a mechanical stirrer and a dropping funnel. From the dropping funnel 5 g. of hydrogen bromide dissolved in 20 ml. of acetic acid was added dropwise with stirring. The mixture was stirred for two days and then evaporated to dryness under nitrogen. It was necessary to exclude oxygen to prevent the oxidation of dihydroquercetin to quercetin. The product was a dark brown viscous gum.

Part of the residue dissolved in hot water; this solution was treated with Norite, filtered, and cooled. A gum-like substance was collected, ground in a mortar, and washed with a small quantity of cold water. The solid was redissolved in hot water, treated with Norite, and cooled slowly. Crystals were obtained and dried in an Abderhalden drying apparatus at reduced pressure (about 3 mm.) and elevated temperature (80°C.)
in the presence of a drying agent (phosphorus pentoxide). When heated rapidly on a Fisher Block, this compound melted at 132-134°C. When the ground solid was heated in a glass capillary tube in a bath of concentrated sulfuric acid, the melting point was 229-230°C. Some of this solid was removed from the capillary and ground with an equal amount of naturally occurring dihydroquercetin; a mixed melting point was taken, m.p. 200-206°C.

The nature of the crystals which melted at 132-134°C. (Fisher Block) was investigated. Analysis by the Parr bomb method showed that no bromine was present in the compound. Some of the crystals (0.4 g.) were added to 10 ml. of twenty per cent sodium bisulfite solution. The mixture was boiled on a hot plate for two hours. A yellow precipitate was formed, 0.2000 g., m.p. 306-308°C. (dec.), uncorrected; mixed melting point with quercetin, 310-312°C.; optical activity: [α]_D^{25°} = 0.0° in acetone-water.

The pentaacetate of the original crystals was prepared by acetylation method (B) as described in the following section. The pentaacetate melted at 138-140°C.
Acetylations

Two general methods were developed for the acetylation of dihydroquercetin. One involved the use of acetic anhydride and sodium acetate (A) and the other was conducted with acetic anhydride in the presence of pyridine (B).

(A) The best procedure was to place 10 g. of dihydroquercetin in a 250 ml. Erlenmeyer flask equipped with a condenser and to add 3.5 g. of sodium acetate and 50 ml. of acetic anhydride. The mixture was heated under reflux on a steam bath for three hours. Then the solution was poured slowly into a beaker containing 250 ml. of cold water with vigorous stirring. A solid formed which was removed by filtration. The crude product (16 g.) was tritaturated with water. A paste was formed which was filtered and dried over potassium hydroxide in a vacuum desiccator. A white solid was obtained, m.p. 88-89°C. Anal. Calcd. for C_{25}H_{20}O_{12}: C, 58.2; H, 4.40. Found: C, 58.0; H, 4.40.

(B) Two g. of dihydroquercetin was added to 20 ml. of anhydrous pyridine. Ten ml. of acetic anhydride was added with shaking and the solution was heated
under reflux for one hour. Then the solution was cooled and poured into 100 ml. of cold water. A solid formed and was filtered, washed with dilute hydrochloric acid (two per cent) and finally washed with water. A white solid was produced, 2.2 g., m.p. 88-89 C.

Methylations

Methylations of dihydroquercetin were conducted using dimethyl sulfate (A) and diazomethane (B). During the course of this investigation a number of trials (27) were conducted in which there were major or minor variations in procedure. These variations involved changes in conditions, reagents, quantities of reagents, process of isolation of product, methods of purification of product, etc. From these experiments five trials judged to be of particular interest and significance have been selected and the results are summarized in Table I. The best procedures for preparing derivatives with dimethyl sulfate and diazomethane are described in detail below.

(A) Sixty g. of dihydroquercetin was dissolved in 400 ml. of warm methyl alcohol and placed in a round-bottom flask equipped with a magnetic stirrer,
Table I

Methylation of Dihydroquercetin

<table>
<thead>
<tr>
<th>Trial</th>
<th>Amt. D.H.Q.*</th>
<th>Methylating Agent</th>
<th>Comments, Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9 g. of D.H.Q. in sodium hydroxide</td>
<td>Dimethyl sulfate</td>
<td>Stirred 6 hrs. Allowed to stand 2 days. Isolated white crystals, m.p. 162-164°C. and two yellow solids. One of these had a melting point of 62-65°C. and the melting point of the other was indefinite.</td>
</tr>
<tr>
<td>2</td>
<td>10 g. of D.H.Q. in sodium hydroxide</td>
<td>Dimethyl sulfate</td>
<td>White powder obtained, m.p. 136-137°C. (using melting point block). Anal. Calcd. for C_{20}H_{22}O_{7}: OCH_{3}, 41.6. Found: 42.0.</td>
</tr>
<tr>
<td>3</td>
<td>60 g. of D.H.Q. in sodium hydroxide</td>
<td>Dimethyl sulfate</td>
<td>Isolated crude product, m.p. 116-119°C. Recrystallized, m.p. 150-153°C. Anal. Calcd. for C_{20}H_{22}O_{7}: C, 64.16; H, 5.9; OCH_{3}, 41.6. Found: C, 64.07; H, 5.9; OCH_{3}, 42.0.</td>
</tr>
<tr>
<td>4</td>
<td>6.4 g. of D.H.Q. in dioxane</td>
<td>Diazomethane</td>
<td>Brown viscous tar-like material obtained.</td>
</tr>
<tr>
<td>5</td>
<td>5.0 g. of D.H.Q. in methyl alcohol</td>
<td>Diazomethane</td>
<td>3.4 g. yellow solid, m.p. 60-70°C. Purified to form a solid, m.p. 130-140°C.</td>
</tr>
</tbody>
</table>

*D.H.Q. - dihydroquercetin
a water-cooled condenser, and two dropping funnels. The entire apparatus was assembled beneath an efficient hood. To the stirred solution 50 ml. of dimethyl sulfate was added from one funnel and 30 ml. of fifty per cent potassium hydroxide was added from the other. An exothermic reaction occurred which caused the solution to reflux. Subsequently, eight alternate additions of dimethyl sulfate (12.5 ml. each) and fifty per cent potassium hydroxide (16 ml. each) were made at such a rate as to maintain a state of vigorous refluxing. When the additions were made as rapidly as possible, this process took about two hours. The solution was then poured at once into a flask containing 1000 g. of ice and water. The mixture was allowed to stand in the refrigerator for twenty-four hours. At the end of this time a fine yellow powder was observed to be floating on the surface of the solution and a clump of brown tarry material had formed at the bottom of the flask.

The powder was removed from the surface of the solution and set aside for future investigation. The gum-like material was separated from the solvent by centrifugation and was allowed to dry. It was then ground to a fine powder (yield, 35 g.), and was separated into a benzene-soluble fraction (25 g.) and into
a fraction that was insoluble in benzene (10 g.). The benzene solution of the product was decolorized with Norite, filtered, and evaporated to dryness. The material that was obtained was triturated with hot petroleum ether, dried, tritutrated with diethyl ether, and dried again. Finally a white solid was obtained, 10.7 g., m.p. 116-119°C. This solid was recrystallized from ethyl alcohol, and fine crystalline needles were formed, m.p. 150-153°C. Anal. Calcd. for C₂₀H₂₂O₇: C, 64.16; H, 5.9; −OCH₃, 41.6. Found: C, 64.07; H, 5.9; OCH₃, 42.0.

Five g. of pentamethyl dihydroquercetin was dissolved in 100 ml. of hot benzene and added to 400 ml. of hot water in a 2 l. round-bottom flask equipped with a condenser, mechanical stirrer, and dropping funnel. Two portions of permanganate were added with stirring over a period of six hours. (The first portion contained 20 g. of potassium permanganate dissolved in 600 ml. of water, and the second consisted of 24 g. of potassium permanganate dissolved in 600 ml. of water.) The mixture was stirred overnight.

At the end of this period the mixture was heated on a water bath until all traces of the characteristic color of permanganate ion had disappeared. The manganese dioxide that had formed was removed by filtration
and washed with 300 ml. of water that had been made slightly basic by the addition of potassium hydroxide. The wash water was added to the original filtrate, and the combined solution was concentrated to 100 ml. Then dilute sulfuric acid was used to neutralize the solution, and it was concentrated once again to 100 ml. The final solution was transferred to a continuous extractor and extracted for four days with diethyl ether. Upon evaporation of the ether 3.0 g. of a sticky solid was obtained which was purified by sublimation. The melting point of the purified sample was 178-180°C.; mixed melting point (with a sample of pure veratic acid) was 177-179°C.

(B) Diazomethane was prepared by the action of potassium hydroxide on nitrosomethylurea (4, p. 165). The nitrosomethylurea was in turn prepared (4, p. 461) from methylamine hydrochloride.

Using the method described by Graham and Kurth (12, p. 412) 5 g. (0.167 mole) of dihydroquercetin was dissolved in 50 ml. of methyl alcohol and treated with 200 ml. of ether containing 5.3 g. (0.13 mole) of diazomethane. The mixture was allowed to stand in the refrigerator for twenty-four hours. The addition of
diazomethane in ether was repeated three times at twenty-four hour intervals. Between each addition the flask was stored in the refrigerator. A yellow solid was formed, 3.4 g., m.p. 60-70°C., which was recrystallized from methyl alcohol, m.p. 130-140°C. Anal. Calcd. for C₁₈H₁₈O₇ (trimethoxy): OCH₃, 27.0. Calcd. for C₁₉H₂₀O₇ (tetramethoxy): OCH₃, 34.6. Found: OCH₃, 29.4. This process was attempted eight times, but in all cases a mixture of the tri- and tetra- methoxy derivatives was obtained.

Two g. of this solid was dissolved in 50 ml. of methyl alcohol and was treated with dimethyl sulfate and potassium hydroxide in the manner described previously. From the resulting mixture 1.0 g. of pentamethyldihydroquercetin, m.p. 115-119°C., was isolated. After repeated recrystallizations from ethyl alcohol, the product melted at 131-132°C. and showed no depression in melting point when a mixed melting point was taken with pentamethyldihydroquercetin (low melting).

Nitrations

A number of attempts were made to nitrate dihydroquercetin with concentrated nitric acid. Various solvents were used, including concentrated sulfuric acid, glacial
acetic acid, acetic anhydride, and nitromethane. Nitrations were attempted at room temperature and also at 0°C. and at -15°C. whenever the nature of the solvent made this procedure possible. Dark brown products which were extremely soluble in water were obtained in all cases. Each reaction in this series was repeated at least once to verify the results obtained.

The nitration of pentamethyldihydroquercetin was accomplished by the procedure which follows. Twenty ml. of concentrated nitric acid (decolorized by boiling with a few crystals of urea) was placed in an Erlenmeyer flask and cooled to 0°C. in an ice-salt bath. The flask was removed from the bath and equipped with a magnetic stirrer. Over a period of one minute 2 g. of finely powdered pentamethyldihydroquercetin was added with stirring. The reaction mixture was poured at once into 250 ml. of water. A purplish solid formed which became brown on standing. The mixture was centrifuged and allowed to stand overnight. The solid was removed by filtration and washed repeatedly until the filtrate was neutral. The solid which remained on the filter paper was dried, 1.9 g., m.p. 155-158°C. (dec.). It was recrystallized using hot alcohol as the solvent, and white crystals were obtained, m.p. 166-167°C.
Anal. Calcd. for C\textsubscript{20}H\textsubscript{21}O\textsubscript{9}N: C, 57.28; H, 5.25; N, 3.34.
Found: C, 57.30; H, 5.05; N, 3.37.

The structure of this nitro derivative was proved by oxidation of the compound to yield a known compound, 6-nitroveratric acid. One hundred and fifty ml. of hot water was placed in a round-bottom flask equipped with a stirrer, condenser, and dropping funnel. A solution containing 1.5 g. of the nitro derivative of pentamethyl-dihydroquercetin and 50 ml. of benzene was placed in the flask also. Then the oxidizing agent was added dropwise to the stirred solution over a period of five hours. The first portion of oxidizing agent that was added contained 8 g. of potassium permanganate dissolved in 400 ml. of water, and the second portion contained 9 g. of potassium permanganate dissolved in 300 ml. of water. The mixture that resulted was stirred and heated under reflux for nine hours and was allowed to stand overnight. The benzene was removed by slow distillation, taking care to prevent excessive heating of the oxidation mixture. Heating and stirring were continued for four hours more. The mixture was allowed to cool by standing overnight and was filtered to remove the manganese dioxide. The filtrate was concentrated to approximately 75 ml.,
acidified with sulfuric acid, and extracted with ether using a continuous extractor over a period of three days. The ether was removed by distillation, and by the process of sublimation a pure product was isolated, m.p. 186-188°. The product was resublimed, and the melting point of this sample was 187-189°C. (The melting point of 6-nitroveratric acid is 188-190°C.) Anal. Calcd. for C9H9O6: N, 6.17. Found: N, 6.20.

Bromination Studies

A series of bromination reactions was conducted using dihydroquercetin, dihydroquercetin pentaacetate, quercetin, and pentamethyldihydroquercetin. Studies were made on the effect of alteration of the molar ratio of bromine and the starting material. The conditions of the reactions, e.g., temperature, quantity of solvent, and reaction time, were also varied. A few trials of particular interest are presented in Table II. Detailed directions for performing three typical bromination reactions are also given in the section that follows. These three reactions are (A) bromination of dihydroquercetin with a limited amount of bromine present, (B) bromination of dihydroquercetin with an excess amount
Table II
Bromination Reactions

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Amount</th>
<th>Reagents</th>
<th>Reaction Time</th>
<th>Comments and Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 g. D.H.Q.</td>
<td>15 ml. of acetic acid; Bromine added until color persists</td>
<td>1 1/2 hrs.</td>
<td>Oil obtained</td>
</tr>
<tr>
<td>2</td>
<td>1 g. D.H.Q.</td>
<td>30 ml. HAc; 0.4 ml. Br₂</td>
<td>1 hr.</td>
<td>Oil obtained</td>
</tr>
<tr>
<td>3</td>
<td>1 g. D.H.Q.</td>
<td>30 ml. HAc; 0.4 ml. Br₂ in 5 ml. of acetic acid</td>
<td>1/2 hr.</td>
<td>Mixture of red crystals, colorless crystals, and red oil.</td>
</tr>
<tr>
<td>4</td>
<td>6.5 g. D.H.Q. in 40 ml. HAc; 2.6 ml. Br₂ in 10 ml. HAc</td>
<td>1 1/2 hrs.</td>
<td>Brown solid obtained, m.p. 154-155°C (dec.)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20 g. D.H.Q.</td>
<td>110 ml. HAc; 7 ml. Br₂ in 10 ml. HAc</td>
<td>17 hr.</td>
<td>Crude product isolated and treated with bisulfite solution in an attempt to form the bromo derivative of quercetin. Yellow-white solid obtained, m.p. 153-158°C.</td>
</tr>
<tr>
<td>Table II (Cont.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5 g. of quercetin, 2 ml. Br&lt;sub&gt;2&lt;/sub&gt; in 5 ml. acetic acid.</td>
<td>17 hrs.</td>
<td>Dibromo product isolated, m.p. 234-236°C.</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1 g. D.H.Q. penta-acetate in 10 ml. HAc, 0.342 ml. Br&lt;sub&gt;2&lt;/sub&gt; in 2 ml. of HAc.</td>
<td>17 hrs.</td>
<td>0.34 g. solid, m.p. 140-145°C.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5 g. D.H.Q. in 100 ml. of HAc, 1.71 ml. bromine.</td>
<td>2½ hrs. for addition. Stood 1 hr. more.</td>
<td>A tar was obtained from which a white solid was isolated, m.p. 196-200°C. (dec.)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>5 g. D.H.Q. 3.42 ml. Br&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Added in 2 hrs. Stood overnight.</td>
<td>9.5 g. of solid obtained, m.p. 130-145°C. from which two compounds were obtained. One had a melting point of 158-160°C. Anal. Calcd. for C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;Br&lt;sub&gt;2&lt;/sub&gt;: C, 33.2; H, 1.67; Br, 44.4. Found: C, 31.8; H, 1.70; Br, 45.9.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>20 g. D.H.Q. 250 ml. of HAc 17.1 ml. Br&lt;sub&gt;2&lt;/sub&gt; in 100 ml. of HAc.</td>
<td>Let stand 48 hrs.</td>
<td>7.2 g. crystals formed, treated with (CH&lt;sub&gt;2&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;SO&lt;sub&gt;4&lt;/sub&gt; and KOH. Yellow solid formed, m.p. 140-145°C. (dec.)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1 g. Pentamethyl-dihydroquercetin</td>
<td>48 hrs.</td>
<td>0.8 g. crude product, m.p. 195-205°C. Recrystallized, m.p. 213-214°C. Anal. Calcd. for C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;Br&lt;sub&gt;2&lt;/sub&gt;: Br, 30.1. Found: Br, 34.5.</td>
<td></td>
</tr>
</tbody>
</table>
of bromine present, and (C) bromination of pentamethyl-dihydroquercetin. Additional studies concerning the preparation and isolation of bromine derivatives are described (D).

(A) Five g. of dihydroquercetin was dissolved in 100 ml. of warm glacial acetic acid. The solution was cooled and to this was added dropwise 1.71 ml. of bromine (measured with a microburet) dissolved in 10 ml. of glacial acetic acid during a period of two and one half hours. Fumes of hydrogen bromide were quite noticable after forty minutes. At the end of three and one half hours, sufficient sodium bisulfite was added to destroy any unreacted bromine; the solution was concentrated by distillation at a pressure of 17 mm. (water pump). In order to prevent oxidation of the product, the capillary which was used to admit air during the vacuum distillation was attached to a nitrogen cylinder and a slow stream of nitrogen was passed through the distillation system. To the contents of the flask 100 g. of ice and water was added, and a red-brown tar-like material was formed. The solid was triturated with water several times and then was dried in air. A light brown powder resulted, 4.5 g.,
m.p. 166-168° C. (dec.). The powder was ground with boiling ether, filtered, and the filtrate was decolorized with Norite and concentrated. Benzene was added slowly with swirling until a point of turbidity was reached. Upon standing a solid was obtained, 3.8 g., m.p. 196-200° C. (dec.). Anal. Calcd. for $C_{15}H_{10}O_7Br_2$: Br, 34.7. Found: Br, 36.4.

(B) The same procedure was followed as described above, but 3.42 ml. of bromine was added over a period of six hours to 5 g. of dihydroquercetin dissolved in 100 ml. of glacial acetic acid. The mixture was stirred for twenty-four hours. An ether soluble solid was obtained, 9.5 g., m.p. 130-145° C. (dec.). This solid was dissolved in hot benzene, filtered while still hot, concentrated, and cooled. A brown gummy precipitate was formed which was removed by filtration. The mother liquor was treated with Norite, concentrated, and cooled. A white solid was obtained, m.p. 158-160° C. The brown gummy precipitate was dried, finely ground, and placed in a Soxhlet, and it was extracted with benzene. Upon concentrating the benzene, a white crystalline solid and a gel were formed. Both the white crystals and the gel were dried and found to be identical with the white solid previously isolated, m.p. 158-160° C.
Anal. Calcd. for C_{15}H_{9}O_{7}Br_{3}: C, 33.2; H, 1.67; Br, 44.4. Found: C, 31.8; H, 1.70; Br, 45.9.

(C) Exactly 0.62 ml. of bromine delivered from a microburet was dissolved in 4 ml. of glacial acetic acid and added dropwise to a solution of 1 g. of penta-methylidihydroquercetin in 20 ml. of glacial acetic acid. The reaction was carried out in a 125 ml. Erlenmeyer flask using a magnetic stirrer. The flask was loosely corked and the solution was stirred for two days. At the end of six hours a precipitate started to form. At the end of two days the mixture was centrifuged; the supernatant liquid was decanted; and the solid was transferred to a Buchner funnel. There the solid was washed once with bisulfite solution and three times with hot water while mild suction was applied. The solid was dried, and 0.8 g. of an orange-red solid was finally obtained, m.p. 195-205°C. (dec.).

This solid was boiled with 25 ml. of ninety-five per cent ethyl alcohol and filtered while still hot. The solid that remained on the filter was deep red in color and melted at 202-205°C. (dec.). The solid was treated with eight more 25 ml. portions of boiling ethyl alcohol. Ninety five mg. of a white solid remained, m.p. 213-214°C (no decomposition). Anal. Calcd. for C_{20}H_{20}O_{7}Br_{2}:
Br, 30.1. Calcd. for C$_{20}$H$_{19}$O$_7$Br$_3$: Br, 39.2. Found: Br, 34.5.

The first three of the filtrates obtained in the above process were combined and concentrated by evaporation to 15 ml. and cooled in a refrigerator. A pink compound was obtained, m.p. 219-222°C. (dec.) Next 85 mg. of this solid was dissolved in 10 ml. of hot ninety-five per cent ethyl alcohol, treated with 8.5 mg. of Norite, and filtered. The filtrate was allowed to stand in the refrigerator for one day. A white solid was obtained, m.p. 222-223°C. (no decomposition). Anal. Calcd. for C$_{20}$H$_{20}$O$_7$Br$_2$: Br, 30.1. Calcd. for C$_{20}$H$_{19}$O$_7$Br$_3$: Br, 39.2. Found: Br, 35.6.

(D) An attempt was made to oxidize the mixture of bromo derivatives obtained in procedure A to quercetin derivatives. One half g. of the bromodihydroquercetin (chiefly dibromo-) was dissolved in 5 ml. of methyl alcohol. To this was added a solution of 0.5 g. of sodium bisulfite and 25 ml. of water. The mixture was heated under reflux for twenty-four hours. The original starting material was recovered.

An attempt was also made to methylate the mixture of bromo derivatives obtained by procedure (B). Five g.
of this solid was dissolved in 100 ml. of methyl alcohol and treated with dimethyl sulfate and potassium hydroxide in the usual manner. The first portion of dimethyl sulfate was 24 ml.; the eight portions that followed were 6 ml. each. The first portion of fifty per cent potassium hydroxide was 14 ml.; the eight portions that followed were 8 ml. each. The period of addition was one hour and fifteen minutes. The solution was poured into 600 ml. of water. A yellowish orange solid was obtained, 4.7 g., m.p. 40-50°C. The solid was partially soluble in hot diethyl ether. The melting point of the ether insoluble fraction was 170-190°C. The solid was also partially soluble in benzene. The portion that dissolved in hot benzene was decolorized with Norite, filtered, and evaporated to dryness. A dark gum was formed which was dried and ground with petroleum ether and dried again. A yellow product was obtained, m.p. 140-165°C. (dec.).

Another attempt was made to obtain a pure derivative by the bromination of pentaacetyldihydroquercetin instead of dihydroquercetin. Ten g. of the pentaacetate was treated with 3.5 ml. of bromine dissolved in 10 ml. of glacial acetic acid according to procedure A.
A dark brown oil was isolated from the reaction mixture, dried, and ground. Various trials were made in order to obtain pure compounds from this chocolate brown solid, but it was obvious that again a mixture of isomers had been obtained.

Bromination of dihydroquercetin was also attempted using pyridine as solvent. Ten g. of dihydroquercetin was dissolved in 50 ml. of pyridine. Bromine (8.6 ml.) was added dropwise to the stirred solution, and stirring was continued for twenty-four hours. The mixture was centrifuged, and the solid that was obtained was washed with bisulfite solution, dilute hydrochloric acid, and water. Washing was continued until the filtrate was neutral to litmus. The product was dried in the presence of phosphorus pentoxide, and the weight of the dry material was 7.3 g., m.p. over 300°C. No ash was formed on burning.

Attempted Preparation of Ketone Derivatives

A number of reactions were carried out in order to determine the possibility of preparing ketone derivatives of dihydroquercetin. Four of these runs are described in Table III. No ketonic derivatives were isolated under the various conditions used. Attempts
Table III

Ketone Derivatives

<table>
<thead>
<tr>
<th>Trial No.</th>
<th>Amt. of D.H.Q.</th>
<th>Reagents</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 g.</td>
<td>0.5 g. of NH₂O₇ HCl</td>
<td>Heat under reflux for eight hours.</td>
<td>Green viscous syrup formed from which only hydroxyl amine hydrochloride could be isolated.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.5 ml. of absolute ethyl alcohol.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5 g.</td>
<td>0.5 g. of NH₂O₇ HCl</td>
<td>Heat under reflux for two hours</td>
<td>Dark brown viscous oil.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 g. KOH</td>
<td>10 ml. of 95% ethyl alcohol.</td>
<td>Poured into 150 ml. of water.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.3 g.</td>
<td>0.22 g. of 2,4-dinitrophenyl-hydrazine</td>
<td>Heat to boiling. HCl added. Heated to boiling again; cooled.</td>
<td>Only solid isolated was the original reagent.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 ml. of 95% ethyl alcohol.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.5 g.</td>
<td>0.2 g. of semicarbazide hydrochloride</td>
<td>Mixture was shaken and cooled.</td>
<td>No product could be isolated.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 g. sodium acetate.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
were also made to prepare ketone derivatives of penta-
methyldihydroquercetin, but no such compounds were
formed.

Treatment with Propylene Oxide

One g. of pure dihydroquercetin was mixed with
10 ml. of water and sufficient 3N-sodium hydroxide was
added to dissolve the mixture. Then 0.2 ml. of
excess base was added, and the solution was poured into
a copper pipe. Propylene oxide (1.7 ml.) was added.
The pipe was sealed and heated in an autoclave (tem-
perature of approximately 100°C.) for eight hours. A
reddish black heterogeneous mass was obtained which
could not be purified.

This reaction was also attempted in neutral
solution. One g. of dihydroquercetin was dissolved in
25 ml. of hot water. The solution was poured into the
copper pipe and cooled to room temperature. Then 1.4
ml. of propylene oxide was added. Heating was
conducted as described previously. Brown crystals
were obtained, which on recrystallization were shown
to be identical with the starting material.
Sulfonations

When dihydroquercetin was treated with chlorosulfonic acid, a charred mass resulted. However, the pentamethyl derivative was treated with some success.

A three-necked round-bottom flask was equipped with a mechanical stirrer, a calcium carbonate tube, and a piece of wide rubber tubing leading to an Erlenmeyer flask containing 5 g. of finely powdered pentamethyldihydroquercetin. Since this reaction must be conducted under anhydrous conditions, care was taken so that all equipment and reagents were absolutely dry. Twenty-five ml. of chlorosulfonic acid was cooled in an ice-salt bath and then transferred rapidly to the round-bottom flask. The pentamethyldihydroquercetin was added to it over a period of two hours. Then 100 g. of ice was added. The mixture was separated by using a centrifuge. An orange-red solid and a yellow solution were obtained. The solid was removed by filtration, washed with water, and dried in a vacuum desiccator. The melting point was found to be 120-130°C (dec.). The dried solid was triturated with hot water, filtered, washed with a very small quantity of boiling acetone, filtered, and dried. The product which was
obtained, m.p. 260-270° C. (dec.), gave a positive test (sodium fusion) for sulfur. Oxidation of 0.21 g. of this product with potassium permanganate according to the procedure previously described on page 18 of this thesis yielded veratric acid (50 mg., m.p. 177-179° C.).

Attempted Replacement of the Hetero Oxygen of Quercetin by Nitrogen

Before this study could be made, it was necessary to prepare pentamethylquercetin. The best procedure for this was to place 12 g. of finely ground quercetin and 780 ml. of methyl alcohol in a round-bottom flask equipped with a condenser and two dropping funnels and to add alternately to this stirred mixture dimethyl sulfate and a fifty per cent solution of potassium hydroxide. The first additions were 144 g. of dimethyl sulfate and 84 ml. of potassium hydroxide solution. The next eight additions were 36 ml. of dimethyl sulfate and 48 ml. of fifty per cent potassium hydroxide. Additions were made at such a rate as to maintain a state of reflux, and the time involved was about two hours. From the basic solution 8.9 g. of yellow solid was isolated. This salt was purified by dissolving it in ninety five
per cent ethyl alcohol, decolorizing with charcoal, concentrating, and cooling. A white solid was obtained, m.p. 151-152°C.; the value reported in the literature (37, p.339) for this melting point was 151-152°C. The yield of the white solid was 5.0 g.

It was next necessary to prepare the salt of pentamethylquercetin. Four g. of finely ground pentamethylquercetin was placed in a flask equipped with a reflux condenser. Forty ml. of freshly distilled dimethyl sulfate (b.p. 88-89°C., 23 mm.) was added. The white solid turned yellow on contact with the dimethyl sulfate and completely dissolved when the solution was heated by an oil bath at 90-110°C. for three and one half hours. The solution was allowed to stand for thirty hours at room temperature. An organic salt, bright orange in color, precipitated and was removed by filtration. The solid was triturated with ether, and 2.7 g. of product, m.p. 192-195°C., was isolated. A small sample of the orange solid (0.1 g.) was treated with dilute sodium hydroxide and white pentamethylquercetin was reformed.

The salt of pentamethylquercetin (2.7 g.) was placed in a separatory funnel and 100 ml. of a
saturated solution of ammonium carbonate was added. Some frothing was observed. Upon shaking a cream colored precipitate appeared which was isolated and dried, 2.22 g., m.p. 154-155°C. The solid was recrystallized using ninety five per cent ethyl alcohol, and 1.0 g. of product, m.p. 155-156°C., was obtained. Analyses for nitrogen were made by both the Dumas method and the Kjeldahl method (omitting digestion). Anal. Calcd. for C_{20}H_{22}O_{6}N: N, 3.62. Found: N, 0.45 (Dumas method); N, 0.00 (Kjeldahl method).

Oxidations and Dehydrogenations

It was necessary to prepare quercetin for various uses during the course of the investigation of compounds related to dihydroquercetin. Three methods were used and are described below, (A) air oxidation, (B) bisulfite oxidation, and (C) dehydrogenation.

(A) After a number of trials the following procedure was developed. Ten g. of dihydroquercetin was dissolved in 1 liter of 1.5 N sulfuric acid and placed in a round-bottom flask equipped with an inlet for compressed air and a condenser. The mixture was
heated on a steam bath while a slow stream of air was passed across the surface of the liquid over a period of twenty-seven hours. The inlet was placed several inches above the surface of the liquid and adjusted so that the surface was as undisturbed as possible. The condenser was tilted so that any drops that were condensed would strike the side of the flask and would not fall directly on the surface of the liquid. By observing these precautions it was possible to obtain a yellow crystalline product which could be removed from the solution by filtration. The solid was transferred to a beaker, washed with water, filtered, and dried at 110°C. Pure quercetin was obtained, 1.85 g., m.p. 316-318°C. (dec.).

(B) Forty-five g. of dihydroquercetin was dissolved in 1 liter of boiling water. Fifty g. of sodium bisulfite was added, and the mixture was heated on an oil bath at 119°C. for three and one half hours. A yellow solid was formed which was removed by filtration and dried. Thirteen g. of crude quercetin was obtained which was washed with water, refiltered, and dried. The above reaction was not influenced by the presence or exclusion of air.
Dihydroquercetin was dehydrogenated by a modification of the method used by Kotake and Kubota (21, p. 270) for the dehydrogenation of ampelopsin. One half g. of dihydroquercetin, 1.2 g. of cinnamic acid, and 0.25 g. of ten per cent palladium on charcoal were placed in a copper pipe and stirred and heated at 170° C. for one and one half hours. It was found that the arrangement of the apparatus was critical for the success of this reaction, and, therefore, a diagram of the apparatus is shown on the following page. The pipe was cooled to room temperature, and the contents were gently emptied into a 50 ml. beaker. No carbon appeared at this point. The mixture in the beaker was boiled and filtered while hot. The precipitate that was collected during this hot filtration was added to 25 ml. of water and extracted with three 25 ml. portions of ether. The aqueous portion was again filtered, and 0.15 g. of quercetin was obtained, m.p. 310-312° C. The pipe was rinsed with acetone, and an additional 0.05 g. of quercetin was obtained by removing the acetone by distillation, washing the residue with boiling water, and crystallizing the quercetin with ninety five per cent ethyl
This was the apparatus used for mechanically stirring and heating the contents of a bomb. The solvent was water at 170°C.
alcohol.

Procedure (C) was used to treat 0.5 g. of the white solid obtained from the bromination of dihydroquercetin with a limited amount of bromine (see pp. 26-27). Quercetin, 0.30 g., m.p. 304-305°, was isolated.

Procedure (C) was used on 0.5 g. of 6,8 dibromoquercetin. Again quercetin was isolated, 0.29 g., m.p. 306-309° C.

Procedure (C) was also used on 0.5 g. of pentamethyldihydroquercetin (m.p. 133-134° C.). The product which was isolated from this reaction was a white solid, m.p. 132-133°. Addition of concentrated hydrochloric acid produced a golden salt and a dark colored supernatant solution. (Note: The salt is a sensitive test for pentamethylquercetin and the colored solution is a sensitive test for pentamethyldihydroquercetin.) The product was a mixture of pentamethylquercetin and pentamethyldihydroquercetin.
Determination of Ultraviolet Spectra

The absorption spectra were determined with a Beckman model D.U. spectrophotometer using silica cells. Each sample was dissolved in ethyl alcohol and rediluted. The spectrum of each compound was observed at intervals of 5 mμ from 225 mμ to 400 mμ. In the region of a maximum the values were recorded at intervals of 1 mμ. The values of the absorption coefficient, k, for each observed point of the spectra were calculated by means of the usual equation, \( k = \frac{2.3a}{cl} \) in which \( a \) is the absorption read from the spectrophotometer; \( c \) is the concentration of the solute in grams per liter; and \( l \) is the width of the silica absorption cell. A typical calculation is given for a point on the spectra of racemic dihydroquercetin (Curve 1).

Racemic dihydroquercetin (1.55 mg.) was placed in a 25 ml. volumetric flask and diluted to volume. The flask was inverted one hundred times to insure uniform mixing. Two ml. of the solution was withdrawn and rediluted to 25 ml. At a wave length of 290 mμ the absorption was 0.307, and hence k was equal to
\[ \frac{2.3 \text{ a}}{\text{cl}} = \frac{2.3 \times 0.307}{\frac{1.55 \times 10^{-3}}{25 \times 25} \times 1.0001} = 144 \text{ l. cm.g}^{-1} \]

Table IV lists the wave length, \( \lambda \), of the maximum and the absorption coefficients for the compounds studied.

The ultraviolet absorption curves numbered 1-14 have never been reported in the literature. Curves 15-20 have been reported in the literature but have been repeated due either to the fact that only the maxima were listed or to the fact that insufficient details of the absorption curves were shown.
### Table IV

**Ultraviolet Absorption Maxima of Flavones and Flavanones**

<table>
<thead>
<tr>
<th>Curve No.</th>
<th>Name of Compound</th>
<th>$\lambda_{max.}$ (m$\mu$)</th>
<th>k</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dl-Dihydroquercetin</td>
<td>290</td>
<td>144</td>
</tr>
<tr>
<td>2</td>
<td>dl-Dihydroquercetin pentaacetate</td>
<td>261</td>
<td>52.5</td>
</tr>
<tr>
<td>3</td>
<td>Low melting form of pentaacetate</td>
<td>313</td>
<td>12.1</td>
</tr>
<tr>
<td>4</td>
<td>High melting form of pentaacetate</td>
<td>341</td>
<td>126</td>
</tr>
<tr>
<td>5</td>
<td>Quercetin pentaacetate</td>
<td>252</td>
<td>90</td>
</tr>
<tr>
<td>6</td>
<td>7,3',4'-Trimethylquercetin</td>
<td>255</td>
<td>156</td>
</tr>
<tr>
<td>7</td>
<td>7,3',4'-Trimethylquercetin diacetate</td>
<td>370</td>
<td>149</td>
</tr>
<tr>
<td>8</td>
<td>3,7,3',4'-Tetramethylquercetin</td>
<td>242</td>
<td>131</td>
</tr>
<tr>
<td>9</td>
<td>3,7,3',4'-Tetramethylquercetin</td>
<td>254</td>
<td>139</td>
</tr>
<tr>
<td>10</td>
<td>Eriodictyol tetraacetate</td>
<td>261</td>
<td>51.4</td>
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<td>11</td>
<td>Epicatechin pentaacetate</td>
<td>270</td>
<td>12.75</td>
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<tr>
<td>12</td>
<td>Catechin pentaacetate</td>
<td>271</td>
<td>9.48</td>
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<tr>
<td></td>
<td>Compound</td>
<td>λmax (nm)</td>
<td>ε (M⁻¹ cm⁻¹)</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------------</td>
<td>-----------</td>
<td>--------------</td>
</tr>
<tr>
<td>13</td>
<td>Tetramethyl-dihydroquercetin</td>
<td>287</td>
<td>129</td>
</tr>
<tr>
<td>14</td>
<td>Tetramethylidihydroquercetin monoacetate</td>
<td>231</td>
<td>132</td>
</tr>
<tr>
<td>15</td>
<td>d-Dihydroquercetin</td>
<td>291</td>
<td>152</td>
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<tr>
<td>16</td>
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<tr>
<td>20</td>
<td>Epicatechin</td>
<td>281</td>
<td>47.2</td>
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</table>
Curve 1: dl Dihydroquercetin
Curve 2: dl-Dihydroquercetin pentaacetate

\( \log K \) vs. wavelength (\( \lambda \) in nm)

Chemical structure of dl-Dihydroquercetin pentaacetate
Curve 3: low melting form of Pentamethyldihydroquercetin
Curve 4: high melting form of Pentamethyldihydroquercetin
Curve 5: Quercetin pentaacetate
Curve 6: 7,3',4' Trimethylquercetin
Curve 7: 7,3',4' Trimethylquercetin diacetate
Curve 8: 3,7,3',4' Tetramethylquercetin

![Graph of 3,7,3',4' Tetramethylquercetin](image-url)
Curve 9: 3',7,3',4' Tetramethylquercetin monoacetate
Curve 10: Eriodictyol tetraacetate
Curve II: Epicatechin pentaacetate

\[ \log K \]

\[ \lambda \text{ mm} \]

\[ 220 \quad 280 \quad 340 \quad 400 \]
Curve 12: Catechin pentaacetate

\[ \log K \]

\[ \lambda \text{ m} \mu \]

\[ 220 \quad 280 \quad 340 \quad 400 \]
Curve 13: 5,7,3',4' Tetramethyl-dihydroquercetin

\[ \log K \]

\[ \lambda \text{ m\text{	extmu}} \]

0 1 2
220 280 340 400

Chemical structure:

![Chemical structure of 5,7,3',4' Tetramethyl-dihydroquercetin](image)
Curve 14: 5,7,3',4' Tetramethyl-dihydroquercetinmonoacetate
Curve 15: d-Dihydroquercetin

\[ \log K \]

\[ \lambda \text{ m}\mu \]
Curve 15: Quercetin

\[ \log K \]

\[ \lambda \text{ nm} \]

[Graph showing a UV-visible spectrum with molecular structure of quercetin]
Curve 17: Penta-methyl-quer cetin
Curve 18: 7 Hydroxy-flavanone
Curve 19: 7,3',4' Trihydroxy-flavanone

\[ \log K \]

\[ \lambda \text{ nm} \]

220 280 340
Curve 20: Epicatechin

\[ \log K \]

\[ \lambda \text{m} \mu \]

![Chemical Structure](image)
V. Discussion of Results of Chemical Studies

Stereochemistry of the Dihydroquercetin Molecule

The dihydroquercetin prepared for use in this investigation was optically active, $[\alpha]_{D}^{20} = 43.0^\circ$, in fifty per cent acetone in water. It is of interest to note that Graham and Kurth (12, p.412) reported a value of $[\alpha]_{D}^{25} = 39.8^\circ$ in the same solvent. Few (33, p.9) found that the product from heartwood had an optical rotation of $[\alpha]_{D}^{20} = 46.0^\circ$. H.L. Hergert (15), Oregon Forest Products Laboratories, has found the value for an exhaustively dried sample to be $[\alpha]_{D}^{20} = 44.2^\circ$.

The fact that the compound is optically active suggests an inquiry into the stereochemistry of dihydroquercetin. In addition to its close relationship with quercetin and eriodictyol, dihydroquercetin bears a resemblance to catechin (XI).

![Chemical Structure](image)
It has been shown by Freudenberg (9, p.1734) that catechin has two asymmetrical carbon atoms giving rise to only four optical isomers which constitute two racemic modifications. All six modifications and only six modifications are known. This result is the one that would be predicted by using the classical "stick and ball" models. However, when the more modern Fisher-Hirschfelder models are employed, it becomes apparent that the hetero ring is puckered, and, therefore, cis and trans (boat and chair) forms should exist. From these it is possible to build two geometrical isomers for each optical isomer mentioned above. This would bring the total number of possible isomers to twelve, rather than to six. Freudenberg (8) attributes the "missing members" of the catechin series to a strained hetero oxygen bond angle. However, no physical chemical proof of this exists other than the fact that the "missing members" are still missing.

In light of the fact that there are at least six possible forms of dihydroquercetin it seemed of interest during the course of this thesis to run paper chromatograms on optically active dihydroquercetin to see whether or not it might be a mixture of
two "d" rotating diastereoisomers. No separation resulted, indicating that there is a single pure active form.

Racemic Dihydroquercetins

The two racemic mixtures have been prepared. One is prepared from aqueous solutions either by racemization of active dihydroquercetin with concentrated hydrochloric acid (33, p.9) or by the reduction of quercetin with sodium hydrosulfite (33, p.9). It melts at 236-238°C. The reduction method was repeated in this laboratory as part of this project. The second racemic product, melting at 132-134°C, was prepared in this laboratory by racemization with hydrogen bromide in glacial acetic acid. It was discovered that the latter isomer could be converted to the former by prolonged heating at 130°C. Evidence that the racemic product that melts at 132-134°C is truly a second racemic form of dihydroquercetin and not a mere hydrate is given by the fact that no change occurred in the melting point of the racemic product upon prolonged drying at 80°C in vacuo over phosphorous pentoxide.
Acetylations

Even before the structure of dihydroquercetin had been elucidated, Graham and Kurth (12, p. 412) acetylated the then unknown flavanone which they had extracted from Douglas-fir bark. They obtained a white granular solid, m.p. 82-85°C., which could not be recrystallized from dilute ethanol, ether, or a mixture of dioxane and water.

The pentaacetate of dihydroquercetin was prepared in the course of the present thesis by treatment of dihydroquercetin with acetic anhydride in the presence of sodium acetate and also with acetic anhydride in the presence of pyridine. The same noncrystalline compound was produced in both cases, the pentaacetyl derivative, m.p. 88-89°C. The sharp melting point and the excellent results obtained upon analysis seemed to establish the purity of this compound.

It is interesting to note that a derivative with different physical characteristics was obtained when racemic dihydroquercetin (synthesized by the reduction of quercetin) was treated with acetic anhydride and pyridine. Geissman (10, p. 3003) obtained
a pentaacetyl derivative, m.p. 149-151°C., which could be recrystallized from alcohol. Again, perfect agreement between theoretical and actual analytical data was reported.

The preparation of this higher melting pentaacetyl derivative from racemic dihydroquercetin was successfully repeated in this laboratory, and it must, therefore, be concluded that two different isomers can be prepared, depending upon the nature of the dihydroquercetin used as the starting material.

Methylations

Dimethyl sulfate and diazomethane were used as methylating agents for preparing methyl derivatives of dihydroquercetin. These reagents have been used in the field of 3-hydroxyflavanones with varying degrees of success.

Treatment with excess dimethyl sulfate and potassium hydroxide generally results in complete methylation of flavanones. Kotake and Kubota (21, p.262) reported the successful preparation of hexamethylampelopsin using dimethyl sulfate and potassium hydroxide with methyl alcohol as the solvent. The totally methylated derivative, tetramethylkaturanin,
was also prepared (36, p.9136h) by this method, but the product that was isolated was in the form of a white oil and could not be purified or recrystallized.

Lindstedt (28, p.778) used dimethyl sulfate and potassium hydroxide with acetone as the solvent for the preparation of the completely methylated compound, trimethylpinobanksin.

The use of diazomethane for the methylation of 3-hydroxyflavanones generally leads to the formation of a mixture of products, the most highly methylated of which contains n - 1 methoxyl groups, where n is the total number of hydroxyl groups originally present in the molecule. It was shown by Kotake and Kubota (21, 254) in the case of ampelopsin that the hydroxyl group which fails to react with diazomethane is the one located in the 3-position, the secondary alcohol group. In the case of ampelopsin a mixture of the tetra- and penta- methyl derivatives was obtained which was separated by fractional crystallization.

Uoda, Fukushima, and Kondo (36, p.9136h) treated katuranin with diazomethane and obtained a mixture of mono-, di-, and tri- methylkaturanin. Of these three
only the monomethyl derivative was obtained in sufficient purity to determine the melting point or other physical properties.

Following the procedure described by Watson (37, p. 339) for the methylation of quercetin with dimethyl sulfate and potassium hydroxide in methyl alcohol, dihydroquercetin was converted to the pentamethyl derivative (XII).

Further proof of the structure of this compound was given by oxidation with neutral permanganate solution. As would be expected, veratric acid (XIII) was isolated.
Pentamethyldihydroquercetin was also converted to pentamethylquercetin using palladium on charcoal with cinnamic acid (this thesis) and also was converted to the pentamethyl ether of epicatechin by Hergert (15) using the Clemmensen reduction.

Dihydroquercetin was next treated with diazomethane. In this case a mixture of the tri- and tetramethoxy compounds was formed, as indicated by analysis of the amorphous solid that was isolated. It was found on treatment of this solid with dimethyl sulfate that pentamethyldihydroquercetin was formed.

Nitrations

Nitration of dihydroquercetin was attempted under a variety of conditions. However, it was found that under no circumstances could the reaction be conducted without severe oxidation and no nitrodihydroquercetin could be obtained. Actually, this result was to be expected, for identical behavior has been reported (6, p.263) for polyhydroxyflavones, e.g., quercetin.

In the case of quercetin successful nitration of the pentamethyl ether has been performed (37, p.340). Here the hydroxyl groups are protected from oxidation.
during the nitration process, and the following reaction occurs:

\[
\begin{align*}
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\end{align*}
\]

The nitration of pentamethyldihydroquercetin was carried out in a similar manner. The mononitro derivative was isolated, purified, and analyzed. Upon oxidation of the mononitro derivative of pentamethyldihydroquercetin, 6-nitroveratric acid was formed, indicating that the following reaction had occurred:

\[
\begin{align*}
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\text{OCH}_3 & \quad \text{OCH}_3 \\
\end{align*}
\]
Brominations

Bromination of dihydroquercetin produces mixtures of compounds, and, hence, this reaction gives rise to tremendous problems of isolation and purification which are, as yet, unsolved. It might be noted that although six 3-hydroxyflavanones have been studied and reported in the literature, not one bromo derivative has been successfully prepared and reported to date. Due to the large amount of time and effort devoted to this phase of the project, progress has been made toward the ultimate solution of this problem.

The initial product isolated from any bromination of dihydroquercetin is generally an oil, a tar, or a gum, and any of these resist purification by the usual method of solution in some new solvent, concentration, and crystallization. A special technique has been developed for handling such substances in order to obtain solid products. This technique involves the repeated trituration of the substance with various solvents, and may be
described by means of the chart given below.

---

**Oil (Mixture of Compounds)**

- **Solvent A**
  - Soluble in Solvent A
  - Insoluble in Solvent A
    - (1) Concentrated. Crystallization attempted. If that failed,
      - Soluble in Solvent B
      - Insoluble in Solvent B
    - Dried, ground, and triturated with Solvent C.
      - Soluble in Solvent C
      - Insoluble in Solvent C

---

The purpose of this procedure was to obtain solids of sufficient purity to repurify by normal crystallization methods. Sometimes it was impossible to obtain crystalline products of this type; only amorphous solids could be isolated. This indicated, of course, that two or more compounds (or two or more isomers of the same compound) with nearly the
same solubility characteristics were present.

Much thought and experimental study were devoted to trying to discover the reasons for this difficulty in obtaining pure bromo derivatives. It is now believed that there are two main factors involved.

The first is fairly obvious, and it is the fact that at least two (and perhaps more) derivatives can be formed which differ in the number of bromine atoms present in the molecule. In the case of quercetin (25, p. 1683)(26, p. 1184) bromination can lead to the formation of two compounds, 6,8-dibromo-quercetin (XIV) and 6,8,3'-tribromoquercetin.

\[ \text{HO} \quad \text{O} \quad \text{OH} \quad \text{OH} \]

\[ \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \quad \text{O} \]

(XIV)

Morin, on treatment with bromine in acetic acid is known (2, p. 794) to form 6,8,3',5'-tetrabromomorin (XV). Bromination of catechin pentamethyl ether
(20, p.4011) results in the formation of a monobromo derivative, 8-bromocatechin pentamethyl ether (XVI).

It has also been shown (37, p.346) that the monobromo derivative is isolated when quercetin pentamethyl ether is brominated.

By analogy with quercetin it seems reasonable that a di- or tribromo derivative might be formed,
or, by analogy with morin or catechin, that even a mono- or tetra- derivative might be postulated. The most likely positions for substitution appear to be 8; 6,8; 6,8,x; 6,8,3',5'. In addition, it must not be forgotten that the 3-position in dihydroquercetin, a flavanone, is a point of reactivity and that either the -H or -OH might be subject to possible reaction with bromine or with hydrogen bromide (the latter is formed during the reaction).

Thus the possible difficulties that might be encountered from varying degrees of substitution have been considered. However, if this were the only problem, surely, as in the cases of the flavones cited, it should be possible to obtain pure bromo-derivatives. Evidently, there is a second factor that is involved in the bromination of flavanones which is not present in the case of flavones. This second factor is isomerism.

It is an established fact (11, p.454) that in the presence of halogens or of halogen acids changes in the stereoisomeric properties of a compound can occur. These changes can involve the optical properties or the geometrical (cis-trans) properties of the
molecule or both. Hence, the number of possible isomers that can be produced by a "simple" bromination process is, indeed, very large.

An interesting experiment was performed which verified this idea. Dihydroquercetin was treated with hydrogen bromide in acetic acid (in an amount equivalent to that formed in the bromination procedure). An exothermic reaction occurred and the product that was isolated melted at 130-132°C., was optically inactive, and contained no bromine. It was readily converted to quercetin by oxidation with bisulfite. Hence, it may be concluded that the product that was isolated was a low melting racemized form of dihydroquercetin.

Thus, it must be realized that the isolation of a bromo derivative must involve the separation of a single isomer from a very large number of isomers and similar compounds. Nevertheless, numerous attempts were made to isolate one or more compounds from the bromination of naturally occurring dihydroquercetin. Literally dozens of trials were made with varying amounts of bromine and solvent and with differences in conditions. Although solid, apparently pure, products were obtained, analytical data always revealed that two or more compounds were present.
Treatment of dihydroquercetin with bromine in a molar ratio of 1:2 gave a derivative that was insoluble in ether at 0°C, yet soluble in boiling ether. This material was found to contain 36.4 per cent bromine. (Dibromodihydroquercetin contains 34.7 per cent bromine, and the tribromo derivative contains 44.4 per cent bromine.) When dihydroquercetin was treated with bromine in a ratio of 1:4, a product was formed which was soluble in either hot or cold ether. Analysis showed that 45.9 per cent bromine was present.

Treatment of pentamethyldihydroquercetin with bromine led to the formation of two different solids, each containing 34.5 per cent bromine. (Pentamethyldibromodihydroquercetin contains 30.2 per cent, and the tribromo derivative contains 39.2 per cent bromine.)

In addition to the work described above, bromination of dihydroquercetin in the presence of pyridine and bromination of dihydroquercetin pentaacetate were attempted. An attempt was made to methylate the bromo derivatives in order to obtain isomers that might vary in physical properties and, hence, be more readily separable. An effort was also made to identify the bromo compounds by oxidation to quercetin derivatives. All of these attempts were unsuccessful.
It has been concluded that there are two remaining avenues to the eventual solution of this problem. One is to use chromatography, but the search for a proper adsorbent from the vast number available and for a proper developer seemed to be beyond the scope of this thesis. The second possibility is to brominate a large quantity (perhaps a kilogram) of dihydroquercetin and to begin a fractional separation of the various compounds by fractional crystallization. Since the products present have solubilities that are nearly identical, this process would be lengthy and also beyond the scope of this thesis.

Attempted Preparation of Ketone Derivatives

Of the six 3,5-dihydroxyflavanones known only one successful preparation of a ketone derivative has been reported. This was in the special case of alpinone (II), and the reason for this occurrence is postulated later in this thesis (in the discussion of the ultraviolet spectra of dihydroquercetin and pentamethyldihydroquercetin).

It was believed that it would be of interest to check the possibility of preparing ketone derivatives of dihydroquercetin. A number of attempts were
made to prepare such compounds as the oxime, hydrazone, phenylhydrazone, and 2,4-dinitrophenylhydrazone. Under no circumstances was a ketone derivative formed. Thus, it may be concluded that there is sufficient chelation between the carbonyl group and the C-5 hydroxyl group in dihydroquercetin to decrease the ketone characteristics of the molecule so that it does not undergo a reaction when treated with ketone reagents. It was also found that the pentamethyl derivative of dihydroquercetin failed to form ketonic derivatives. The lack of ketone properties in this compound may be attributed to tautomerism. The conclusions reached about the lack of ketone properties in dihydroquercetin are supported by studies of the ultraviolet spectra (to be discussed later in this thesis).

Propylene Oxide Studies

Treatment of dihydroquercetin with propylene oxide in the presence of base at elevated temperature and pressure resulted in severe degradation. Actually, this result might have been predicted from the work of Kotake and Kubota (21, p.262) on the degradation of ampelopsin by alkali fusion and the cleavage of
hexamethylampelopsin in alcoholic potassium hydroxide.

When the reaction was attempted in neutral solution, only starting material was recovered, i.e., no reaction occurred.

Sulfonations

When dihydroquercetin was treated with chlorosulfonic acid, severe decomposition occurred. When the pentamethyl derivative was treated with chlorosulfonic acid, a solid sulfur containing derivative was obtained. Upon oxidation of this product, veratric acid was produced.

Attempted Replacement of the Hetero Oxygen of Quercetin by Nitrogen

It was suggested that it would be of interest to prepare a compound related to quercetin in which
the hetero oxygen atom is replaced by a nitrogen atom (XVII), because such a compound might possess significant antimalarial activity. Among the known antimalarial agents of this type is cincophen (XVIII).

\[ \text{(XVII)} \]

\[ \text{(XVIII)} \]

The method used was patterned after that employed by von Baeyer (3, pp. 2337-2342) in the following conversion:
The reaction with quercetin was carried out and the product obtained was analyzed by the Dumas method. Nitrogen (0.45 per cent) was found to be present. Analysis by the Kjeldahl method (without digestion) proved that this was not ammonia nitrogen but nitrogen within an organic compound. Thus, a small yield of the nitrogen containing compound was obtained.

Oxidations and Dehydrogenations

The oxidation and dehydrogenation of dihydroquercetin were investigated for two reasons. First, it was necessary to obtain quercetin for experimental work (e.g., the attempt to replace the hetero oxygen by nitrogen). Second, it was hoped that a method could be found which would be suitable for converting
dihydroquercetin derivatives (e.g., the bromo derivatives) to quercetin derivatives. Since the structures of a number of quercetin derivatives are known, this reaction would be a valuable tool for the identification of new dihydroquercetin derivatives.

It was realized that any conversion of dihydroquercetin to quercetin must be carried out under extremely mild conditions, for upon vigorous oxidation the dihydroquercetin molecule undergoes cleavage, and a variety of lower molecular weight oxidation products is formed.

At the start of this investigation only one method was known, the air oxidation of dihydroquercetin in dilute sulfuric acid (33, p. 7). Conditions were carefully established to obtain pure samples of quercetin for experimental use in this laboratory.

While this study was in progress, Dr. Kurth of Oregon State College was working on the use of sodium bisulfite for the conversion of dihydroquercetin to quercetin. The method was repeated successfully. It was decided that it would be of interest to study the mechanism of this reaction. The question had arisen as to whether or not oxygen (air) was necessary for the
oxidation of dihydroquercetin with bisulfite ion (as, indeed, it is essential for the sulfuric acid oxidation).

By experiment it was shown that identical yields of quercetin were obtained by bisulfite oxidation in the presence of air or in the presence of nitrogen (air excluded). Hence, the bisulfite oxidation is independent of oxygen, and is probably due to the reduction of bisulfite ion to some lower oxidation state.

When either of these two methods (air oxidation or bisulfite oxidation) was used to oxidize a bromodihydroquercetin derivative, severe oxidation took place and no quercetin derivative could be isolated. Therefore, one other method of conversion was attempted.

It was known that pentamethylampelopsin could be dehydrogenated by treating it with a mixture of cinnamic acid and palladium black (21, p. 270).
With considerable revision of the original method it was possible to convert dihydroquercetin to quercetin and pentamethyldihydroquercetin to pentamethylquercetin. However, the method was unsuccessful when applied to bromo derivatives of dihydroquercetin, for it was found that under the conditions of this reaction that the bromo derivatives were debrominated. Debromination of 6,8-dibromoquercetin also took place under these same conditions, indicating that the bromine atoms might have been attached to the aromatic ring.
VI. Discussion of Results of Ultraviolet Absorption Spectra

Theoretical Significance of Ultraviolet Spectra

As a result of recent developments in both the theory and techniques of ultraviolet absorption spectra, there has been an increasingly wider application of this tool to many chemical problems of structural and analytical nature. Theoretical advances have been made by application of quantum-mechanical principles in an approximate manner, particularly by Mulliken (30, p. 265) and Sklar (35, p. 689). Technical improvements have been highlighted by the development of ultraviolet sensitive photoelectric cells and by the manufacture of inexpensive, ultraviolet-transmitting, high silica glass. Thus, these new aids make available relatively simple and accurate methods of quantitatively determining the ultraviolet absorption spectra of organic compounds in solution.

The absorption spectra of molecules, as in the case of atoms, are to be attributed to transitions, upon absorption of energy in the form of radiation, from states of low energy content to those of high energy
content. In contrast to sharp-line atomic spectra, however, molecular spectra are broad, that is, spread over a relatively wide range of wave lengths. In atoms separate levels arise from the different energies associated with different distributions of electrons. In molecules, in addition to the different energies accompanying various distributions of the electrons, there is also associated with any given electronic arrangement, various possible modes of vibrations between the atoms, each with an attendant difference in energy. For each mode of vibration there is a number of possible modes of rotation also accompanied by differences in energy. Rotational energy differences are small in comparison with vibrational energies, and the latter, in turn, are of a lower order of magnitude relative to differences in energy of the electronic state. As a result a complex energy-level diagram is obtained for molecules and many transitions are possible in which the differences in energy are very close. According to the Bohr relation the frequency (\(\nu\)) of the light absorbed is directly proportional to the difference in energy, \(E_2 - E_1\), between the two states involved in the transition, i.e.

\[ h \nu = E_2 - E_1 \]
where \( h \) is Planck's constant. Consequently, with molecules, large numbers of lines in close proximity are observed. In most cases these lines are so close that they cannot be distinguished with an ordinary spectrograph and hence appear as a broad region of absorption called a band.

It is primarily in the region of 200 \( \mu \)m to 400 \( \mu \)m that simple techniques and fruitful interpretation are available. If a molecule is to absorb radiation near or above 200 \( \mu \)m, it must, in general, possess a number of possible resonance forms, the interaction of which leads to the different electronic energy states described previously. In general, the normal or ground state can be represented by resonance among nonionic structures, whereas, the high energy, excited state of the molecule is composed of contributions from ionic structures. For example, in the case of ethylene, the ground state can be represented essentially by a single nonionic form, \( \text{H}_2\text{C} = \text{CH}_2 \), while the excited states arise from resonance primarily between the ionic forms, \( \text{H}_2\text{C}^+\cdot\text{CH}_2 \) and \( \text{H}_2\text{C}^-\cdot\text{CH}_2 \). The difference in energy between the normal state and the first excited state corresponds to the energy of the radiation (180 \( \mu \)m) absorbed by ethylene and other simple olefins.
The greater the resonance between a set of structures, the lower will be the energy of the molecules in the state made up of these structures, i.e., the greater will be the stabilization of the state. Resonance is increased by increasing the number of possible configurations of a molecule. A substituent which modifies a molecule so as to increase its possible resonance forms will, in general, increase the number of configurations contributing to the excited state to a greater extent than it will increase the number contributing to the ground state. Consequently, the excited state is stabilized more than the ground state, and the difference in energy between the two is decreased. Thus, according to the Bohr relation, $h\nu = E_2 - E_1$, the radiation corresponding to a transition between these two states will be shifted to smaller frequencies or longer wave lengths, i.e., towards the visible, in comparison to the absorption of the unsubstituted molecule. A shift of a maximum towards the visible is called a bathochromic shift as contrasted with a shift away from the visible which is called a hypsochromic shift.

An example of a bathochromic shift is shown by the series of compounds listed in Table V as
calculated from the spectra reported by Hausser, Kuhn, and Seitz (14, p. 392).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Wave Length of Maximum Absorption</th>
</tr>
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<tbody>
<tr>
<td>$C_6H_5-(CH = CH)-C_6H_5$</td>
<td>326 m$\mu$</td>
</tr>
<tr>
<td>$C_6H_5-(CH = CH)_2-C_6H_5$</td>
<td>355 m$\mu$</td>
</tr>
<tr>
<td>$C_6H_5-(CH = CH)_3-C_6H_5$</td>
<td>381 m$\mu$</td>
</tr>
<tr>
<td>$C_6H_5-(CH = CH)_4-C_6H_5$</td>
<td>408 m$\mu$</td>
</tr>
</tbody>
</table>

Table V

Series of Compounds Showing a Bathochromic Shift

With increasing conjugation the number of resonance configurations contributing to the excited state is greatly increased, in comparison to the ground state, and the maxima in the absorption spectra are shifted progressively toward higher wave lengths.

In contrast to conjugated systems, if two absorbing groups are insulated from each other, the absorption curve will be approximately that given by the summation of the absorptions of the two constituents. Thus, Carr, Pickett, and Stucklen (5, p. 261) report
that 1,5 hexadiene, \( \text{CH}_2\text{C}H\text{-CH}_2\text{-CH}_2\text{-CH}\text{C}H\text{=CH}_2 \), has an absorption spectrum in the ultraviolet which is almost identical with that of pentene-1, \( \text{CH}_3\text{-CH}_2\text{-CH}_2\text{-CH}\text{C}H\text{=CH}_2 \), except that the intensity in the former case is approximately twice that in the latter.

Since the ultraviolet absorption spectra are so sensitive to the constitution of the molecule, it is often difficult to correlate absorption with particular functional groups. However, this may be done for a family of compounds by comparing the spectra of substituted and unsubstituted compounds.

Specific Purposes of Studies

The ultraviolet spectra of dihydroquercetin and of a number of flavanones and flavones related to dihydroquercetin were studied not only to make analytical data available for these substances but also to ascertain information of fundamental nature concerning the electronic character of the structures of flavanones and flavones.
Interpretation of the Absorption Curve of Dihydroquercetin

The curve showing the absorption spectrum of d-dihydroquercetin (see Curve 15) was first reported by Graham and Kurth (12, p.41l) without interpretation of the shape of the curve. Utilizing the absorption data obtained during the course of this investigation, it is now possible to present a full explanation.

There are three characteristics of this curve that must be interpreted, and they are as follows: (1) the region of high absorption that occurs at 200-210 μm, (2) the maximum at 291 μm, and (3) the "hidden" maximum at 318 μm. The term, "hidden" maximum, is used to describe a maximum which appears as an inflection point on the curve.

The region of high absorption that occurs at 200-210 μm is common to all flavanones and flavones and is changed very little with substitution of groups into these nuclei. This fact has been well established (1, p.562) and will not be discussed here.

The maximum that occurs at 291 μm and the "hidden" maximum at 318 μm can be explained through
deductions based on data appearing in the literature and on the experimental work performed in this laboratory. Skarzynski (34, p.150) reported that flavanone (XIX) has two maxima in the ultraviolet range, 253 μ and 320 μ. Addition of electron doning phenolic groups to the aromatic rings causes an increase in the number of resonance forms, as in the case of phenol,

and thus a bathochromic shift in the maxima is produced. This shift was reported by Herzog and Hillmer (17, p.206)
for benzene (200 m\(\mu\), 255 m\(\mu\)) and phenol (215 m\(\mu\), 275 m\(\mu\)). A determination of the ultraviolet absorption curves of 7-hydroxyflavanone (XX) and 7,3',4'-trihydroxyflavanone (XXI) in the present study revealed a similar bathochromic shift in the maximum that occurs at 253 m\(\mu\) in flavanone. The new maxima are 276 m\(\mu\), 311 m\(\mu\) and 278 m\(\mu\), 311 m\(\mu\), respectively. The curve of epicatechin (XXII), a compound which differs from dihydroquercetin in the absence of the 4-carbonyl group was determined and this
compound shows only one maximum (280 μ)(Graph I). The slight bathochromic shift is due to the two additional hydroxyl groups. The lack of absorption in the region of 310-320 μ is therefore due to the absence of the carbonyl group. It is concluded that absorption in the 280 μ region is due to the flavanone nucleus and that absorption due to the ketone group in dihydroquercetin should occur in the 310-320 μ area.

At this point two questions still remained to be answered about the spectrum of dihydroquercetin. What was the cause of the bathochromic shift in the 280 μ band and what was the cause of the increased absorption which hides the 318 μ carbonyl band? Since neither compounds XIX, XX, or XXI showed these effects, it became apparent that the effect must be associated with the 3-hydroxyl group, the 5-hydroxyl group, or both.
The spectrum of the pentaacetate of racemic dihydroquercetin was determined, and the curve confirmed this hypothesis by showing two distinct maxima of lowered intensity at 261 m\(\mu\) and 313 m\(\mu\).

Patterson and Hibbert (32, p.1862) reported two distinct maxima (271 m\(\mu\), 311 m\(\mu\)) for 2-hydroxy-1-(3,4-dimethoxyphenyl)-1-propanone (XXIII). Hergert

\[
\begin{align*}
\text{CH}_3\text{O} & \quad \text{H} \\
\text{CH}_3\text{O} & \quad \text{H} \\
& \quad \text{H} \\
& \quad \text{OH}
\end{align*}
\]

(XXIII)

Kurth (16) have attributed the shift in the infrared frequency of the carbonyl group in eriodictyol to chelation between the carbonyl group and the hydroxyl group located in the five position. From these data it is concluded that the bathochromic shifts in the 280 m\(\mu\) and 313 m\(\mu\) bands are due to the resonance forms shown on the following page.
These would enhance the 280 m\(\mu\) (flavanone) band and thus explain the much greater intensity and shift to 291 m\(\mu\) in the spectrum of dihydroquercetin. It should also be noted that due to the contribution of the carbonyl group in the above forms the intensity of absorption due to the carbonyl is somewhat lessened and thus is readily hidden. It is significant to note that carbonyl derivatives of dihydroquercetin have not been prepared successfully.

An Observation Concerning Acetylated Flavanones

Many interesting facts arise from the study of the pentaacetates of dihydroquercetin, quercetin, and epicatechin and the tetraacetate of eriodictyol (Graph II). The first is that the ultraviolet spectra of acetylated flavanones are the same as the parent
flavanones (i.e., \(-\text{OCOCH}_3\) replaced by \(-\text{H}\)). This observation is a successful extension of an effect noted by Wilds and his coworkers (38, p. 1985) for substituted benzophenone. The maxima of acetophenone (XXIV)

![XXIV]

(220 \(\text{m}\), 286 \(\text{m}\)) undergo a bathochromic shift upon addition of a p-hydroxyl group (235 \(\text{m}\), 323 \(\text{m}\)) or p-methoxyl group (232 \(\text{m}\), 318 \(\text{m}\)). Both of these groups are electron donors. A hypsochromic shift occurs when p-hydroxyacetophenone is converted to the acetyl derivative (XXV). The maxima produced

![XXV]

(220 \(\text{m}\), 290 \(\text{m}\)) are almost identical with unsubstituted acetophenone. It can thus be concluded that the
electron attracting power of the acyl group is approximately equal to the electron releasing power of the hydroxyl and methoxyl groups.

This same effect is observed from the data obtained in this laboratory in the flavanone family as shown in Table VI.

Table VI
Ultraviolet Maxima of Certain Flavanones

<table>
<thead>
<tr>
<th>Name</th>
<th>Maxima</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flavanone</td>
</tr>
<tr>
<td>Flavanone</td>
<td>253 μm</td>
</tr>
<tr>
<td>Dihydroquercetin pentaacetate</td>
<td>261 μm</td>
</tr>
<tr>
<td>Eriodictyol tetraacetate</td>
<td>261 μm</td>
</tr>
</tbody>
</table>

Further proof of the flavanone and the carbonyl bands is given by the single maximum of epicatechin pentaacetate (270 μm).

Observations Concerning Quercetin and Quercetin Pentaacetate

A second interesting observation is made in the case of quercetin and quercetin pentaacetate. The
Pentaacetate (XXVI) shows a broad maximum in the region of 295-305 μm. This is due to the fact that this is the region for double bonds conjugated to the benzene ring as well as for ketones alpha to the benzene ring. This is a drastic hypsochromic shift of the same band in quercetin (370-380 μm).

Hergert and Kurth (16) reported a bathochromic shift in the infrared carbonyl band upon acetylation of quercetin. They offer no explanation of this observation. This effect can not be attributed to resonance forms, for these are fewer in number in the acetylated compound. It appears that this might be the starting point for an investigation of the basic differences between infrared and ultraviolet data.
Resonance Forms of Quercetin and Its Derivatives

Deductions regarding the resonance forms present in quercetin and in methylated or acetylated derivatives of quercetin can be made from the experimental data obtained in this thesis. When the 7, 3', 4' hydroxyl groups in quercetin are methylated, only a slight hypsochromic shift occurs in the main band. Further shifts occur when the 3- and 5- hydroxyl groups are methylated (Table VII)(Graph III).

Table VII

<table>
<thead>
<tr>
<th>Name</th>
<th>Main Band</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,3',4'-Trimethylquercetin</td>
<td>374 μm</td>
</tr>
<tr>
<td>3,7,3',4'-Tetramethylquercetin</td>
<td>354 μm</td>
</tr>
<tr>
<td>3,5,7,3',4'-Pentamethylquercetin</td>
<td>340 μm</td>
</tr>
<tr>
<td>Quercetin pentaacetate</td>
<td>300 μm</td>
</tr>
</tbody>
</table>

The surprising observation is that a large shift is noted on methylation of the 3-hydroxyl group. This points to the importance of a tautomeric form suggested by Hattori (13, p.4816) and shown on page 109 (XXVII).
The fact that destruction of chelation by methylation of the 5-hydroxyl group does not cause as drastic a change as by acetylation indicates that chelation of the carbonyl by the 5-hydroxyl group is not the major contribution to this band.

In the case of flavones, the band is due mostly to the resonance form (XXVIII) shown below.
This form is ordinarily not important in flavanones, but the fact that this form is important in quercetin is further borne out by the maxima of the diacetate of 7,3',4'-trimethylquercetin (332 mµ) and the resulting maxima from the curve of the monoacetate of 3,7,3',4'-tetramethylquercetin (340 mµ).

It should be noted that the latter has exactly the same maximum as pentamethylquercetin. It is seen quite clearly that the 3-hydroxyl and 5-hydroxyl groups do not play the major role in the spectrum of quercetin. However, when the 4'-hydroxyl group is acetylated, thus blocking the previously described resonance form, a drastic hypsochromic shift occurs. (Graph IV)

The Structure of Pentamethyldihydroquercetin

From experimental data certain deductions may be drawn regarding the structure of pentamethyldihydroquercetin. It was noted that the spectrum of pentamethyldihydroquercetin is remarkably similar to that of pentamethylquercetin (Graph V). Since this involves a large bathochromic shift, it suggests that a new effect is involved in pentamethyldihydroquercetin, differing from that of unsubstituted dihydroquercetin.
The new resonating structure must account for the following facts: (1) it does not occur in dihydroquercetin; (2) it does not occur in the pentaacetate of dihydroquercetin; (3) it does not occur in 5,7,3',4'-tetramethyldihydroquercetin nor in the monoacetate derivative of this compound; and (4) it does occur in pentamethyldihydroquercetin.

The following rearranged structure (XXIX) might be suggested,

![Structure](image)

but was ultimately ruled out by chemical evidence. Pentamethyldihydroquercetin has been converted to pentamethylquercetin (this thesis) and has also been converted to the pentamethyl ether of epicatechin by Hergert (15).

It has, therefore, been concluded from the facts available at the present time that the 3-methoxyl
group must enter into the resonance or tautomeric structure which would, of course, enhance the resonance of pentamethyldihydroquercetin. A suggested "solution" to this problem which is sterically sound is given in formula XXX below and involves a no bond resonance analogous to that found in toluene.

![Chemical Structure](XXX)

This "chelation" might very well be conjugated also and thus be made stable by further no bond resonance as shown below (XXXI).

![Chemical Structure](XXXI)
From steric considerations it is not possible for the 5-methoxyl group and the carbonyl group to show this effect.

VII. Summary

Optically active dihydroquercetin, obtained by the extraction of Douglas-fir bark, was treated with numerous reagents to determine the fundamental properties of this compound.

The phenolic and alcoholic hydroxyl groups were esterified with acetic anhydride and were converted to ether groups by methylation. Substitution occurred upon nitration of pentamethyldihydroquercetin or by bromination of dihydroquercetin or the pentamethyl derivative. The lack of ketonic character of dihydroquercetin and of pentamethyldihydroquercetin was demonstrated and explained. Studies were conducted using propylene oxide and chlorosulfonic acid. An attempt was made to replace the hetero oxygen of quercetin by a nitrogen atom. Several experiments were conducted involving the oxidation and dehydrogenation of dihydroquercetin.
The ultraviolet spectra of dl-dihydroquercetin and of a number of related flavanones and flavones were determined for the first time. Certain deductions were drawn regarding the structures of dihydroquercetin, quercetin, and pentamethyldihydroquercetin.
VIII. Bibliography


10. Geissman, Theodore Albert and Harold Lischner. Flavanones and related compounds, VII. The formation of 4,6,3',4'-tetrahydroxy-2-benzylcoumaranone-3 by the sodium hydrosulfite


15. Hergert, Herbert L. Private communication.


