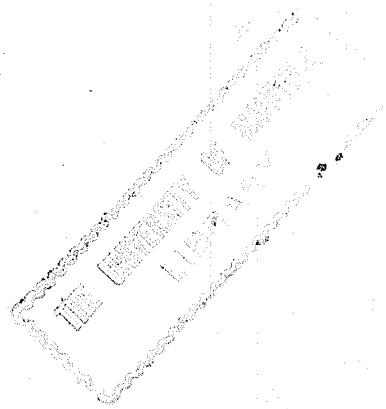
I. THE ATTEMPTED SYNTHESIS OF 6-HYDROXYMORINIDIN AND OF GOSSYPETINIDIN

II. OXIDATION PRODUCTS OF QUERCETIN AND SYNTHESIS OF HYDROXYQUERCETIN

by

Mitchell Borkowski



Being a Thesis Presented to the Committee on Post-Graduate Studies of the University of Manitoba in Partial Fulfillment of the Requirements for the Degree of Master of Science.

September, 1948

University of Manitoba

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CONTENTS

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CONTENTS (cont.)

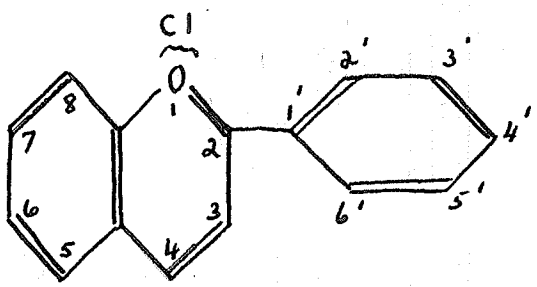
I. THE ATTEMPTED SYNTHESIS OF 6-HYDROXYMORINIDIN AND OF GOSSYPETINIDIN (8-HYDROXYCYANIDIN)

Anthocyanidins deviating from the normal types have been synthesized by Robinson and Charlesworth (9) who, by coupling antiarolaldehyde with appropriate acetophenone derivatives followed by demethylation of the products, prepared and characterized the 6-hydroxyderivatives of some of the chief anthocyanidins, namely, 6-hydroxygalanginidin chloride, 6-hydroxypelargonidin chloride, 6-hydroxycyanidin chloride and 6-hydroxydelphinidin chloride. One of the objects of the present work was to complete this series by the synthesis of the 6-hydroxyderivative of morinidin. Morinidin chloride, the anthocyanidin corresponding to the flavonol morin, and not yet identified as a naturally occuring pigment, has been prepared by Robinson's method of synthesis by condensing O-benzoylphloroglucinaldehyde with fisetol (8).

The synthesis of the anthocyanidin analagous to the flavonol gossypetin, which occurs as a glucoside in Egyptian cotton flowers, has not hitherto been effected in the laboratory. Therefore it was decided to undertake the preparation and characterization of this pyrylium pigment, gossypetinidin or 8-hydroxycyanidin.

INTRODUCTION

Anthocyanins are coloring matters which are responsible for the red, violet and blue pigmentations of flowers, fruits, leaves and stems of plants. They are glycosides and may be hydrolyzed by boiling with dilute mineral acids or, in particular instances with alkalies, to yield one or more sugar compounds and an aglycone or anthocyanidin; frequently there is also present a third constituent, an organic acid such as p-hydroxybenzoic, malonic, p-hydroxycinnamic or p-coumaric, which may esterify the hydroxyl groups of the anthocyanidin or those of the sugar component. The pioneer researches of Willstätter and his co-workers from 1913 to 1916 have shown that the numerous individual anthocyanin plant pigments contain similar nuclei. The fundamental parent substance of the entire group is the oxygen heterocyclic skeleton known as 2-phenylbenzopyrylium chloride (I) which Decker and von Fellenberg (10) formulated on the basis of the oxonium theory:



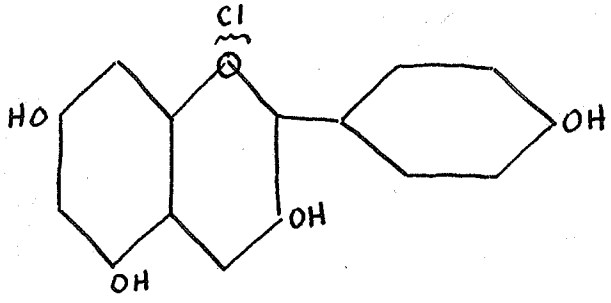
I

2-Phenylbenzopyrylium Chloride
(Flavylium Chloride)

The wide variations in color are due to slight alterations in the molecule which do not affect the basic molecular skeleton, to differences in pH of the cell sap and to the presence of co-pigments such as tannin, gallic acid, etc. which possess the ability to intensify or modify the color.

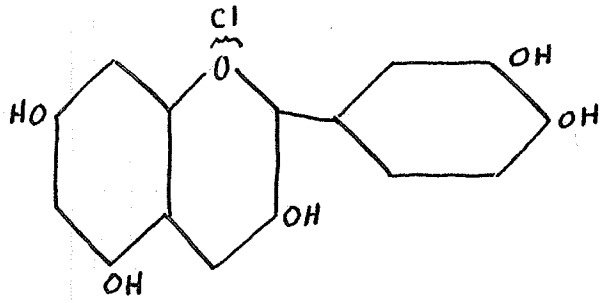
For a long time, all the anthocyanins found in nature fell into three fundamental types, pelargonidin (II), cyanidin (III) and delphinidin (IV). These all belong to the flavonol group and carry a hydroxyl group on carbon atom 3. More recently an anthocyanin gesnerin, with the aglucone gesneridin (V) (29) has been found which lacks this feature. Again, a nitrogenous anthocyanin, betanin (33), occurs in the red beet, and a similar compound appears in Bougainvillaea (27). A few other abnormal types are known.

At present, therefore, four basic types are known; trihydroxy- (gesneridin), tetrahydroxy- (pelargonidin), pentahydroxy- (cyanidin) and hexahydroxy- (delphinidin) flavylium derivatives.



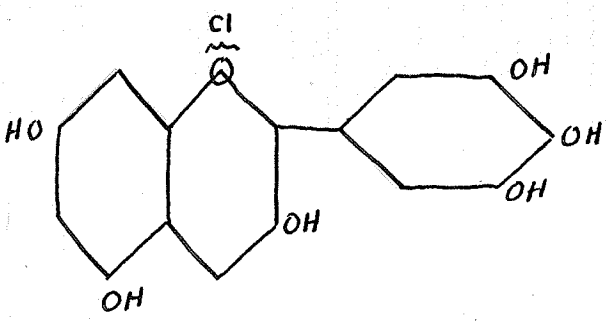
II

Pelargonidin Chloride



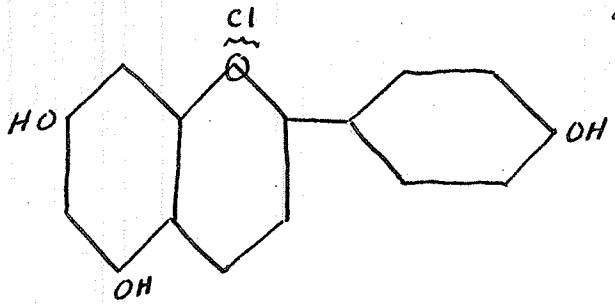
III

Cyanidin Chloride



IV

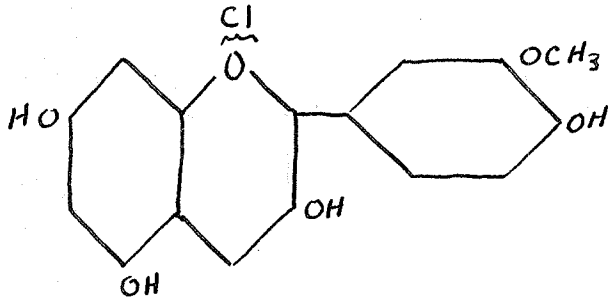
Delphinidin Chloride



V

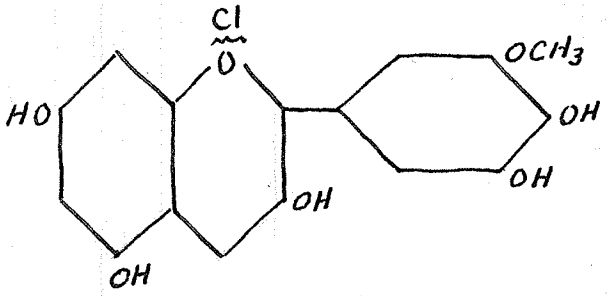
Gesneridin Chloride

Methyl ethers of these types also occur in nature. Peonidin (VI), a 3'-methyl derivative of cyanidin is present as the 3,5-diglucoside in the peony. Also, three methyl derivatives of delphinidin, petunidin (VII), malvidin (syringidin) (VIII) and hirsutidin (IX) have been found in the plant kingdom.



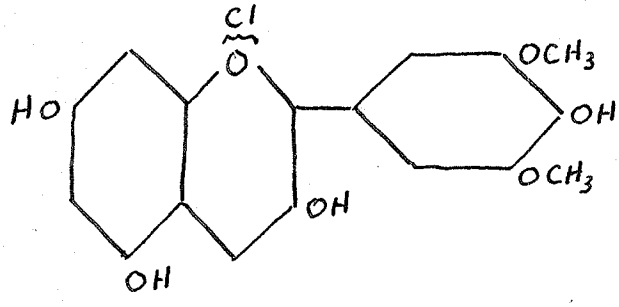
VI

Peonidin Chloride



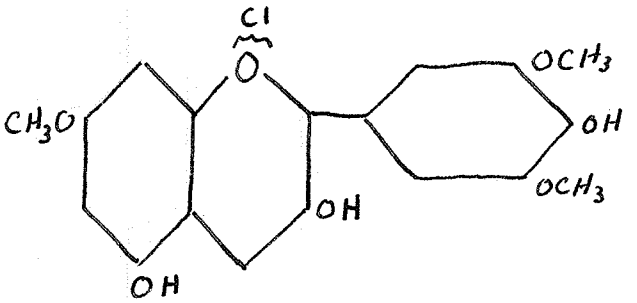
VII

Petunidin Chloride



VIII

Malvidin Chloride

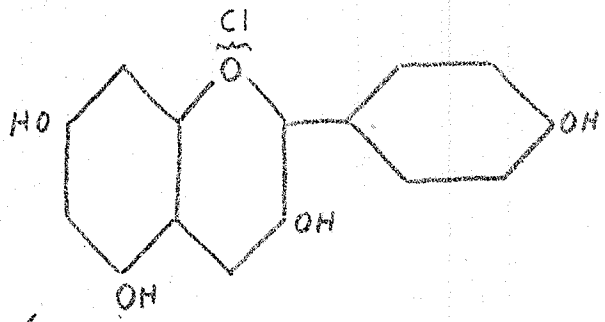


IX

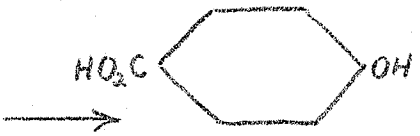
Hirsutidin Chloride

These pigments are written here as chlorides as they are usually isolated in this form, although they exist in the plant in association with plant acids. All the type groups have been synthesized by Robinson and his co-workers through methods to be discussed later which leave no doubt as to the validity of their structure.

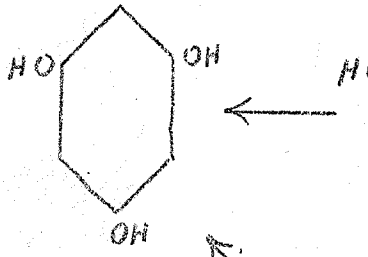
The constitutions indicated in the formulas given above were originally established by degradative methods. On fusion with alkali, phenolic and acid fission products are obtained. The degradation is illustrated below in the case of the three important parent classes, pelargonidin (II), cyanidin (III) and delphinidin (IV). The phenolic compound isolated from each of the three is the same, namely, phloroglucinol (X). The second decomposition product obtained from pelargonidin is p-hydroxybenzoic acid (XI) while cyanidin and delphinidin furnish under the same conditions protocatechuic acid (XII) and gallic acid (XIII) respectively.



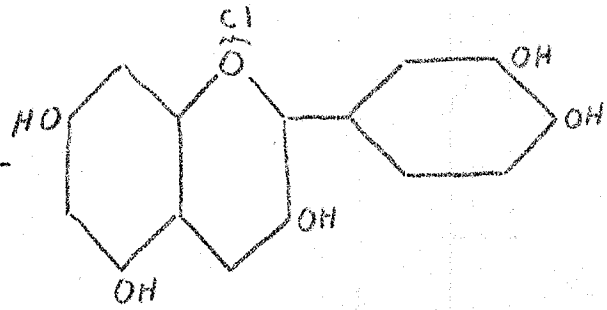
II
Pelargonidin



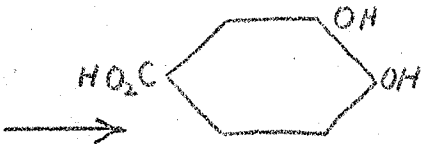
XI
p-Hydroxybenzoic Acid



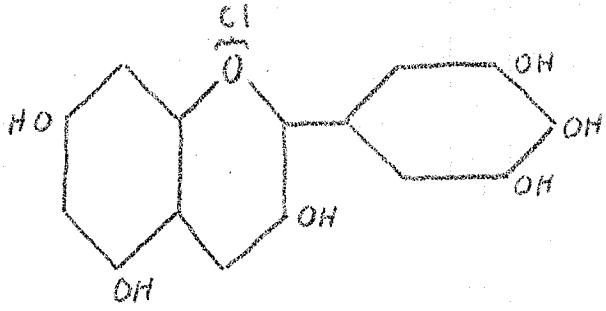
X
Phloroglucinol



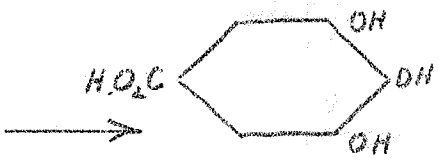
III
Cyanidin



XII
Protocatechuic Acid

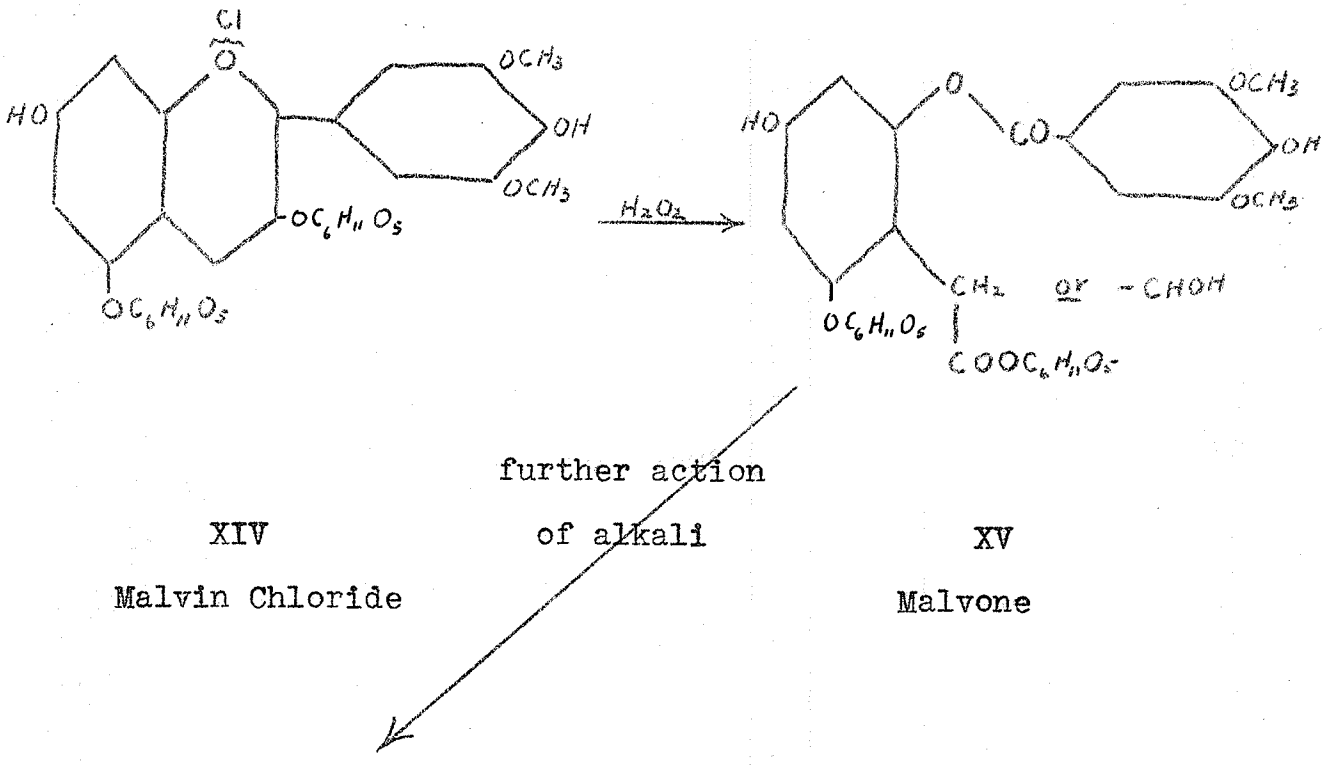


IV
Delphinidin



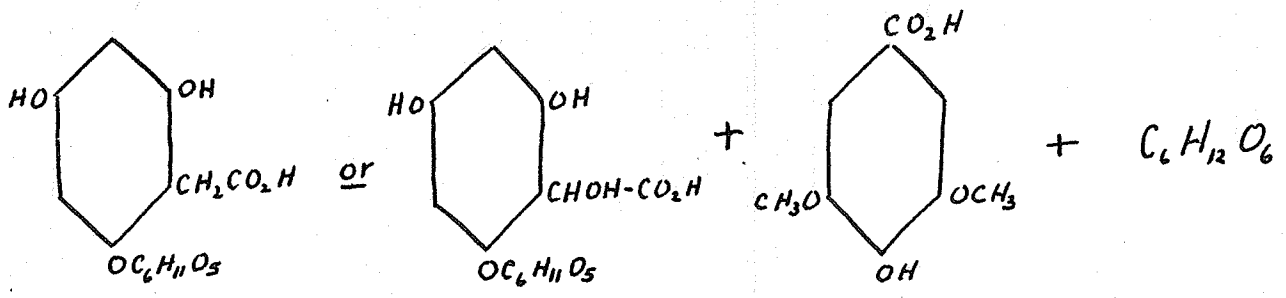
XIII
Gallic Acid

For the determination of the structure of the methylated anthocyanins, the alkali fusion is frequently too drastic, since it is accompanied by extensive demethylation. Degradation of the sugar-free pigments with dilute barium or sodium hydroxide in an atmosphere of hydrogen possesses the advantage that, while the fission is similar, the methoxyl groups are not hydrolyzed and their orientation in the anthocyanidin molecule may consequently be deduced from the structure of the methoxy acids formed. The heterocyclic ring of the anthocyanins can also be opened by oxidative degradation with 15 per cent hydrogen peroxide without removing either the sugar residue or the methoxyl groups. Subsequent hydrolysis of the resulting intermediate yields the corresponding methylated phenolic acid. This mode of attack can be illustrated with the diglucoside, malvin chloride (XIV):



XIV
Malvin Chloride

XV
Malvone



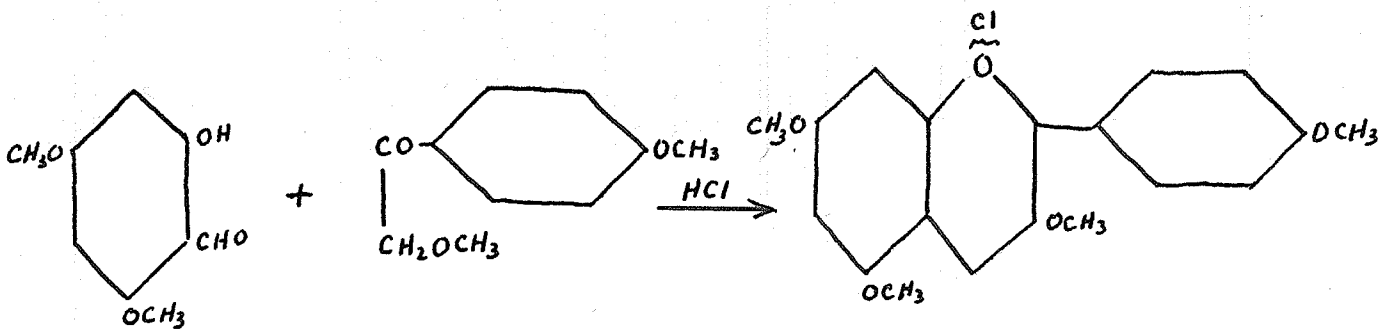
XVI XVII

Syringic Acid

The hydrolytic fragment (XVI) containing the phloroglucinol nucleus has not yet been isolated, so that it is still impossible to decide whether malvone contains the $-CH_2-$ or -CH(OH)- group.

The conclusions drawn from the above degradative evidence are completely confirmed by the synthesis of the anthocyanins themselves. The resulting synthetic specimens have been carefully compared and identified with the natural products.

The most widely used synthetic method is that of Robinson (25) which consists in the condensation of o-hydroxybenzaldehydes with appropriate acetophenone derivatives. The reaction is typified by the following scheme of the condensation between 2-hydroxy-4,6-dimethoxybenzaldehyde (XVIII) and ω,4-dimethoxyacetophenone (XIX) which, in the presence of hydrogen chloride, results in the formation of the chloride of pelargonidin tetramethyl ether (XX). Demethylation of this product yields pelargonidin.

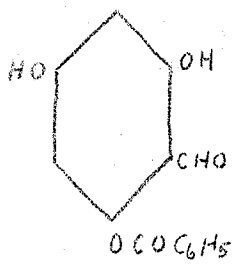


XVIII XIX XX

Chloride of Pelargonidin Tetramethyl Ether

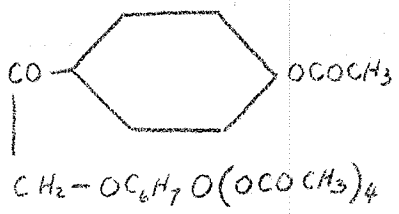
This method is not without its difficulties. The readiness with which phloroglucinalhyde condenses with itself, and the fact that polyhydroxybenzaldehydes are not easily condensed with ketones, made it necessary for Robinson to use the methylated aldehydes. The drawback of this procedure is that demethylation of the methoxy flavylium salt yields products which are difficult to purify. A search for groups that would afford the maximum protection to the hydroxyl groups combined with the minimum difficulty in their removal led to the introduction first of the acetyl derivatives and later of the benzoyl derivatives.

By this same general method anthocyanins may be obtained. Thus, starting with 2-O-benzoylphloroglucinaldehyde (XXI) and ω-(tetraacetyl-β-glucosidoxy)-4-acetoxyacetophenone (XXII), callistephin (XXIII), which occurs in summer aster and carnations, is formed (26):

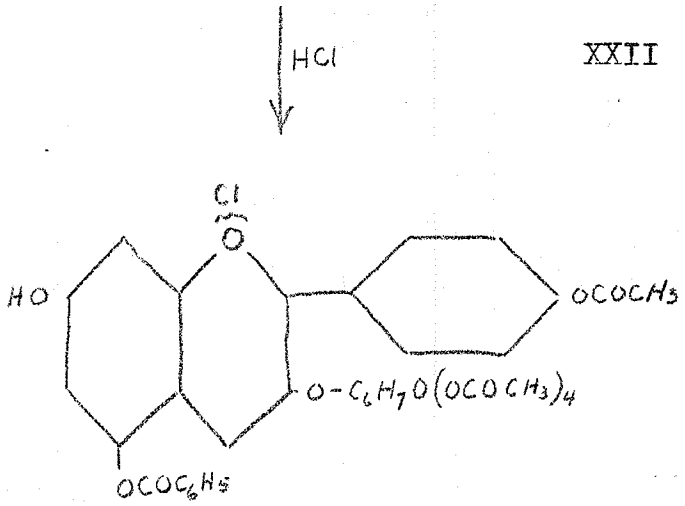


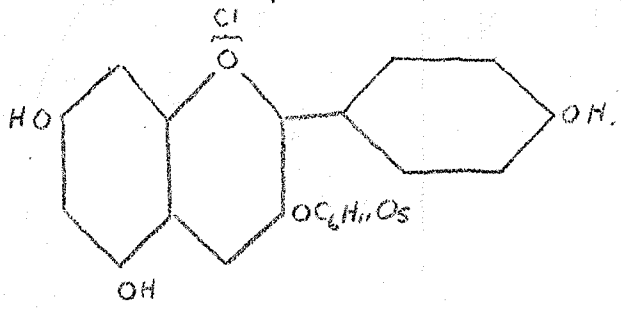
XXI

+



XXII

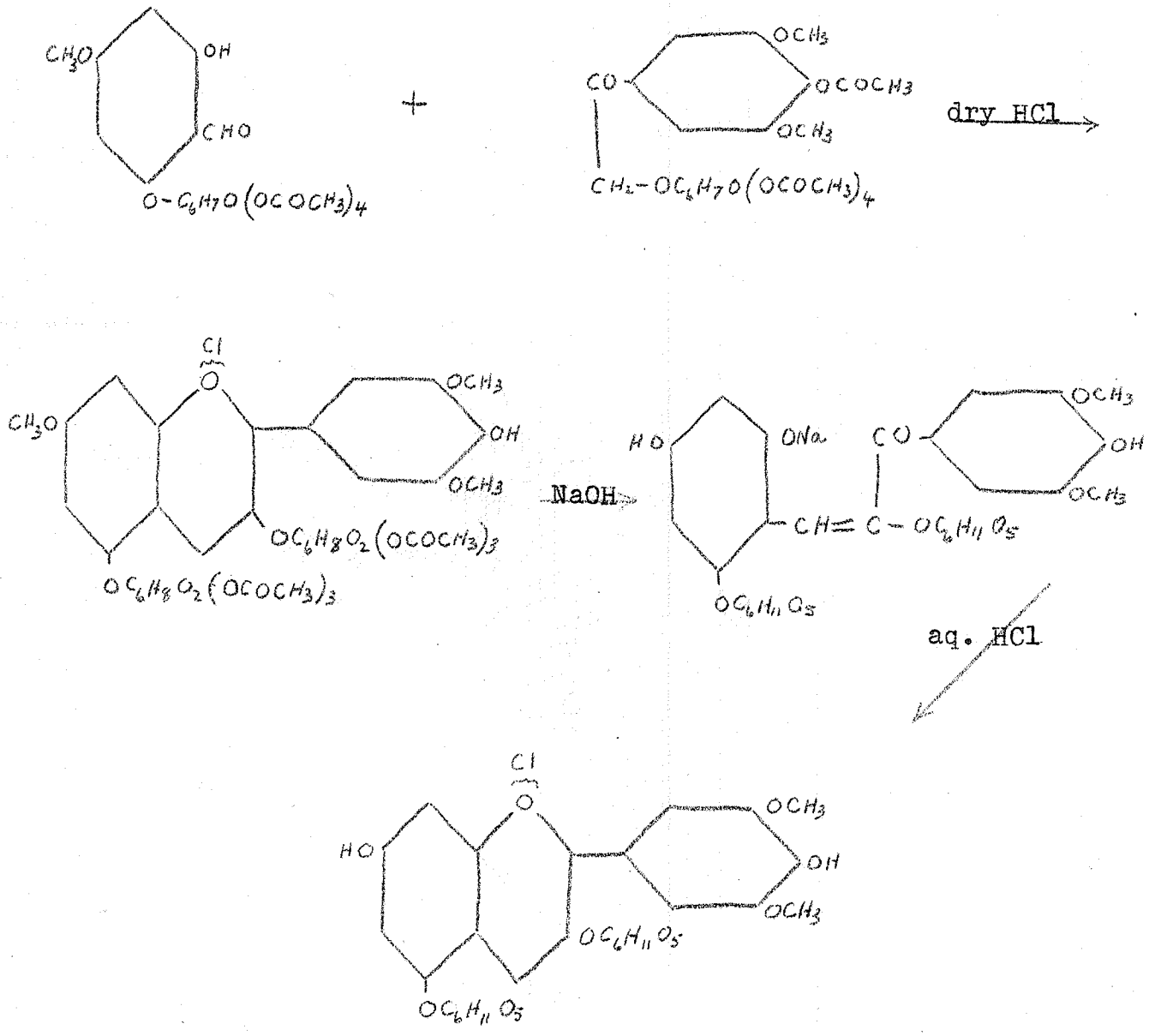




XXIII

Callistephin Chloride

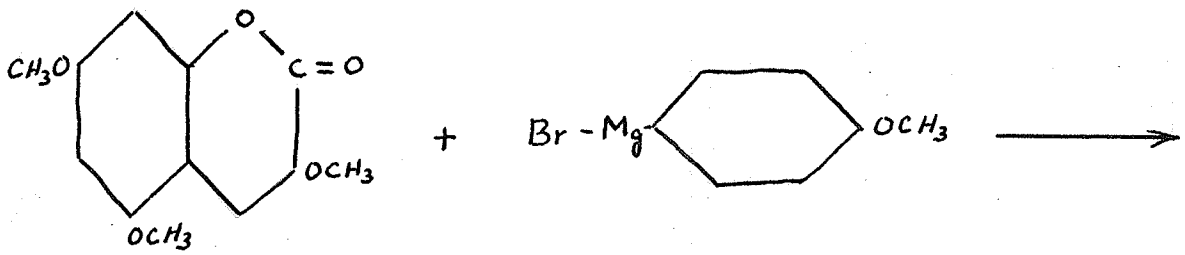
Diglycosides may be obtained in a similar manner, as is instanced by the following synthesis (30) of the 3,5-diglucoside hirsutin (XXIV):



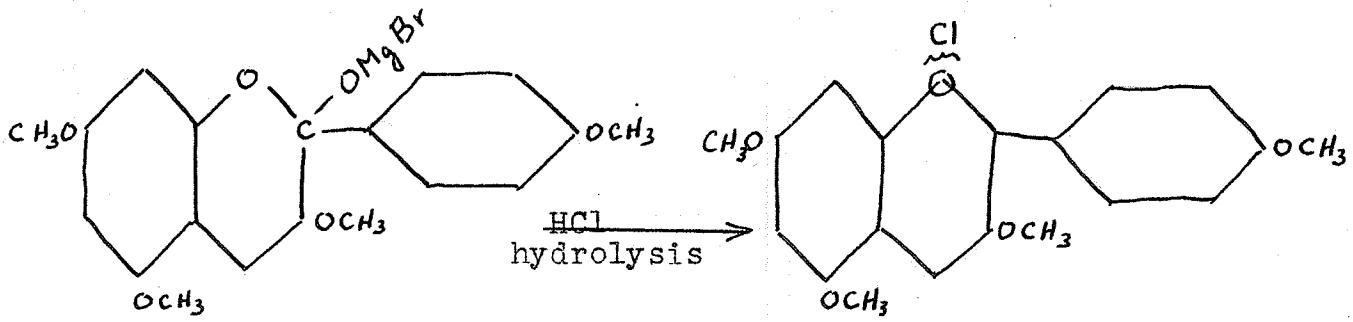
XXIV

Hirsutin Chloride

Certain anthocyanidins may be synthesized by Willstätter's method (38) which involves the interaction of methoxycoumarins with aryl Grignard reagents. Hydrolysis of the intermediate formed by the action of p-anisyl magnesium bromide (XXVI) on 3,5,7,-trimethoxycoumarin (XXV) yields the chloride of pelargonidin tetramethyl ether (XX):

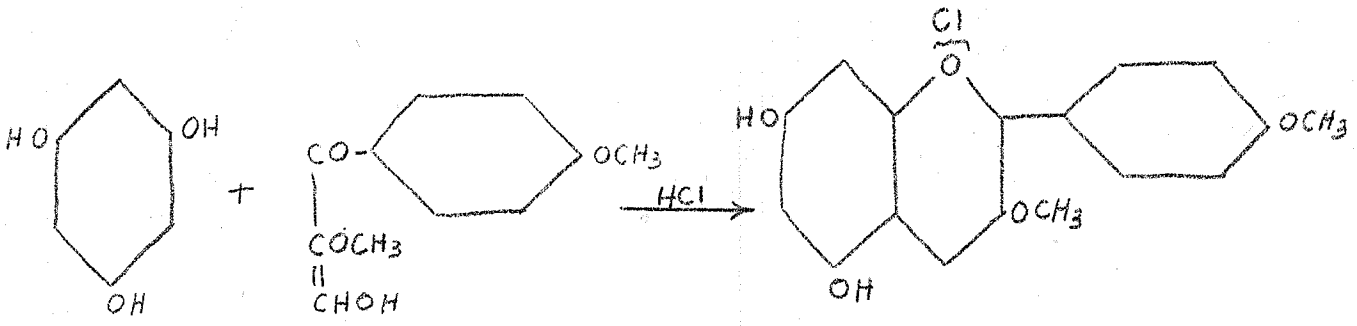


XXV XXVI



XX

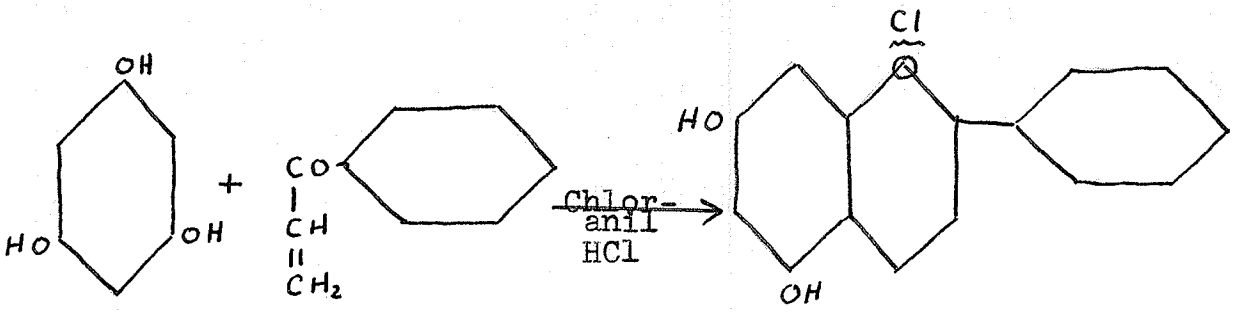
An older synthesis introduced by Bulow and his collaborators (6) utilizes the condensation of β-diketones or hydroxymethylene ketones with reactive phenols by means of hydrogen chloride. As an illustration of this method, the condensation of phloroglucinol (X) and anisyl α-methoxy-β-hydroxyvinyl ketone (XXVII) leads to the formation of 5,7-dihydroxy-3,4'-dimethoxyflavylium chloride (XXVIII):



X XXVII XXVIII

5,7-dihydroxy-3,4'-dimethoxy-flavylium chloride

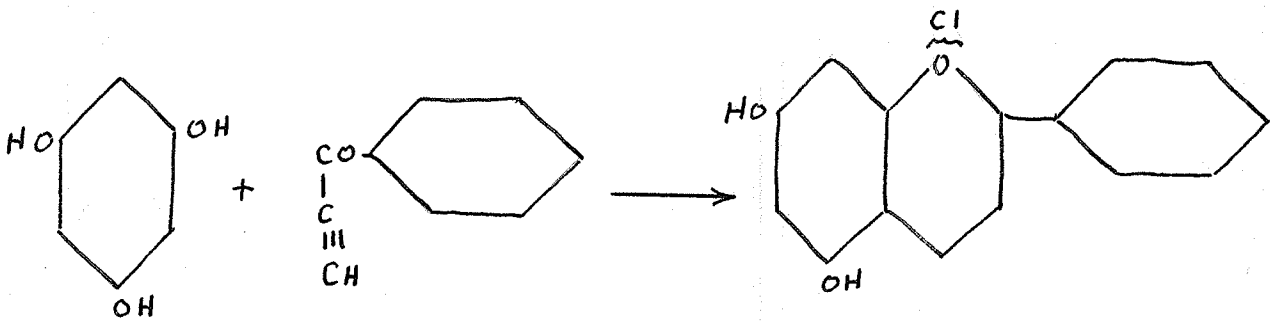
Another synthesis which may be regarded as an extension of Bulow's method has been described by Robinson and Walker (31). It involves the condensation of a reactive phenol with an α,β-ethylenic aldehyde or ketone in acid solution in the presence of an oxidizing agent. The particular agent employed was chloranil. Its application to the preparation of chrysinidin (XXX) (a pyrylium salt corresponding to the anthoxanthidin chrysin, which has not yet been found in nature) from phloroglucinol (X) and phenyl vinyl ketone (XXIX) is shown below.



X XXIX XXX

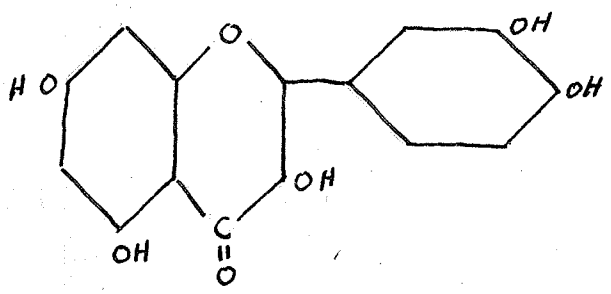
Chrysinidin Chloride

Recently flavylium salts have been synthesized by Johnson and Melhuish (14) employing a new reaction which consists of the interaction of phenols with α/β-ethynyl ketones in the presence of mineral acids. The general nature of the reaction is illustrated by the condensation of phenyl ethynyl ketone (XXXI) and phloroglucinol (X) to yield chrysinidin chloride (XXX):



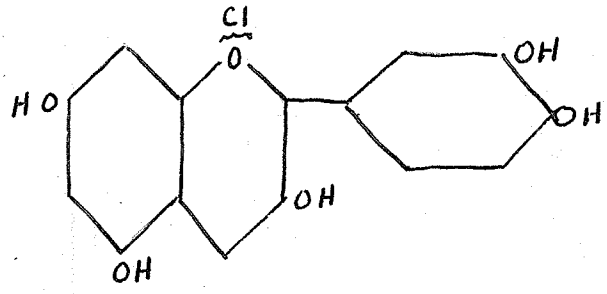
X XXXI XXX

From the standpoint of the degree of oxidation the anthocyanidins represent a class of substances intermediate between the flavonols and the catechins. This relationship may be appreciated from a comparison of the following structural formulas of the anthocyanidin cyanidin (XXXIII), the flavonol quercetin (XXXII) and d,l-epicatechin (XXXIV):



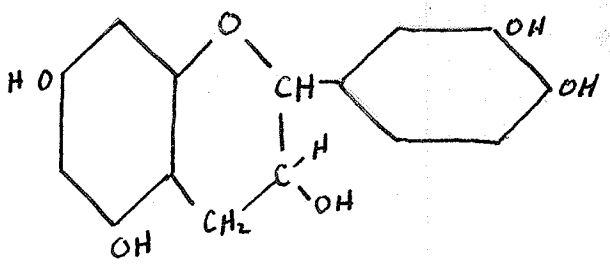
XXXII

Quercetin



XXXIII

Cyanidin



XXXIV

d,l-Epicatechin

This relationship has been proved by the conversion of quercetin to cyanidin as well as other flavone derivatives into anthocyanidins by chemical or electrolytic reduction (4). The reduction of cyanidin to d,l-epicatechin has been realized by Freudenberg and his co-workers (12). The reverse conversion of flavylium salts into flavonols has also been effected in the laboratory (15).

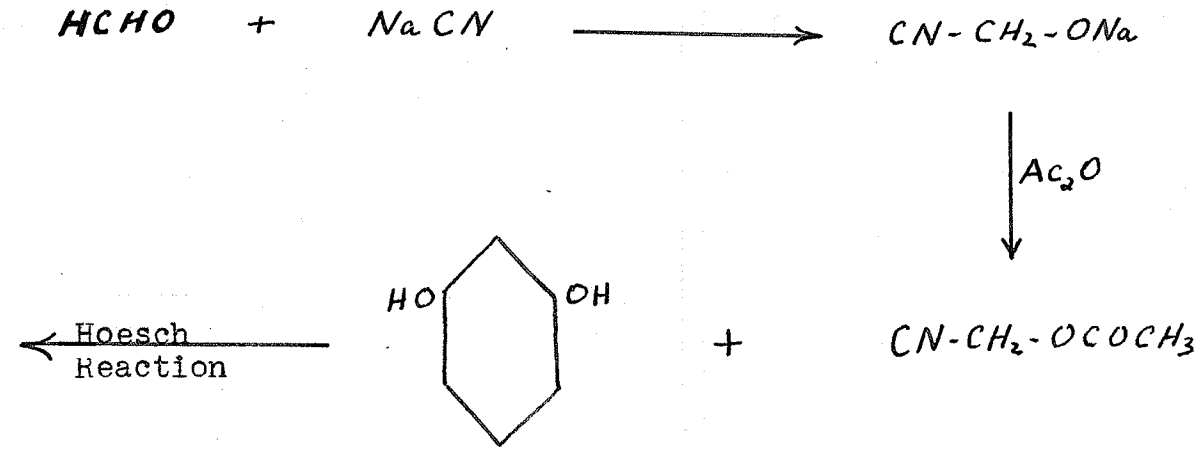
The wide distribution of flavone dervatives in the plant kingdom and the successful conversion of flavonols to anthocyanidins led to the view that similar reactions occur in the plant and that the flavones are the precursors of the anthocyanins. Investigations on this point have in fact shown that the maximum formation of flavones is always observed before the occurrence of the anthocyanins, and that a rise in the content of the anthocyanin is always accompanied by a corresponding decrease in flavone, their sum remaining practically constant.

It is now believed that anthocyanins are formed in plants in at least two different ways, through reduction of flavones or flavonols by ultraviolet light (4) or through hydrolysis of what are called leuco-anthocyanins (28). The latter reaction is often, but apparently not necessarily, accompanied by oxidation. There may be other ways of developing anthocyanins in plants, but this has not yet been proved definitely for any anthocyanin in any leaf, flowers or fruit. Whether the synthesis of anthocyanins in the plant involves the reduction of flavone derivatives or the decomposition of leuco-anthocyanins with or without accompanying oxidation, the reaction is undoubtedly an enzyme reaction because no ordinary reducing agent is known that the plant could use which would transform a flavone to an anthocyanin, and because it seems unlikely that there could be sufficient acidity to effect the hydrolysis of a leuco-anthocyanin without the presence of an enzyme.

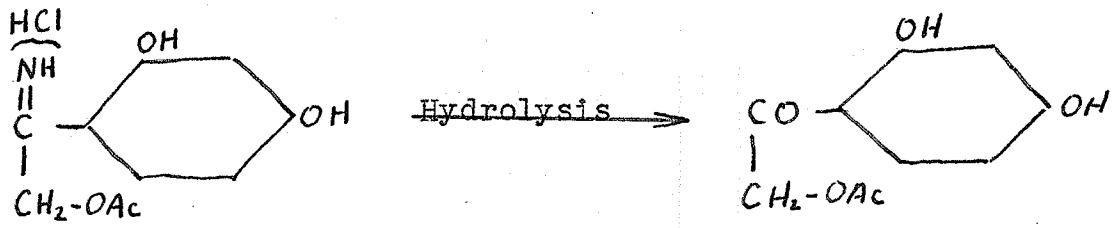
Although present knowledge is not sufficiently adequate to reveal positively the mode of formation of anthocyanins in plants, it seems probable that if anthocyanidins exist in nature which have not yet been isolated they will be found to correspond to known flavonols or derivatives of these, and some will be identified with the flavylium salts 6-hydroxymorinidin and gossypetinidin whose attempted synthesis is described in this report.

DISCUSSION

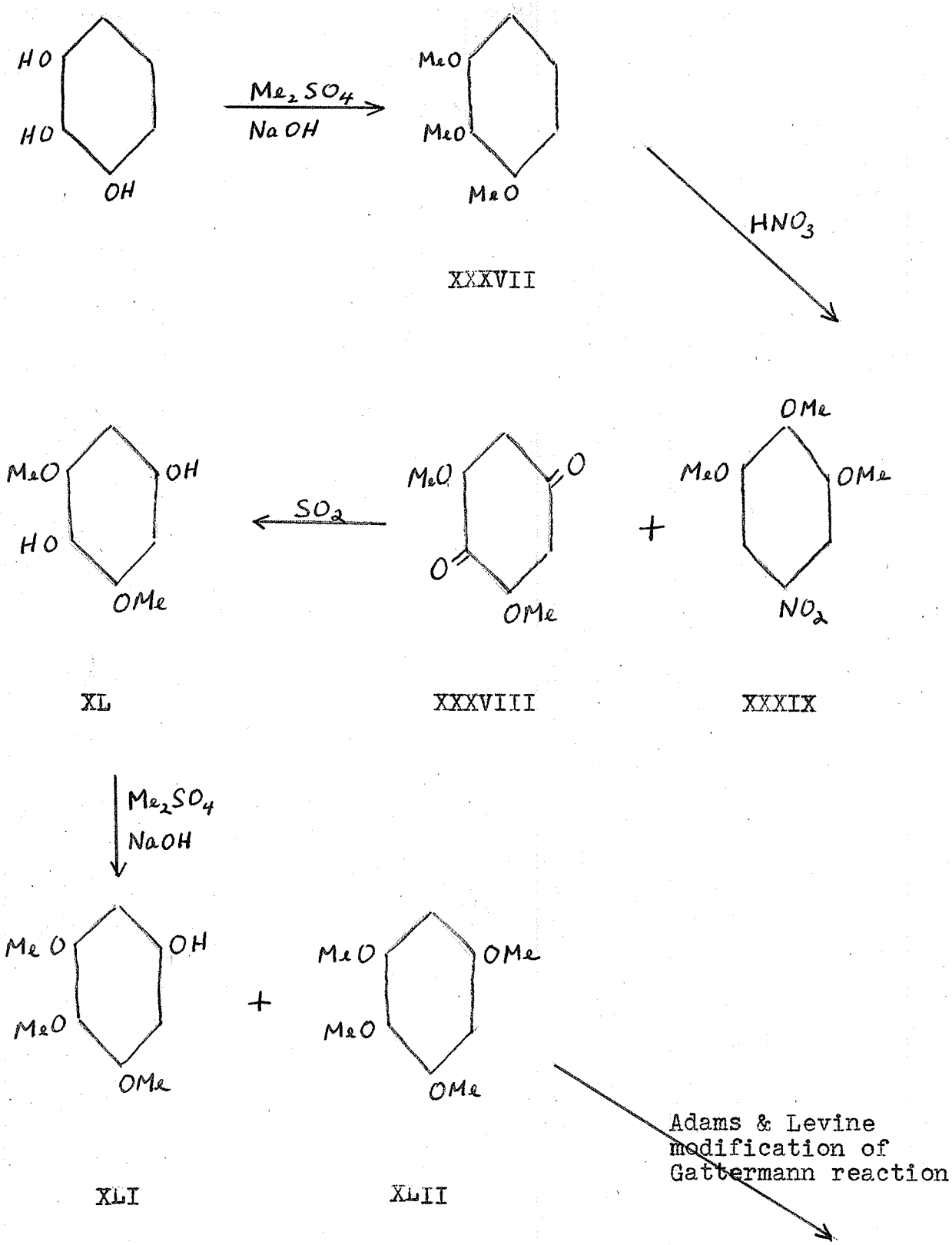
The scheme illustrated below was followed in the attempted synthesis of 6-hydroxymorinidin (XLV). Antiarolaldehyde (XLIII), synthesized from pyrogallol by the series of reactions indicated, was condensed with ω-acetoxy-2,4-dihydroxy-acetophenone (XXXVI) in ethyl acetate solution with the help of hydrogen chloride. The acetophenone derivative was prepared by the application of the Hoesch reaction using acetoxyacetonitrile (XXXV) and resorcinol. The trimethyl ether (XLIV) on demethylation with hydriodic acid in the usual manner should give the chloride of 6-hydroxy-morinidin.

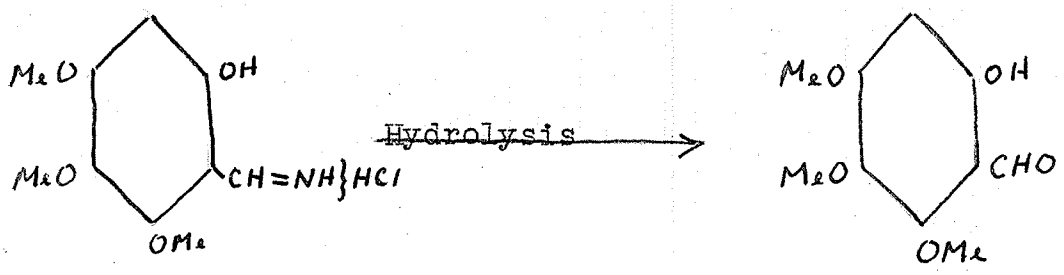


XXXV

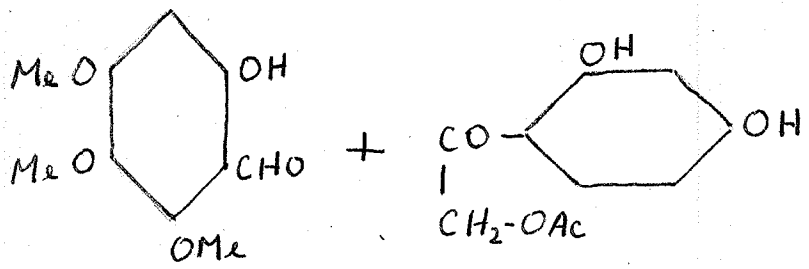


XXXVI

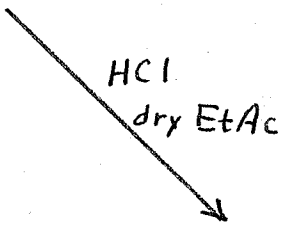

HO
HO
OH
Me2SO4
NaOH
MeO
MeO
MeO
HNO3
XXXVII
MeO
OH
HO
OMe
XL
SO2
MeO
O
O
OMe
XXXVIII
+
OMe
MeO
OMe
NO2
XXXIX
Me2SO4
NaOH
MeO
OH
MeO
OMe
XLI
+
MeO
OMe
MeO
OMe
XLII
Adams & Levine
modification of
Gattermann reaction

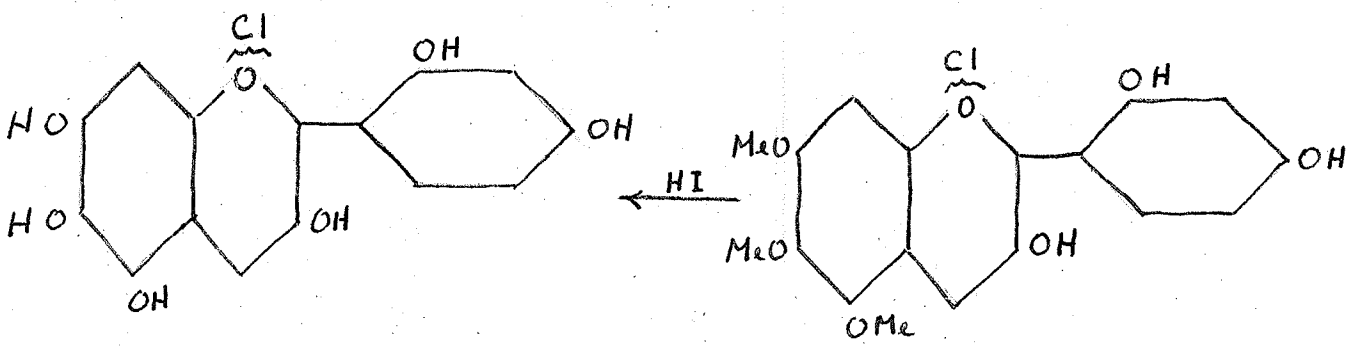


XLIII



XLIII XXXVI

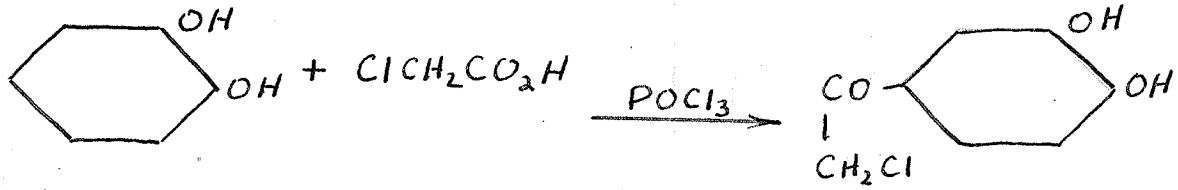




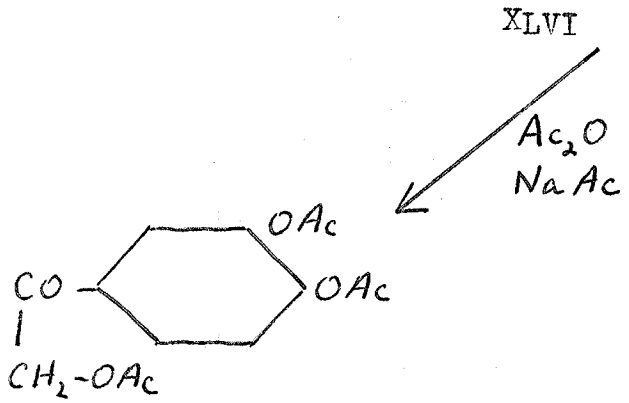
XLV XLIV

6-Hydroxymorinidin

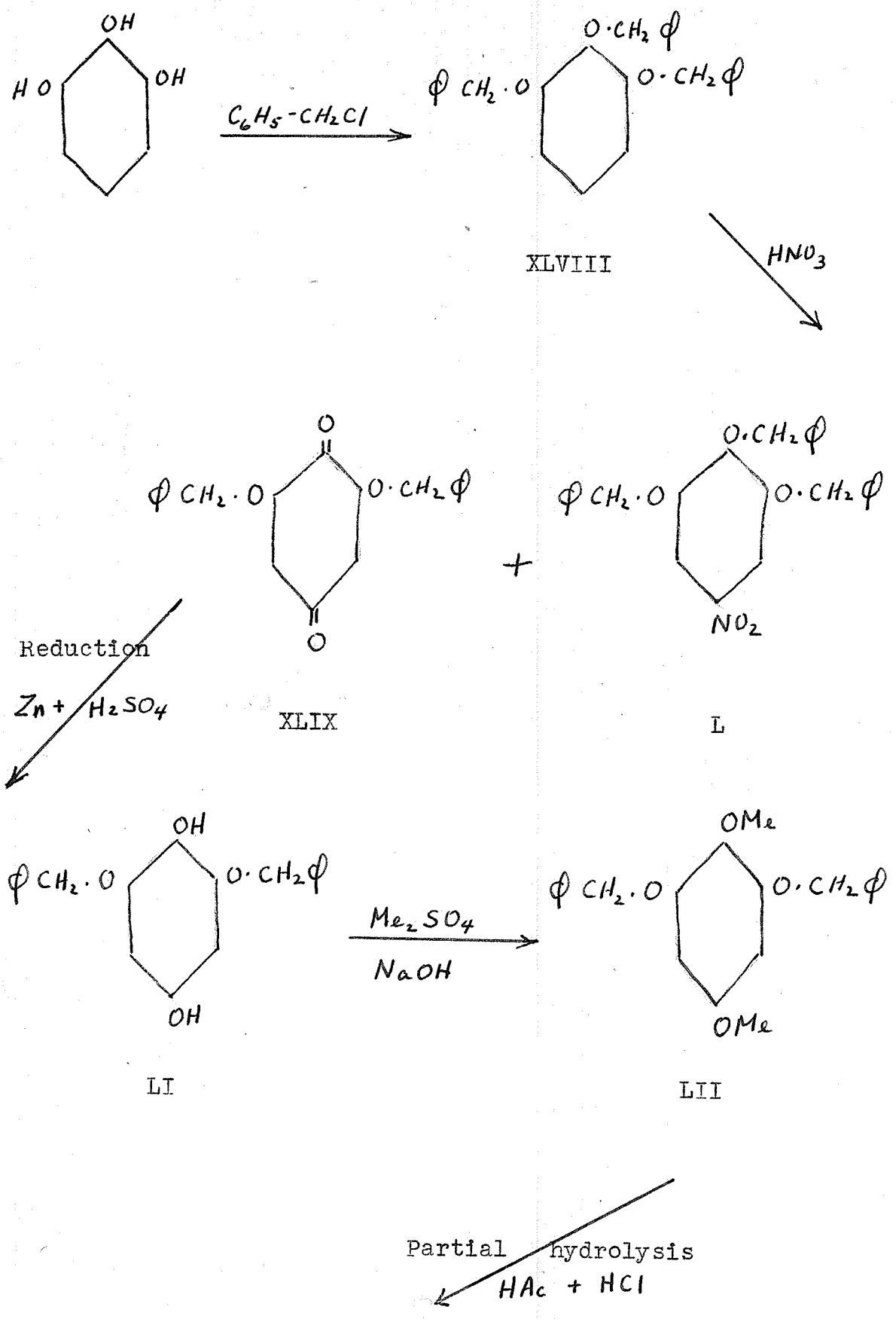
The method proposed for the synthesis of gossypetinidin or 8-hydroxycyanidin (LVI) is similar to that suggested for the preparation of 6-hydroxymorinidin. 2,4-Dihydroxy-3,6-dimethoxybenzaldehyde (LIV) was prepared from pyrogallol through the stages shown schematically below. ω-Chloro-3,4-dihydroxyacetophenone (XLVI), formed by the interaction of catechol and chloracetic acid, was converted to ω,3,4-triacetoxyacetophenone (XLVII). Demethylation of the product obtained from the condensation of this acetophenone derivative with 2,4-dihydroxy-3,6-dimethoxybenzaldehyde should produce the desired compound, gossypetinidin chloride (LVI).

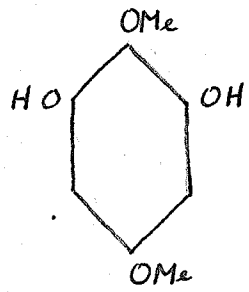


XLVI



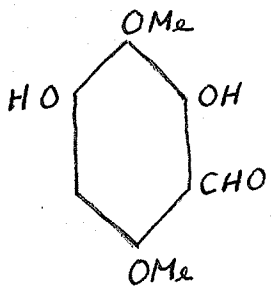
XLVII


OH
HO
OH
$C_6H_5-CH_2Cl$
$\phi CH_2 \cdot O$
$O \cdot CH_2 \phi$
$O \cdot CH_2 \phi$
XLVIII
HNO_3
O
$\phi CH_2 \cdot O$
$O \cdot CH_2 \phi$
O
XLIX
+
$O \cdot CH_2 \phi$
$\phi CH_2 \cdot O$
$O \cdot CH_2 \phi$
NO_2
L
Reduction
$Zn + H_2SO_4$
OH
$\phi CH_2 \cdot O$
$O \cdot CH_2 \phi$
OH
LI
Me_2SO_4
$NaOH$
OMe
$\phi CH_2 \cdot O$
$O \cdot CH_2 \phi$
OMe
LII
Partial hydrolysis
$HAc + HCl$

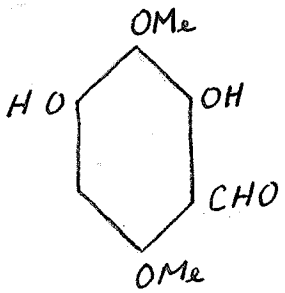


LIII

Adams and Levine modification of the Gattermann reaction

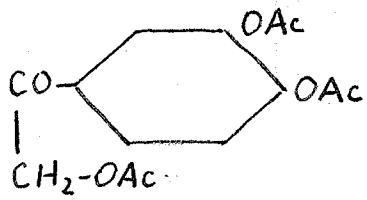


LIV



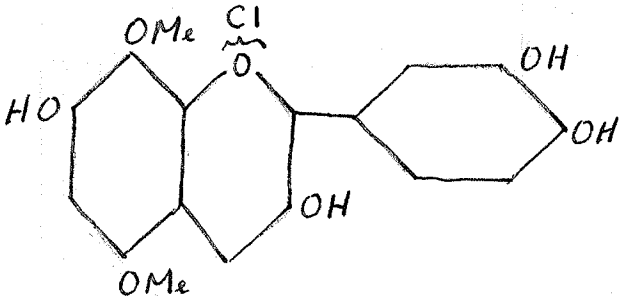
LIV

+



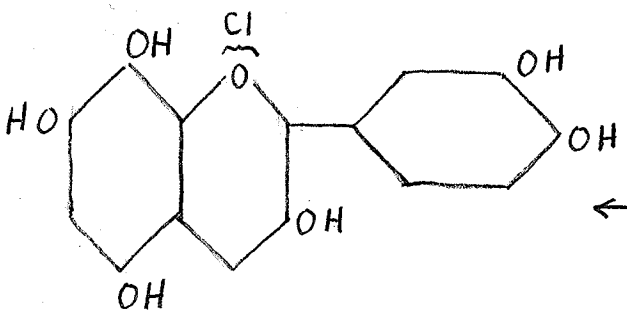
XLVIII

HCl
dry EtAc



LV

HI



LVI

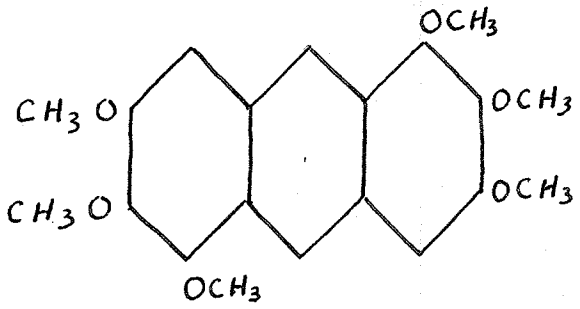
Gossypetinidin

Antiarolaldehyde (XLIII) has been previously synthesized from antiarol (XLI) by Chapman, Perkin and Robinson (7) who employed the Gattermann synthesis of hydroxy aldehydes to effect this conversion. To avoid the use of anhydrous hydrogen cyanide this compound was obtained in good yields by using the modification of this general reaction introduced by Adams and Levine (1). Antiarol was treated in anhydrous ether with zinc cyanide and then dry hydrogen chloride as described in the experimental section. By this procedure, anhydrous hydrogen cyanide was formed in the reaction mixture and condensed with the hydrogen chloride and phenol to give an aldimine hydrochloride which was hydrolyzed to the required aldehyde. The zinc chloride which was produced at the same time acted as an effective condensing agent.

2,4-Dihydroxy-3,6-dimethoxybenzaldehyde (LIV), which has hitherto not been reported in the literature, was also prepared by this method. The 2,5-dimethoxyresorcinol used as the starting material according to Baker, Nodzu and Robinson (3) has two molecules of water of crystallization associated with it. It is possible that the yield might be improved somewhat by employing the anhydrous phenol which they report melts at 86-88°.

Attempts to obtain the condensation product of antiarolaldehyde (XLIII) with ω-acetoxy-2,4-dihydroxyacetophenone (XXXVI) in a crystalline condition were unsuccessful. Although the color reactions of this substance with aqueous sodium carbonate and

sodium hydroxide would seem to indicate that it is a flavylium salt, its behavior towards ether is peculiar in that contrary to experience with other anthocyanidins this material is not readily precipitated with ether but appears to be somewhat soluble in this reagent. The results obtained on analysis of the crystalline picrate produced by treating the condensation product with picric acid do not agree with the values required by the formula 3,2,'4'-trihydroxy-5,6,7-trimethoxyflavylium picrate which is the expected product. Evidently the principal reaction did not proceed in the suggested direction but it is difficult to suggest an alternative view of its nature. As a result of the condensation of two molecules of antiarolaldehyde it is possible that a derivative of anthracene with the formula (LVII) was produced:



LVII

The picrate complex of the substance with this formulation ($C_{20}H_{22}O_6 \cdot CH_2(NO_2)_3OH$) would have the composition: C, 53.16%; H, 4.29%; N, 7.14% which is in better agreement with the analysis found experimentally.

Instead of using ω-acetoxy-2,4-dihydroxyacetophenone it is possible that the condensation of an acetoxy or methoxy derivative of this compound might yield the desired flavylium salt. The condensation may also be assisted by a change of solvent. By employing a variety of solvents such as glacial acetic acid, ether, formic acid and ethyl formate the reaction may be induced to proceed in the normal manner. Time was not available for building up a further supply of antiarolaldehyde and the acetophenone derivative necessary for these trials.

Lack of time did not permit the synthesis of gossypetinidin to be completed. ω,3,4-Triacetoxyacetophenone (XLVII) and 2,4-dihydroxy-3,6-dimethoxybenzaldehyde (LIV) have been prepared as described in the experimental section but the condensation of these two compounds has not been attempted. The preparation of 2,4-dihydroxy-3,6-dimethoxybenzaldehyde was the critical step in this synthesis since past experience has shown that the synthesis of hydroxy aldehydes of this type by means of the Gattermann reaction is not always successful. This step having been successfully accomplished, it is likely that the condensation would proceed normally. This statement may seem unusual in view of the fact that the condensation between antiarolaldehyde and ω-acetoxy-2,4-dihydroxyacetophenone did not yield the expected product. However, it is felt that the failure of this reaction to go smoothly is associated with the 2'-hydroxyl group since similar condensations to yield derivatives of morinidin have been reported to proceed with difficulty.

EXPERIMENTAL

SYNTHESIS OF 6-HYDROXYMORINIDIN (XLV)

Acetoxyacetonitrile (XXXV)

This substance was prepared by the method of J.J. Chavan (unpublished material). Sodium cyanide (84 g.) was dissolved in water (200 cc.) in a 2-litre round bottom flask equipped with a reflux condenser, thermometer, dropping funnel and a mechanical stirrer. Formaldehyde solution (120 cc., 37-41%) was added in small amounts during the course of about one hour, the temperature of the flask being cooled to below 15° by means of an ice bath. After standing a short time a solution of acetic anhydride (formed by distilling 270 g. acetic anhydride into 400 cc. dry ether) was added gradually to the mixture, whose temperature rises to a maximum of about 45°. To get as intimate as possible a mixture between the two layers it was agitated by means of a stirrer. After about an hour the addition is complete and the ether solution was separated after the addition of a little salt, the aqueous layer extracted with fresh portions of ether, and the combined extracts dried over anhydrous sodium sulfate. The ether was distilled off on a water bath and, on fractionation of the residue, the portion (50 g.) boiling between 174-177° at ordinary pressure was collected.

ω-Acetoxy-2,4-dihydroxyacetophenone (XXXVI)

The method described by Charlesworth, Chavan and Robinson was employed (8). The Hoesch synthesis proceeds in the normal manner with resorcinol and acetoxyacetonitrile, the acetyl group not being hydrolyzed under these conditions. A mixture of resorcinol (33.6 g., dried at 100°), acetoxyacetonitrile (30 g.) and dry ether (250 cc.) was saturated at 0° with hydrogen chloride. Separation of the pink colored ketimine hydrochloride commenced after about two hours. The salt was freed from hydrogen chloride by keeping in a vacuum desiccator over potassium hydroxide, and then heated on the water bath with 600 cc. of water until all passed into solution. Immediately following the disappearance of the solid, the hydrolyzed product began to separate as an oil which rapidly changed to a crystalline solid. The product was recrystallized from hot water to yield 30 g., m.p. 166-167°.

Pyrogallol Trimethyl Ether (XXXVII)

This ether was obtained in good yields by the methylation of pyrogallol (7). Aqueous sodium hydroxide (150 cc. containing 60 g. NaOH) was added slowly over a period of three hours to a mixture of pyrogallol (42 g.), methyl sulfate (140 cc.) and alcohol (100 cc.) agitated by means of a gas-tight stirrer and contained in a flask from which air was excluded by propane. During the addition the flask was kept cool by standing in an ice bath. After the completion of the reaction the addition of water (150 cc.) caused the separation of the ether (49 g.) which was crystallized from dilute alcohol.

2,6-Dimethoxybenzoquinone (XXXVIII)

This quinone is formed by the action of nitric acid on pyrogallol trimethyl ether along with 5-nitropyrogallol trimethyl ether (XXXIX) (13). Nitric acid (200 cc., sp.gr. 1.2) was added to a solution of pyrogallol trimethyl ether (40 g.) in alcohol (200 cc.) contained in a 1-litre round bottom flask equipped with a reflux condenser. On warming, a vigorous reaction began, followed by the separation of the quinone (33 g.) which was filtered off after cooling to room temperature, the nitro-derivative remaining in the alcoholic mother liquor.

2,6-Dimethoxyquinol (XL)

This compound was produced by the reduction of 2,6-dimethoxy-benzoquinone with sulfur dioxide (36). 75 g. of the quinone and 525 cc. of water were heated on the steam bath and sulfur dioxide gas was passed in till all the quinone dissolved and the color of the solution turned from dark to pale brown. After cooling, the colorless crystals of the quinol (65 g.) were immediately filtered off, washed with SO_2 water and then recrystallized from water.

Antiarol (XLI)

The semi-methylation of 2,6-dimethoxyquinol to yield antiarol together with 1,2,3,5-tetramethoxybenzene (XLII) was carried out according to the conditions given by Chapman, Perkin and Robinson (7). Asolution of sodium hydroxide (20 g. NaOH in 140 cc. water) was added to 2,6-dimethoxyquinol (34 g.) in a 1-litre round bottom

flask fitted with a dropping funnel, a mechanical stirrer with a mercury seal and inlet and outlet tubes for propane which was used to exclude air. Methyl sulfate (32 g.) was then introduced and the mixture stirred for an hour. The flask was cooled in ice and the solid material which had separated was filtered off and recrystallized from alcohol by the addition of water. This substance was found to be identical with 5-nitropyrogallol trimethyl ether (XXXIX) produced originally by the action of nitric acid on pyrogallol trimethyl ether and isolated at this stage due to incomplete separation from the quinone. No 1,2,3,5-tetramethoxy-benzene was isolated as described by Chapman, Perkin and Robinson, any which formed probably being lost during the recrystallization of the nitro-derivative. Acidification of the alkaline filtrate with HCl caused the separation of antiarol (15 g.) which was recrystallized from ten times its weight of water.

Antiarolaldehyde (XLIII)

To avoid the use of anhydrous hydrogen cyanide the Adams and Levine modification of the Gattermann synthesis of hydroxy-aldehydes (1) was employed in the conversion of antiarol to antiarolaldehyde (compare Chapman, Perkin and Robinson's method, (7)). The preparation of the zinc cyanide required for the reaction according to the directions given by Adams and Levine invariably yielded a sticky mass which was difficult to filter even though an excess of zinc chloride over sodium cyanide was used. A suitable product was obtained by using 95% instead of 50% ethyl

alcohol for the solution of the zinc chloride and allowing the precipitate of zinc cyanide to stand for a while before filtering. Also it was found necessary to use double the quantity of magnesium chloride suggested by Adams and Levine in order to precipitate the impurities of sodium hydroxide and sodium carbonate from the sodium cyanide.

Antiarol (4.5 g.) in anhydrous ether (225 cc.) was placed in a wide-mouth bottle fitted with a stopper holding a mechanical stirrer with a mercury seal, a reflux condenser and a wide-mouth inlet tube extending nearly to the bottom of the bottle. To this inlet tube was attached a safety bottle and a sulfuric acid bottle leading to a generator producing hydrogen chloride. To the top of the condenser was connected a tube leading into a wash bottle containing sulfuric acid from which a tube lead to a safety bottle and then to the surface of a sodium hydroxide bottle. Zinc cyanide (4.5 g.) was introduced, the stirrer started, and dry hydrogen chloride passed in rapidly. After about ten minutes the ethereal solution turned yellow-green in color and the yellow powdery aldimine hydrochloride commenced to deposit. At the end of about three hours the ether was decanted from the solid material and the aldimine hydrochloride decomposed by heating on the steam bath with water (25 cc.) for a few minutes. The aldehyde, which separated as an oil and crystallized on cooling (4.5 g.), was recrystallized by the addition of water to its solution in hot methyl alcohol.

Condensation of Antiarolaldehyde with ω-Acetoxy-2,4-dihydroxyacetophenone

A moderate stream of dry hydrogen chloride was passed through an ice-cold solution of (a) antiarolaldehyde (4.7 g.) and ω-acetoxy-2,4-dihydroxyacetophenone (4.7 g.) in ethyl acetate (130 cc. dried over calcium chloride and phosphorus pentoxide) and absolute ethyl alcohol (10 cc.), (b) antiarolaldehyde (4.6 g.) and ω-acetoxy-2,4-dihydroxyacetophenone (4.6 g.) in ethyl acetate (110 cc.) and glacial acetic acid (5 cc.). The outlet for the hydrogen chloride gas was protected from atmospheric moisture by a calcium chloride tube. A change in color of the liquid from light orange to blood-red accompanied by a large increase in volume was observed. At the end of five hours the flask (a) was stoppered and kept for four days at room temperature and two days in the refrigerator. The hydrogen chloride was passed through solution (b) for seven hours and the stoppered flask was allowed to stand in the refrigerator for ten days. During this time there separated a small amount of semi-solid material. On addition of much ether a heavy viscous liquid separated whose solution in methyl alcohol gave a blue coloration with aqueous sodium carbonate and a violet color with aqueous sodium hydroxide.

All attempts to obtain this substance in a crystalline condition were unsuccessful so the following treatment was adopted. It was dissolved in methyl alcohol-hydrochloric acid and a saturated aqueous solution of picric acid added to the hot solution. On cooling rust-red powdery picrate separated. The substance is

fairly soluble in such solvents as ethanol, methanol, acetone, ethyl acetate, glacial acetic acid and 1,4-dioxane but not readily soluble in water, chloroform, ligroin, petroleum ether, xylol and benzene. Both the picrate and the chloride dissolve in concentrated sulfuric acid to a dark red solution which however exhibits no fluorescence. The crude picrate was recrystallized from ethyl alcohol. The purified material which starts to sinter at 193° and melts at 197-199° gave the following results on analysis:

	% H	% C	% N	% Ash
After drying in a vacuum	5.10	53.54	5.17	0.62
desiccator at room temp.	4.82	53.79	5.02	0.70
After drying at 110° in	4.48	53.02		0.48
vacuo	4.56	53.16		0.62

The small ash residue is of yellow color. Although the difference in the above two sets of figures is rather small, it seems that the sample has changed after the drying procedure. 3,2',4'-Trihydroxy-5,6,7-trimethoxyflavylium picrate, ($C_{24}H_{19}O_{14}N_3$), requires: C, 50.26%; H, 3.34%; N, 7.33%.

The picrate was changed to chloride by passing dry hydrogen chloride into a hot saturated solution in absolute ethyl alcohol protected from the atmosphere by a calcium chloride tube. Addition of a large volume of ether to the dark red solution caused the separation of a gelatinous flocculent brown-red material which was

not retained by filter paper. The crude chloride was separated from the supernatent liquid by decantatation and finally by the use of a separatory funnel. After dissolving in absolute alcohol the above treatment was repeated. Efforts to induce the crystallization of the compound were unsuccessful. On standing in the open laboratory and then in a vacuum desiccator over potassium hydroxide for some time there remained a brown-red solid residue which does not melt below 350° and whose solution in ethyl alcohol gives a violet coloration with aqueous sodium carbonate and sodium hydroxide.

SYNTHESIS OF GOSSYPETINIDIN (LVI)

ω-Chloro-3,4-dihydroxyacetophenone (XLVI)

The method of Dzierzgowsky (11) as modified by Murakami, Robertson and Robinson (19) was employed in the preparation of this compound. A mixture of equal quantities (100 g.) of catechol, chloracetic acid, phosphorus oxychloride and toluene was heated on the steam bath for 1½-2 hours. The toluene was removed by steam distillation and the residue (100 g.) was treated with animal charcoal. On recrystallization from water a product melting at 174-5° was obtained in 55 per cent yield.

ω,3,4-Triacetoxyacetophenone (XLVII)

A mixture of ω-chloro-3,4-dihydroxyacetophenone (20 g. dried at 110°), freshly fused and powdered sodium acetate (20 g.) and acetic anhydride (100 cc.) was heated on the steam bath for three hours and then refluxed for one-half hour. The reaction mixture was then treated with animal charcoal and poured on ice. The separated product was recrystallized from methyl and ethyl alcohol (35).

1,2,3-Tribenzyloxybenzene (XLVIII)

The method described by Baker, Nodzu and Robinson (3) was employed for the benzylation of pyrogallol. Benzyl chloride was gradually added to a mixture of pyrogallol (50 g.), anhydrous potassium carbonate (240 g.) and dry acetone (300 cc.) which was refluxed in an atmosphere of propane for thirty hours. The

mixture was then heated for a further ten hours after which time the portion of the reaction product that was insoluble in water was dissolved in ether and washed with aqueous sodium hydroxide. The solvent and unchanged benzyl chloride were removed by distillation, finally in steam. The brown gummy residue was crystallized from alcohol.

2,6-Dibenzyloxy-p-benzoquinone (XLIX)

1,2,3-Tribenzyloxybenzene was oxidized to the quinone following the directions given by Baker, Nodzu and Robinson (3). 1,2,3-Tribenzyloxybenzene (80 g.) was dissolved in glacial acetic acid (800 cc.) and nitric acid (40 cc., sp.gr. 1.2) was added to the solution which was kept at 40°. The mixture was then allowed to stand at room temperature for four hours and the 5-nitro-1,2,3-tribenzyloxybenzene (L) filtered off. A further quantity of nitric acid (40 cc.) was added to the reddish-brown filtrate and after about fifteen minutes the quinone began to separate out. After standing overnight the product was filtered (30 g.) and recrystallized from acetone. A further small quantity was recovered from the filtrate by the addition of water.

2,6-Dibenzyloxyquinol (LI)

The reduction of the foregoing quinone to give the quinol in yields 93 per cent of the theorectical was accomplished by means of zinc and sulfuric acid (3). Sulfuric acid (30 cc. of 25%) was introduced during the course of three hours to a gently boiling

mixture of 2,6-dibenzyloxy-p-benzoquinone (40 g.), alcohol (400 cc.) and zinc dust (80 g.) contained in a 3-necked flask fitted with a water condenser, dropping funnel and a mechanical stirrer with a mercury seal. At the end of this time the liquid was filtered hot and dilute sulfurous acid was added to the filtrate. The quinol which separated was dissolved in alcohol and recrystallized by the addition of sulfurous acid.

2,6-Dibenzyloxy-1,4-dimethoxybenzene (LII)

The methylation of 2,6-dibenzyloxyquinol was carried out according to the conditions described by Baker, Nodzu and Robinson (3). Dibenzyloxyquinol (12 g.) and alcohol (120 cc. of 95%) were placed in a 3-necked flask equipped with a reflux condenser, a mechanical stirrer passing through a mercury seal, a dropping funnel, and an inlet tube for propane gas. Aqueous sodium hydroxide (12 g. of a solution of 60 g. of sodium hydroxide in 150 cc. of water) and then methyl sulfate (16 g.) were added to this mixture. In order to keep the solution weakly alkaline, a further quantity (7 g.) of the sodium hydroxide solution was introduced in the course of nine hours. The temperature was then raised to 45° for an hour. After standing at room temperature for about 12 hours water (50 cc.) was added and the product, obtained in 80 per cent yield, was collected and recrystallized from acetone.

2,5-Dimethoxyresorcinol (LIII)

Considerable difficulty was encountered in effecting the partial hydrolysis of 2,6-dibenzyloxy-1,4-dimethoxybenzene according to the

method of Baker, Nodzu and Robinson (3). A mixture of dibenzyloxydimethoxybenzene (47.8 g.), glacial acetic acid (335 cc.) and hydrochloric acid (143 cc., sp.gr. 1.16) was kept at 65-70° for an hour. A large volume of water (about 1000 cc.) was added and the solution evaporated down to a syrup under diminished pressure. The syrup was extracted with hot water and the aqueous solution was treated with animal charcoal, then evaporated down to a small volume (about 75 cc.) under reduced pressure. On further evaporation of the extract in a vacuum desiccator over sulfuric acid, light brown crystals separated out together with a small amount of darker brown material. It was found necessary to keep the desiccator in a cool place, otherwise the solution turned dark red in color and evaporated down to a syrup without the separation of a solid. After further cooling in the refrigerator the crude product (13 g.) was filtered off and crystallized from benzene in fine faintly pink tinged crystals melting at 59°.

2,4-Dihydroxy-3,6-dimethoxybenzaldehyde (LIV)

This compound was prepared by the Adams and Levine modification of the Gattermann synthesis of aromatic aldehydes. The procedure employed is similar to that described for the synthesis of antiarolaldehyde (page 30). Zinc cyanide (4.0 g.) was introduced into the wide-mouth bottle containing 2,5-dimethoxyresorcinol (4.5 g.) and anhydrous ether (200 cc.) and dry hydrogen chloride was passed in for 2½ hours. The zinc cyanide gradually disappeared, the reaction mixture became green in color, and a thick brown oil

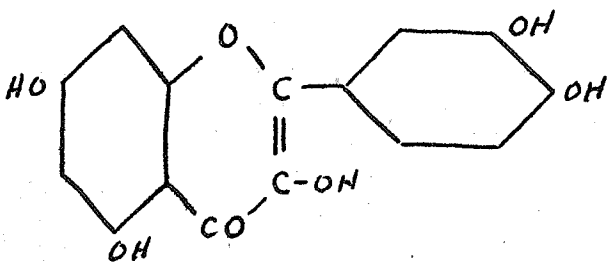
began to separate after about 10-15 minutes. In about half an hour yellow powdery solid commenced to deposit. The ether was decanted from the solid material and the imide hydrochloride then decomposed with water (30 cc.) to yield 4.5 g. of orange powdery aldehyde after filtration and drying. A further quantity was recovered from the filtrate. By crystallization from water with the addition of animal charcoal very fine glistening needle-shaped crystals with a slight yellow tinge and m.p. 196° were obtained which on analysis gave: C, 54.62%; H, 5.15%. $C_9H_{10}O_5$ requires C, 54.54%; H, 5.09%. The compound gave a positive test with both Tollen's reagent and Fehling's solution.

Oxime of 2,4-dihydroxy-3,6-dimethoxybenzaldehyde

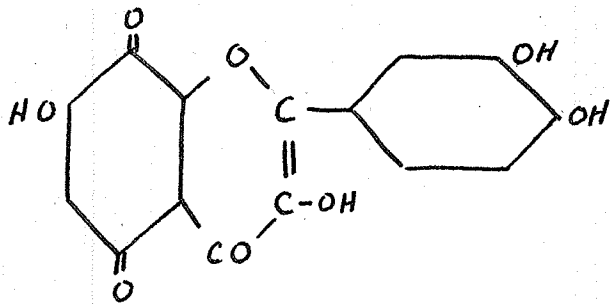
Hydroxylamine hydrochloride (0.5 g.) was dissolved in water (3 cc.); sodium hydroxide solution (2 cc. of 10%) and 2,4-dihydroxy-3,6-dimethoxybenzaldehyde (0.2 g.) were then added. Since the aldehyde is not very soluble in water, just sufficient alcohol was added to give a clear solution. The mixture was warmed on the steam bath for 10 minutes and then cooled in an ice bath. Almost colorless glistening crystals of the oxime (0.2 g.) separated. Crystallization from water yielded fine needle-shaped crystals melting at 182°.

II. OXIDATION PRODUCTS OF QUERCETIN AND SYNTHESIS OF HYDROXYQUERCETIN

Nierenstein and Wheldale (22) subjected quercetin (I) to chromic acid oxidation and obtained quercetone to which they assigned the p-quinone structure II.

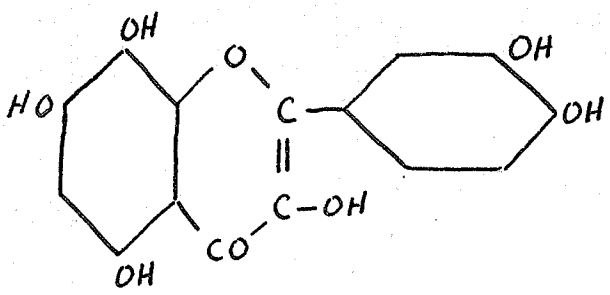


I

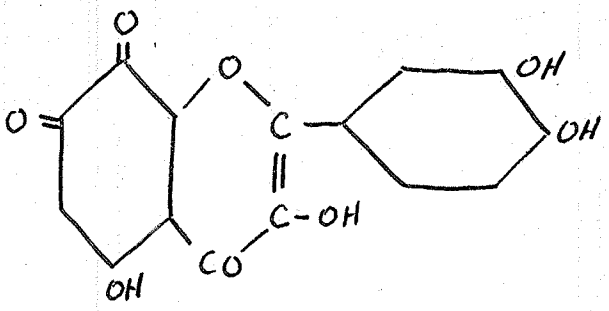


II

The reduction of this oxidation product with acetic anhydride and zinc dust followed by hydrolysis resulted in the formation of the hydroxyquercetin, 3,5,7,8,3',4'-hexahydroxyflavone (III). This flavone is isomeric with gossypetin, quercetagetin and myricetin, yet quite different from these pigments. Oxidation of hydroxyquercetin (III) by means of p-benzoquinone (20) gave a product, isoquercetone (IV), to which Nierenstein assigned the constitution of an ortho-quinone. Isoquercetone is converted back to hydroxyquercetin on acetylation and reduction with acetic anhydride and zinc dust and subsequent hydrolysis.

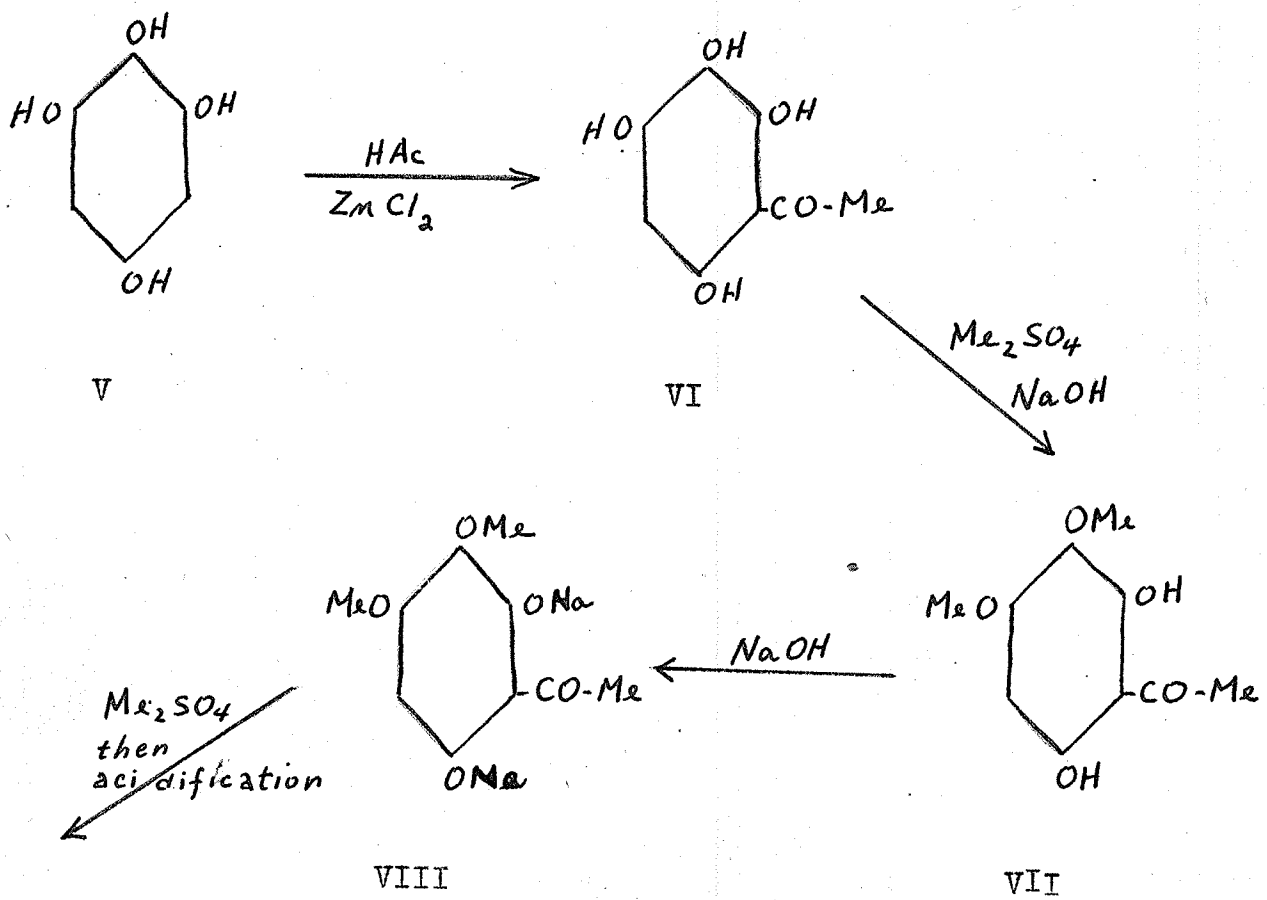


III

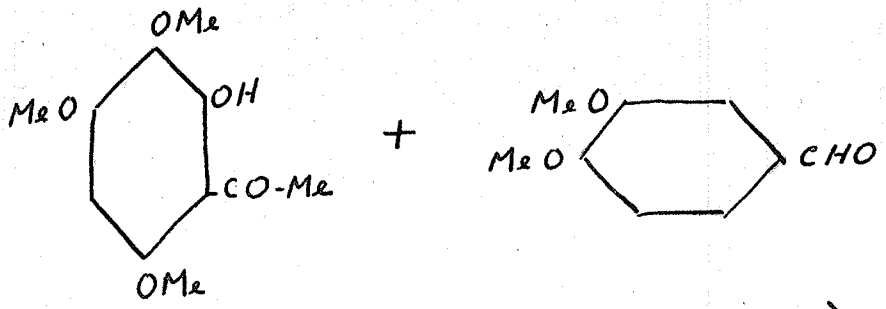


IV

In order to verify the structure assigned to hydroxyquercetin (III) Nierenstein undertook the synthesis of this compound (21). The method he employed is similar to that used for the synthesis of quercetin by Kostanecki, Lampe and Tambor (16) and is indicated schematically below.

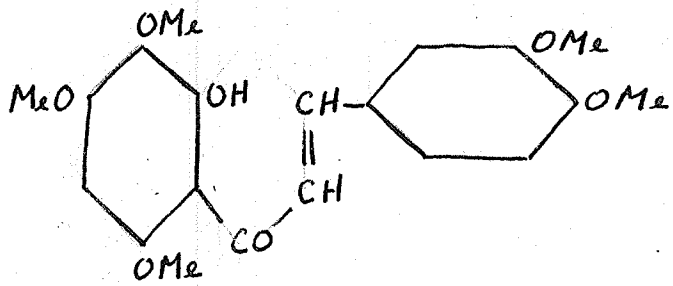


V VI VII VIII



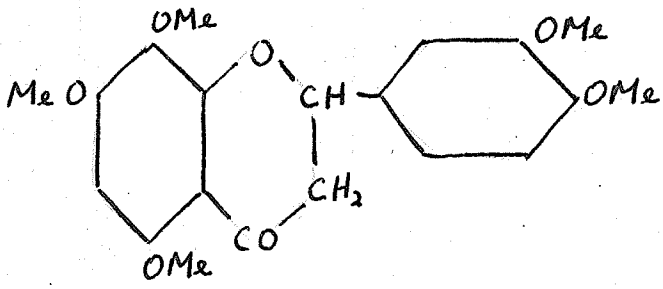
IX X

NaOH
followed by
acidification



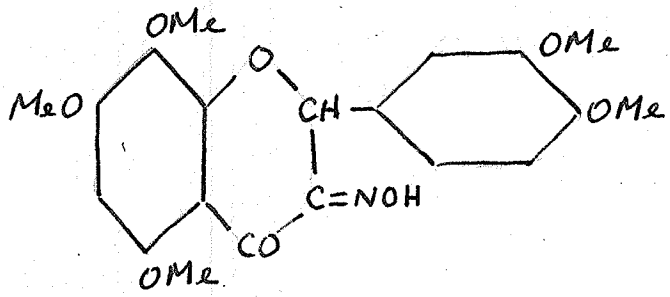
alc. HCl

XI

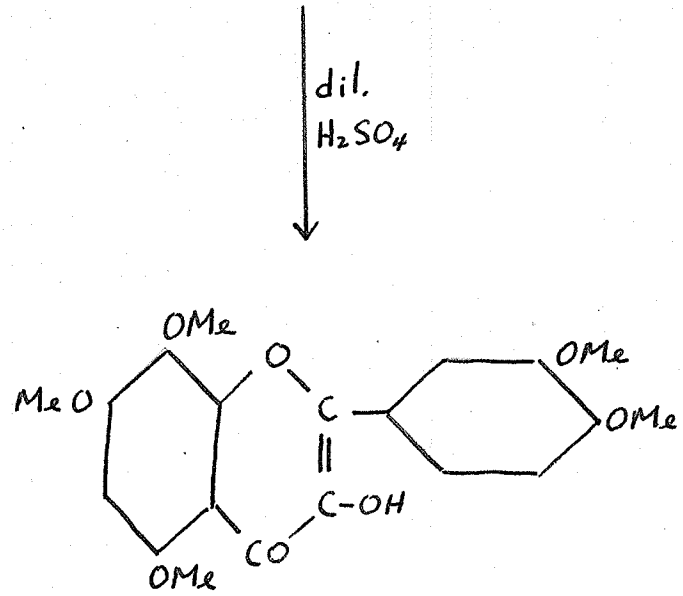


XII

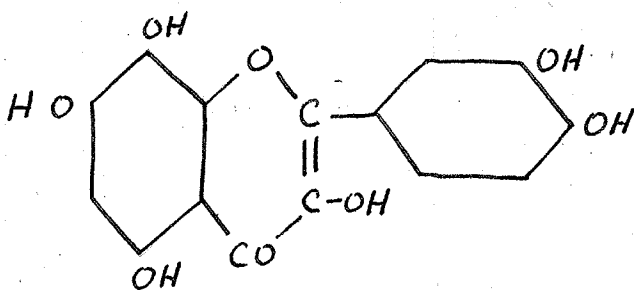
amyl nitrite
HCl



XIII



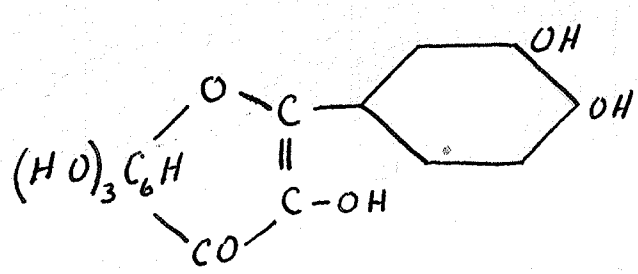
XIV



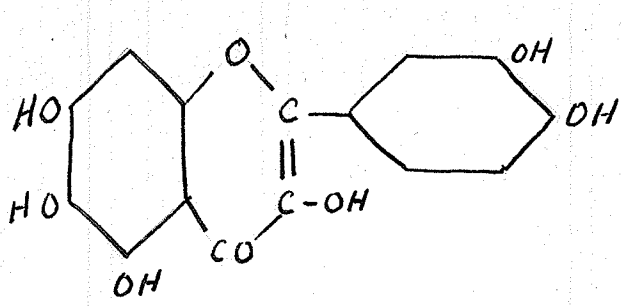
III

1,2,3,5-Tetrahydroxybenzene (V) was converted to 2,3,4,6-tetrahydroxyacetophenone (VI) by heating its solution in glacial acetic acid with granulated zinc chloride, and this on methylation gave 2,6-dihydroxy-3,4-dimethoxyacetophenone (VII). On methylation and subsequent acidification the disodium salt of 2,6-dihydroxy-3,4-dimethoxyacetophenone (VIII) formed 2-hydroxy-3,4,6-trimethoxyacetophenone (IX) which was condensed with veratraldehyde (X) to yield 2-hydroxy-3,4,6-trimethoxyphenyl-3,4-dimethoxystyryl ketone (XI). This was refluxed with alcoholic hydrochloric acid with formation of 5,7,8,3',4'-pentamethoxyflavanone (XII), which on treatment with amyl nitrite and concentrated hydrochloric acid was converted to the isonitroso derivative (XIII). 5,7,8,3',4'-Pentamethoxyflavonol (XIV) was obtained by heating the isonitroso compound with dilute sulfuric acid under reflux. By the action of concentrated hydriodic acid the pentamethoxyderivative was demethylated to give the free hydroxyquercetin (III).

The isomeric pigments gossypetin and quercetagetin were examined by A.G. Perkin (24) who found that both substances were represented by the formula XV. However he was unable to fix the position of the hydroxyl groups in the tetrahydroxybenzene nucleus.



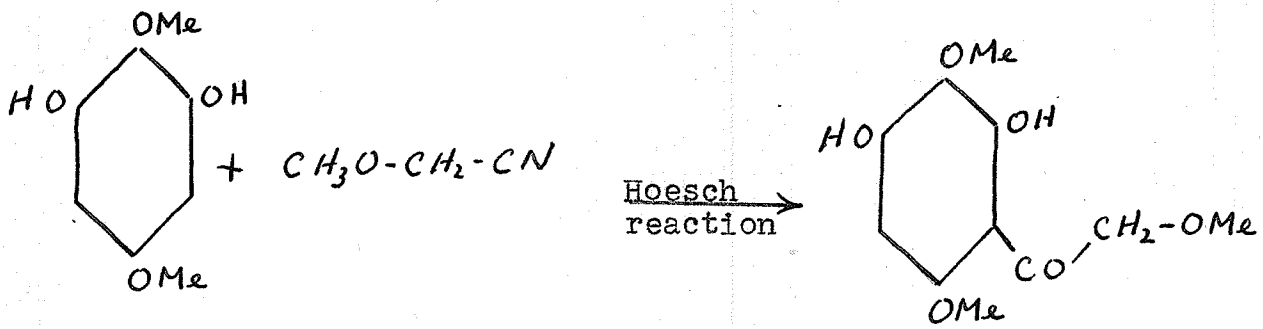
XV



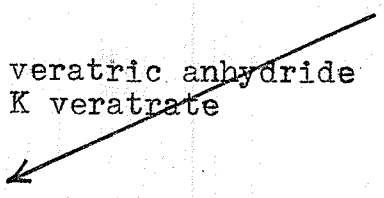
XVI

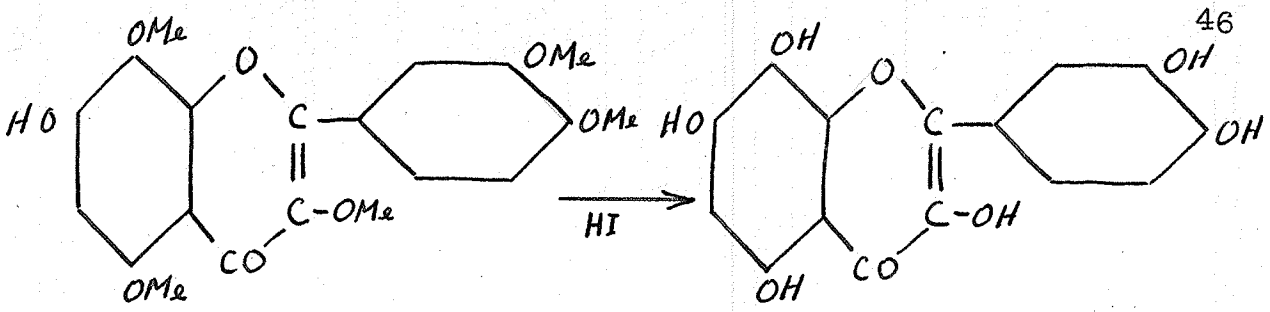
The suggestion was made that these flavonols were hydroxy derivatives of quercetin with the structure III and XVI. But this idea conflicted with the work of Nierenstein described above since he had shown that the substance with the formula III was not identical with either gossypetin or quercetagetin.

The synthesis of both gossypetin and quercetagetin, identical in all respects with the natural coloring matters, was effected in 1929 by Baker, Nodzu and Robinson (3). They were able to show that both these pigments were derivatives of 1,2,3,5-tetrahydroxybenzene and that the structures of gossypetin and quercetagetin conformed to formulae III and XVI respectively. Their synthesis of gossypetin is based on the following series of reactions:



XVII XVIII XIX





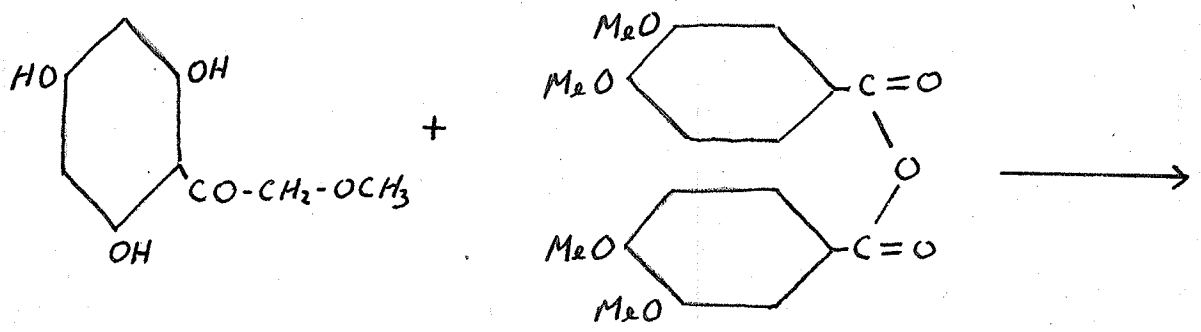
XX III

2,5-Dimethoxyresorcinol (XVII) was prepared as described in the first section of this report. The application of the Hoesch reaction, using methoxyacetonitrile (XVIII), led to the formation of ω,3,6-trimethoxy-2,4-dihydroxyacetophenone (XIX) and the veratroylation of this ketone gave 7-hydroxy-3,5,8,3',4'-pentamethoxyflavone (XX). The hexahydroxyflavone (III) obtained on demethylation was proved to be identical with gossypetin.

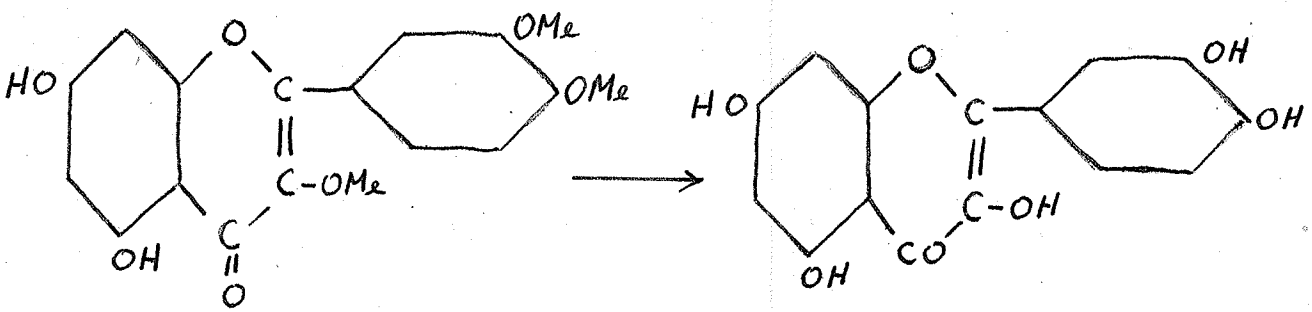
The results of Baker, Nodzu and Robinson are in complete agreement with the work of Perkin but in contradiction with that of Nierenstein. Nierenstein assigns formula III to a substance which is not identical with either gossypetin or quercetagetin. According to his results the flavonol possessing this formula can be obtained by the reduction of either of the two isomeric quinones, quercetone or isoquercetone. He furnishes confirmatory evidence for this view by synthesizing a hydroxyquercetin identical with that obtained by reduction using a method which seems clearly to establish the structure of the final product. Comparison of derivatives confirms this identity. Robinson, on the other hand, effects the synthesis of a flavonol which he shows to be identical with gossypetin employing a method which apparently proves that it has the constitution III.

In order to bring this conflicting evidence into agreement it was proposed to go over some of the above work. In view of the fact that discrepancies have been reported in other papers published by Nierenstein, it was decided to undertake the repetition of his work on the oxidation products of quercetin and the synthesis of hydroxyquercetin.

This requires the preliminary preparation of quercetin which has been synthesized by Allan and Robinson (2) by the veratroylation of ω-methoxyphloracetophenone (XXI) followed by demethylation of the resulting quercetin trimethyl ether (XXIII):

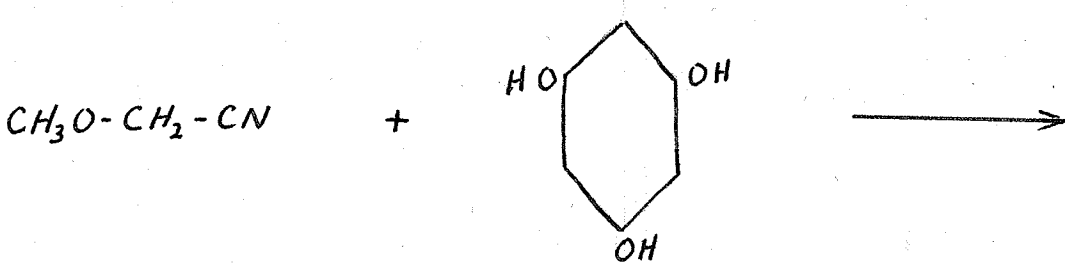


XXI XXII

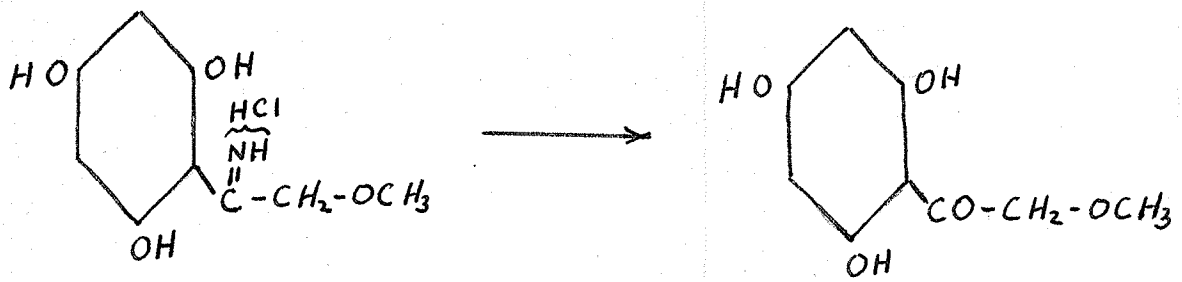


XXIII I
Quercetin

ω-Methoxyphloracetophenone was obtained from the condensation of methoxyacetonitrile (XXIV) and anhydrous phloroglucinol (XXV) (34):

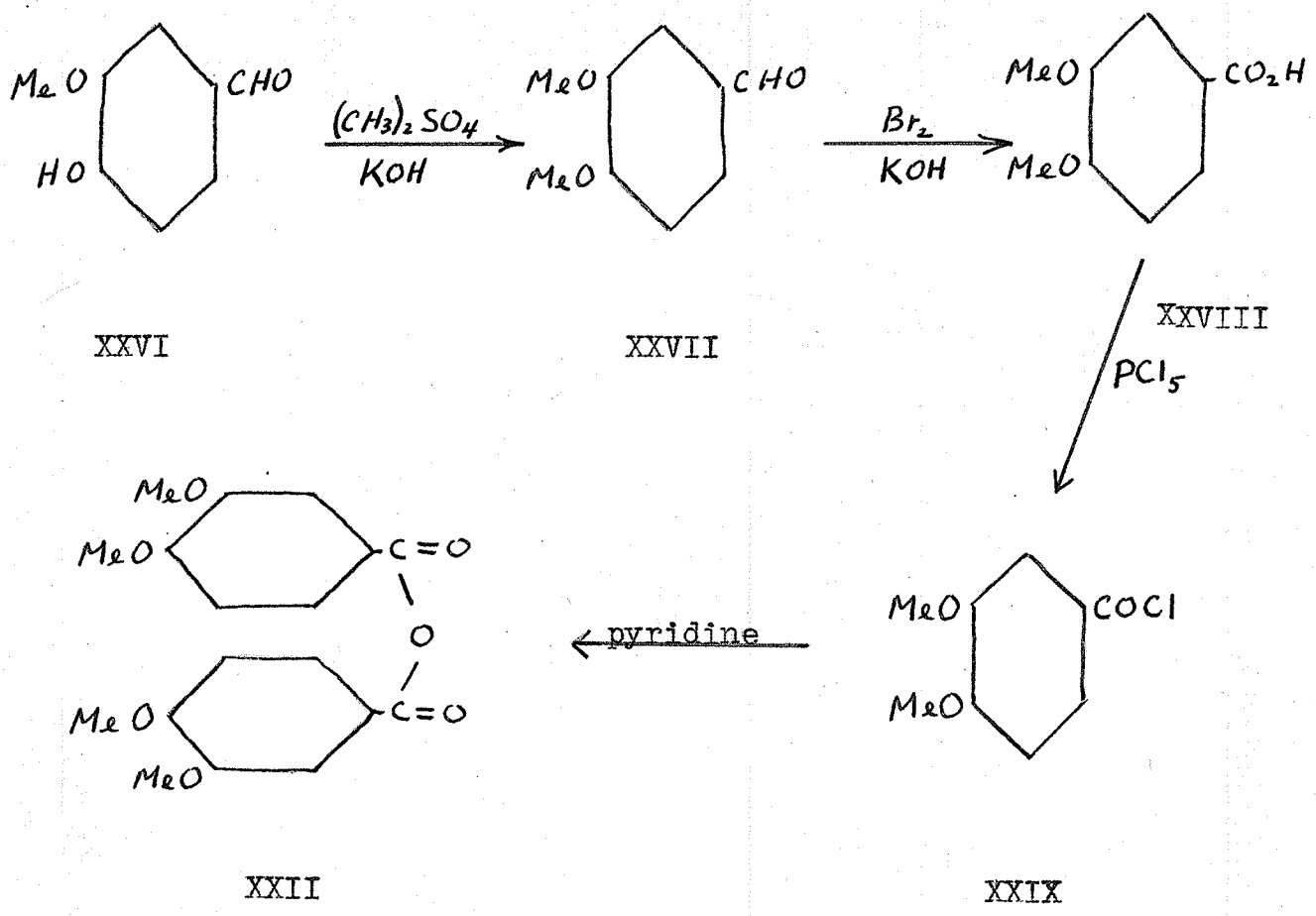


XXIV XXV



XXI

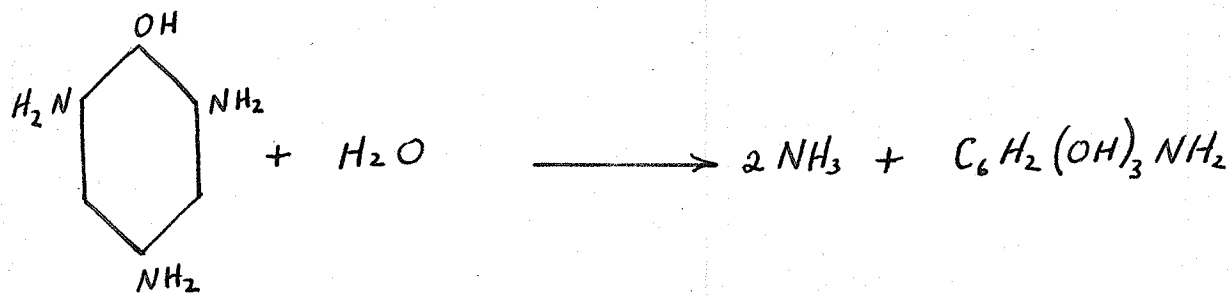
The preparation of veratric anhydride (XXII) was effected through the action of pyridine on veratoyl chloride (XIX) (2) which was synthesized from vanillin (XXVI) by the series of reactions indicated below:



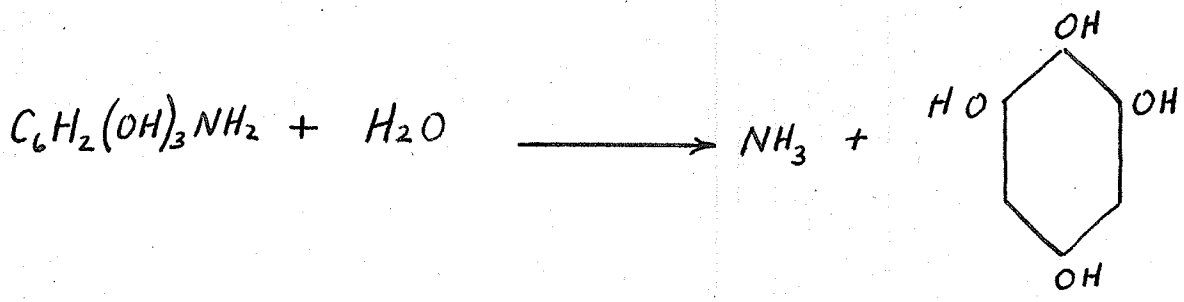
Lack of time did not permit more than a start to be made on this problem. The preliminary preparation of quercetin has been partially completed as described in the experimental section. Investigation of this problem is being continued by A. Giesinger.

1,2,3,5-Tetrahydroxybenzene (V), the starting material in the series of reactions leading to the synthesis of hydroxyquercetin, was prepared by Nierenstein according to Oettinger's method (23). 2,4,6-Triaminophenol (XXX) on hydrolysis is converted

to trihydroxyaniline (XXXI) which is subsequently transformed to 1,2,3,5-tetrahydroxybenzene as indicated in the following equations:



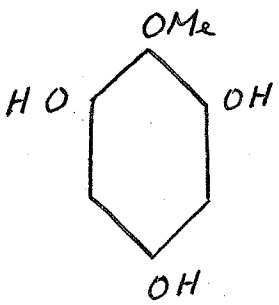
XXX XXXI



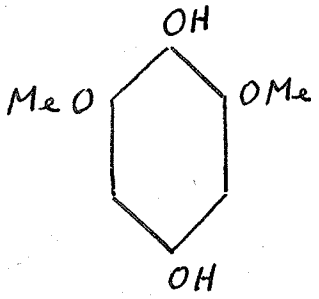
XXXI V

Oettinger reported that the tetrahydroxybenzene is very unstable and the small quantity he was able to isolate was insufficient to prepare derivatives. Nierenstein however claims that a slight modification of the method employed by Oettinger enabled him to produce this compound on a large scale. Laire and Tieman (18) prepared 1,2,3,5-tetrahydroxybenzene by demethylating iretol (XXXII) but their efforts to isolate it in a crystalline form were unsuccessful. A. Giesinger has made attempts to synthesize this substance by the demethylation of 2,6-dimethoxyquinol (XXXIII),

which is one of the intermediates in the synthesis of 6-hydroxy-morinidin described in the first section of this report, but at the time of writing of this thesis has found it impossible to isolate the product in a crystalline condition. It is possible that the crude amorphous material may be induced to undergo the transformation to 2,3,4,6-tetrahydroxyacetophenone without further purification.



XXXII



XXXIII

EXPERIMENTAL

Methoxyacetonitrile (XXIV)

This nitrile was prepared by methylating the product resulting from the action of sodium cyanide on paraformaldehyde (32). Paraformaldehyde (60 g.) was added in small amounts to a mixture of pulverized sodium cyanide (98 g.) and water (200 cc.) which was agitated by means of a mechanical stirrer. When the sodium cyanide had dissolved and the temperature rose to 20-25° the flask was surrounded by a freezing mixture and the temperature was kept below 25° during the introduction of the remaining paraformaldehyde. Methyl sulfate (200 cc.) was placed in a dropping funnel and, when the temperature inside the flask had dropped to 13°, a 20-30 cc. portion of the sulfate was added. An exothermic reaction set in. When the temperature began to fall the remainder of the methyl sulfate was admitted at such a rate as to keep the temperature at 12-15°. When the addition was complete the mixture was stirred an additional 40 minutes during which time the temperature dropped to about 5°. The oily upper layer was separated, dried with anhydrous sodium sulfate, and distilled under diminished pressure. The portion boiling below 70° at 15 mm. was redistilled at atmospheric pressure and the fraction coming over at 118°-122° collected. The lower aqueous layer was returned to the flask and methylated as before.

Veratraldehyde (XXVII)

Good yields (93 per cent of theoretical) of this aldehyde were obtained by the methylation of vanillin (5). Vanillin (152 g.) was melted by warming on a steam bath in a 1-litre, 3-necked, round bottom flask equipped with a mechanical stirrer, a reflux condenser and two separatory funnels. With vigorous stirring a solution of potassium hydroxide (92 g. KOH in 150 cc. water) was run in at the rate of 2 or 3 drops per second. About 20 seconds after this was started, the addition of methyl sulfate (120 cc.) was begun at about the same rate. The external heating was stopped after a few minutes and the mixture continued to reflux from the heat of reaction. At the end of about 20 minutes the reaction was complete and the mixture was poured into a large beaker, covered with a clock glass and allowed to stand overnight. The crystalline mass of veratraldehyde was removed, ground in a mortar with ice-water (300 cc.), filtered with suction and dried in a vacuum desiccator.

Veratric Acid (XXVIII)

Kostanecki and Tambor's method (12) was employed in the synthesis of this acid. Veratric aldehyde (10 g.) was heated for an hour on a sand bath with bromine in potassium hydroxide solution (14 g. KOH and 200 cc. water). The solution was filtered and sodium bisulfite added to the filtrate. Acidification caused the separation of the acid (8 g.) which was filtered off.

Veratroyl Chloride (XXIX)

This substance was prepared from veratric acid using the method described by Kostanecki and Tambor (17). A mixture of veratric acid (10 g.), carbon disulfide (20 g.) and phosphorus pentachloride (13 g.) was heated on a water bath for about an hour until it ceased to evolve hydrogen chloride. On fractionation, the portion coming over between 270-277° was collected.

SUMMARY

1. Antiarolaldehyde, which had been previously prepared by the Gattermann reaction employing anhydrous hydrogen cyanide, has been synthesized by the Adams and Levine modification of this reaction.

2. The synthesis of 2,4-dihydroxy-3,6-dimethoxybenzaldehyde and its oxime, which have hitherto not been reported in the literature, has been effected.

3. The condensation of antiarolaldehyde with ω-acetoxy-2,4-dihydroxyacetophenone did not yield the expected derivative of 6-hydroxymorinidin. The constitution of the product obtained is uncertain. It is suggested that the reaction may be induced to proceed in the normal manner by a change of solvent.

4. A quantity of ω,3,4-triacetoxyacetophenone has been prepared but lack of time did not permit trial of its condensation with 2,4-dihydroxy-3,6-dimethoxybenzaldehyde to yield a methoxy derivative of gossypetinidin.

5. The work of Nierenstein is irreconcilable with that of Baker, Nodzu and Robinson and it is believed that this difference can be cleared up by the repetition of Nierenstein's investigation of the oxidation products of quercetin and his synthesis of hydroxyquercetin. The preliminary preparation of quercetin has been partially completed.

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