Part I

The Synthesis of Hydrastic Acid

Part II

Studies on the Degradation of Picropodophyllin

Part III

Studies on the Synthesis of Podophyllotoxin

by
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of the University of Maryland in partial
fulfillment of the requirements for the
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FOREWORD

Part I of this thesis deals with the synthesis of hydrastic acid and determination of some of its physical constants. The preparation of the intermediates involved in the synthesis are also described. Hydrastic acid was prepared for use as an intermediate in the synthesis of 3,4-methylenedioxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid.

Part II concerns some degradation studies carried out on picropodophyllin that were designed to gain more conclusive evidence concerning the position of the free hydroxyl group.

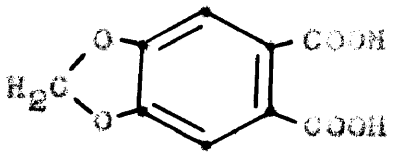
Part III deals with the attempted synthesis of podophyllotoxin. Reactions leading to the preparation of 6,7-methylenedioxy-4-(3,4,5-trimethoxyphenyl)-3-carbomethoxynaphthol-1 (an intermediate in the proposed synthesis) are described. Further reactions of the naphthol-1 ester are also included.

PART I

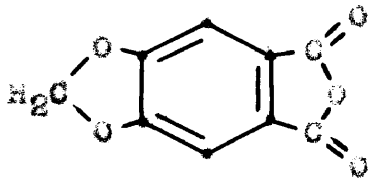
THE SYNTHESIS OF HYDRASTIC ACID

INTRODUCTION

The purpose of this phase of the research was to develop a method of preparing hydrastic acid (I) and hydrastic anhydride (II) in overall yields large enough to enable their use as an intermediate in the preparation of 4,5-methylenedioxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid (III).



I



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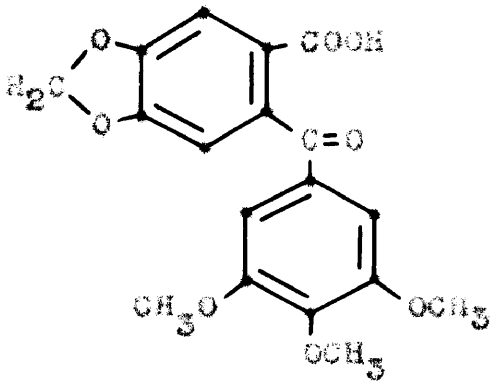
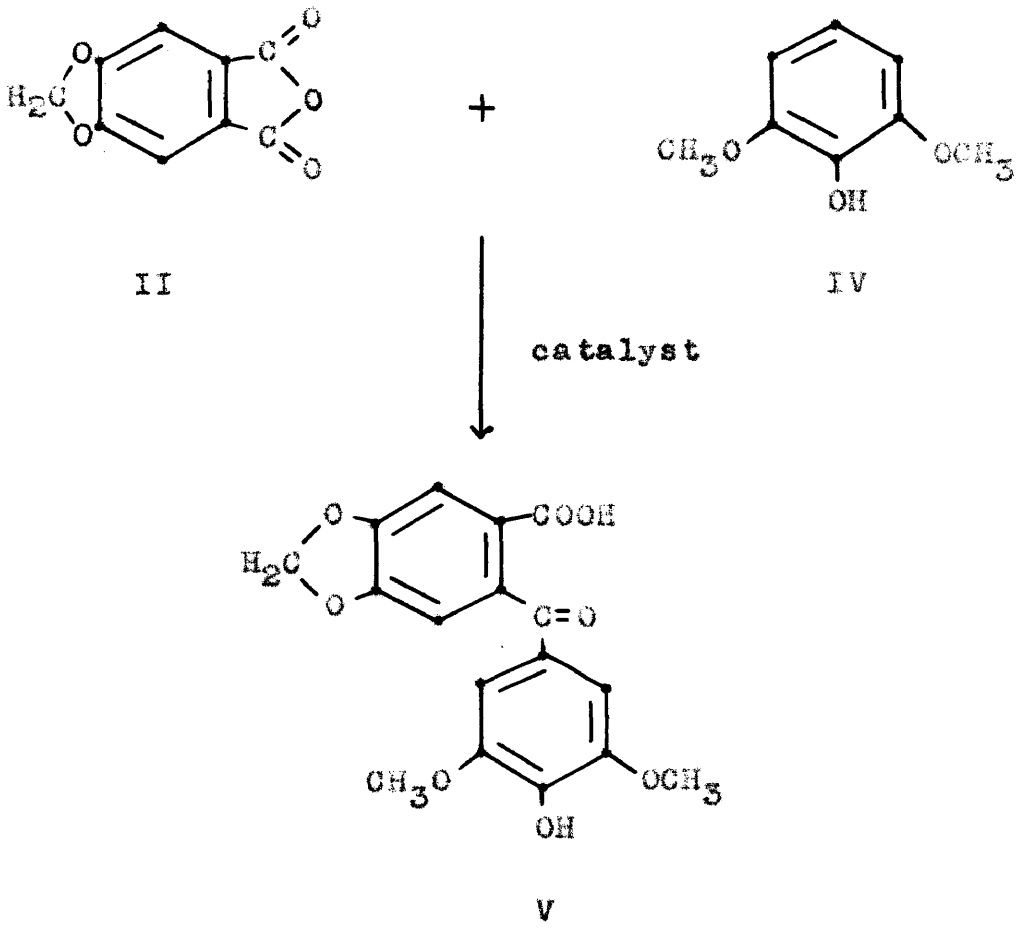


III

An earlier attempt by Sterling[19] to prepare compound III failed. Later, Bareckson[3] did prepare small amounts by the oxidation of a disubstituted dihydroisoquinoline. Utilization of hydrastic anhydride (II) in the synthesis of III would involve a Friedel-Crafts condensation with compound IV to yield 4,5-methylenedioxy-2-(3,5-dimethoxy-4-hydroxybenzoyl)-

benzoic acid (IV). A subsequent methylation of the free phenolic group should then yield the keto acid (III)



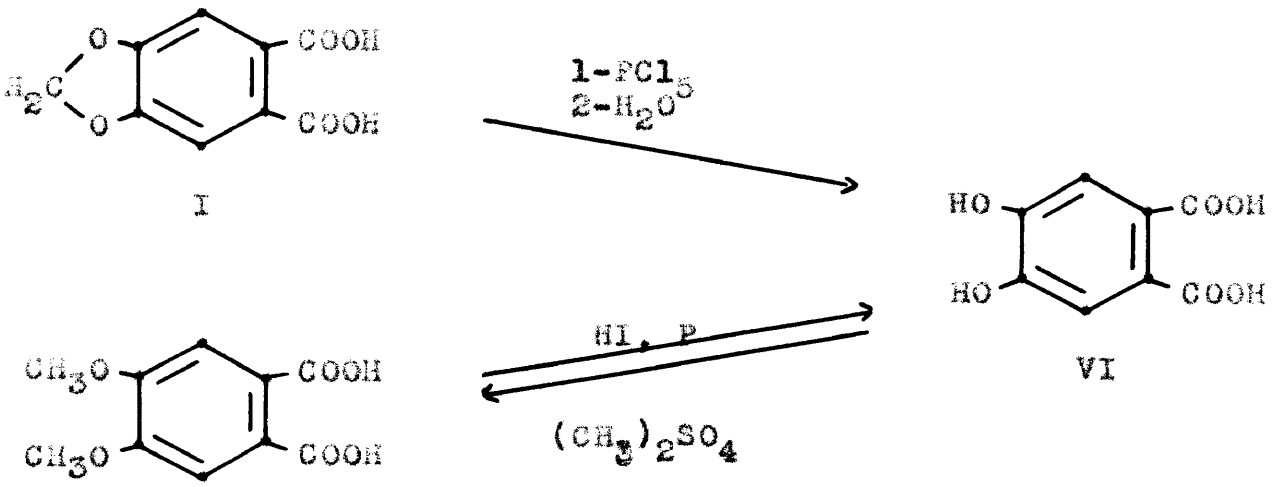
II IV

V

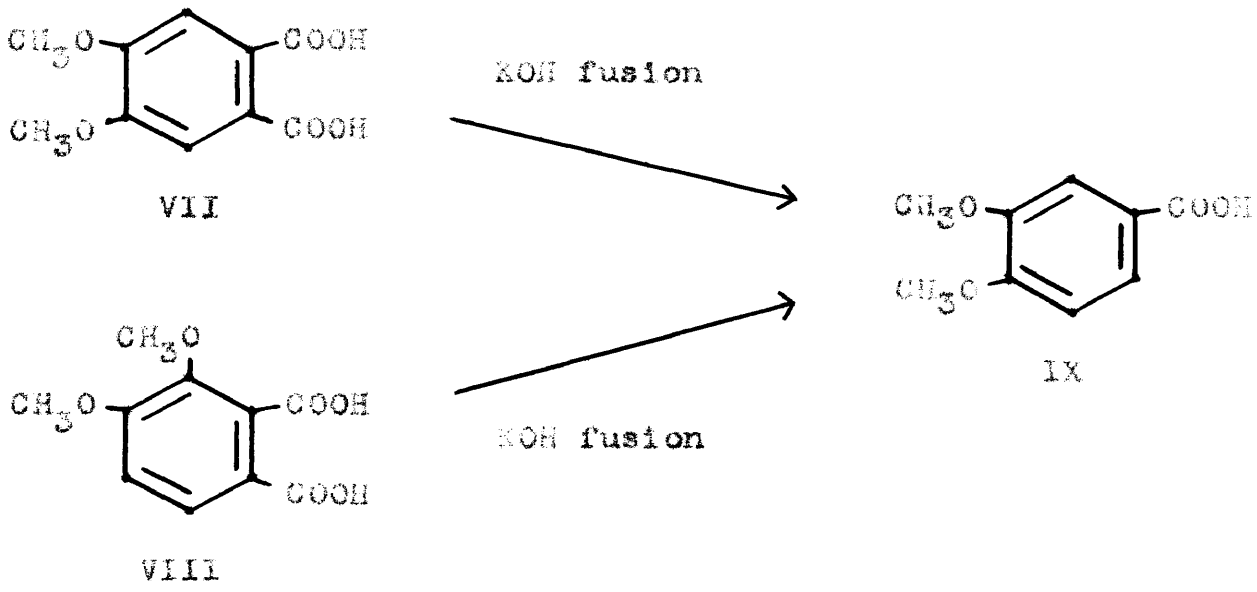
The main objective has been realized although the subsequent use of hydrastic acid (I) and hydrastic anhydride (II) to prepare the keto acid (V) has been dropped in favor of another approach described in a latter section of this thesis.

Hydrastic acid (I) was first obtained by Freund and Lachmann[4] by the oxidation of hydrastine with potassium permanganate. Subsequent treatment of hydrastic acid (I) with phosphorous pentachloride followed by an aqueous hydrolysis produced a compound that was identified as

normetahemipinic acid[5] (VI); the latter being obtained from metahemipinic acid (VII) by treatment with hydriodic acid and red phosphorous[18]. The structure of metahemipinic acid as given in figure VII was based by Goldschmiedt[6] on its



relationship to hemipinic acid (VIII); the latter having been previously determined by Wegschneider.[21] Both on potassium hydroxide fusion give protocatachuic acid (IX). This could only have occurred if the two were positional isomers. Wegschneider's proof of structure for hemipinic acid (VIII) was based on the number of isomers possible for the monomethyl ester. As can be seen, there is only one half ester possible for figure VII but two possible for figure VIII. Both half esters of hemipinic acid were prepared with the result that structure VIII was assigned. The alpha ester (X) was obtained by the oxidation of the methyl ester of opianic acid (XII), while the beta ester (XI) was formed by treating

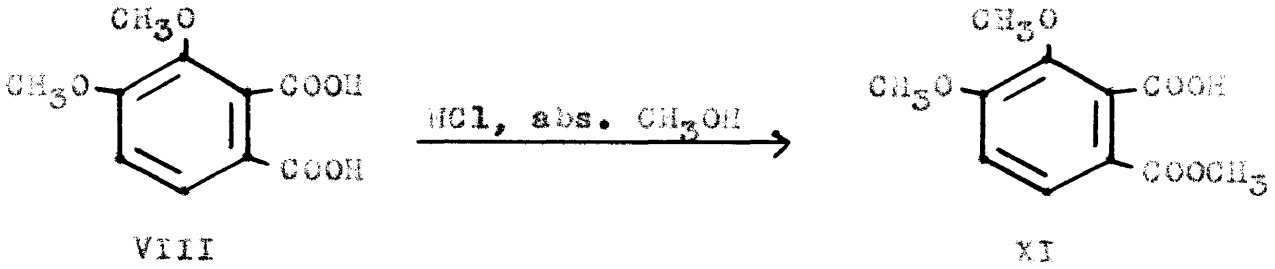


VII

VIII

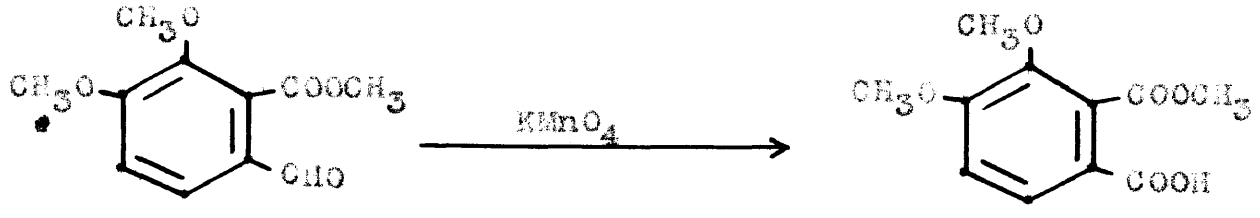
IX

hemipinic acid in absolute methanol with anhydrous hydrogen chloride. The two half esters have different crystal forms, melting points and water solubilities. In this round about manner, the structure for hydrastic acid (I) was assigned by Freund[5].



VIII

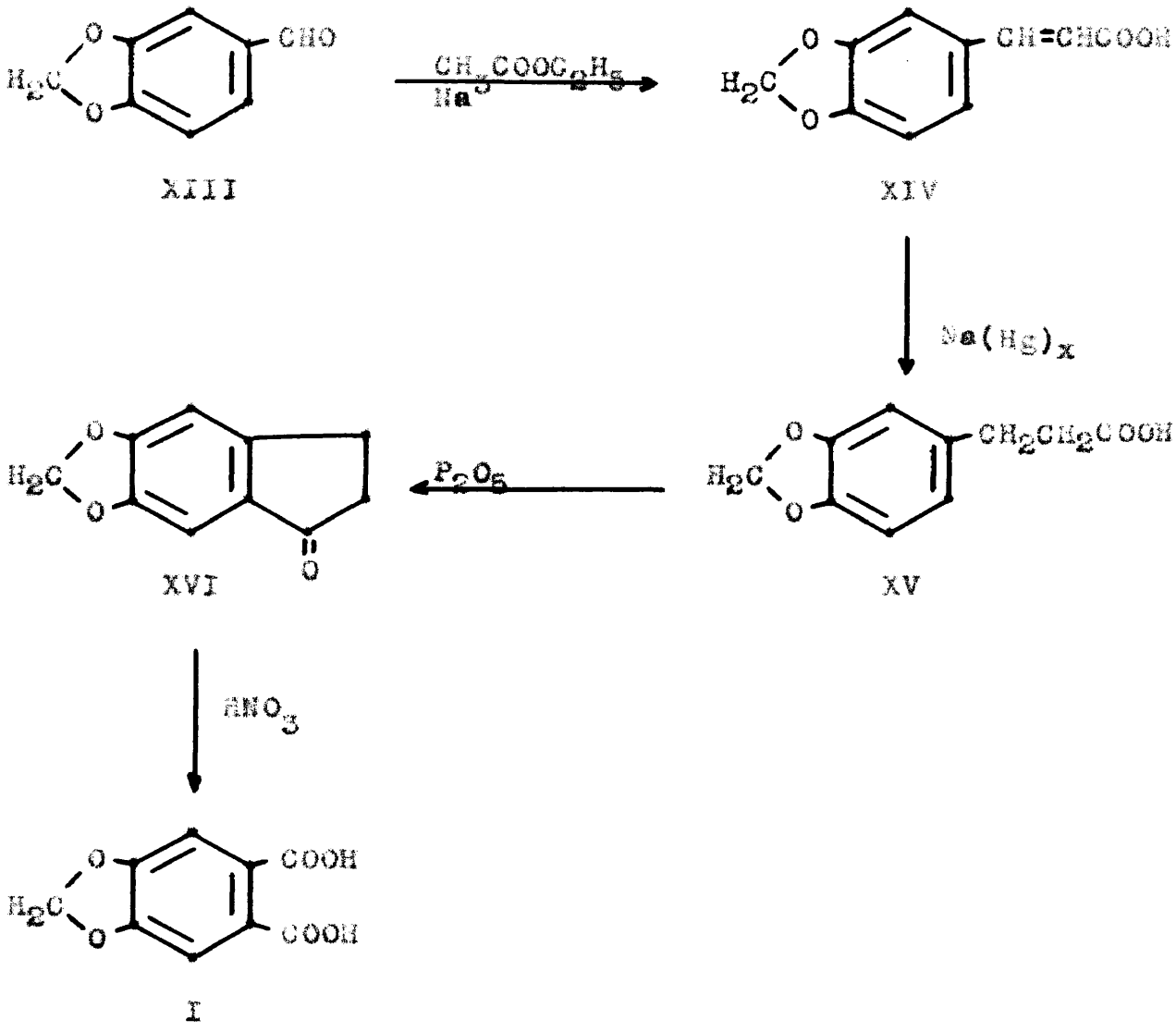
XI



XII

X

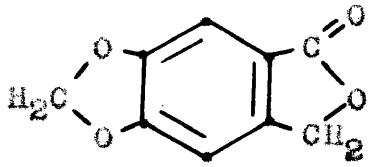
The following year, Perkin[12] obtained a dibasic acid with properties similiar to those advanced by Freund for hydrastic acid (I) by the oxidation of Berberine with potassium permanganate. He subsequently prepared hydrastic acid (I) through a four step procedure that utilized piperonal (XIII) as the starting material[13].



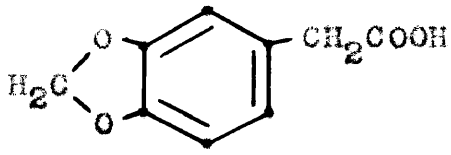
XIII XIV XV XVI I

Since that time, hydrastic acid (I) has been obtained from the natural alkaloid Tazettine by Späth and Kahovec[18];

from alpha methylprotopin by Lanckwortt[2]; from podophyllotoxin by Späth[17] and synthetically prepared by Konek and Janovics[10], Oertly and Pictet[11], and Stevens and Robertson[20]. Konek and Janovics[10] followed Perkins'[13] procedure to compound XVI and then oxidized the oxime of this with nitric acid to give hydrastic acid (I) in unstated yield. An alkaline potassium permanganate oxidation of 4,5-methylenedioxyphthalide (XVII) enabled Stevens and Robertson[20] to obtain small amounts of hydrastic acid (I). Compound XVII was obtained by a neutral



XVII

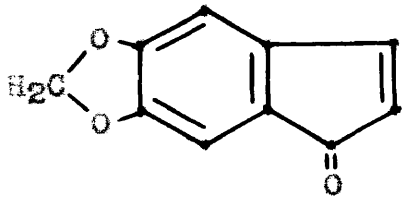


XVIII

potassium permanganate oxidation of a lactone formed by the action of formaldehyde on homopiperonylic acid (XVIII). Oertly and Pictet[11] used a six step procedure starting with piperonal (XIII) that required an oxidation to piperonylic acid followed by nitration to 6-nitropiperonylic acid; its subsequent reduction to the amine; conversion to the nitrile through the diazo reaction and cuprous cyanide; and then a two step hydrolysis to the end product. No yield is given for one critical step, which suggests that the overall yield is small.

DISCUSSION

The initial attempt to synthesize hydrastic acid (I) involved the preparation of 5,6-methylenedioxyhydrindenone-1 (XIX) by the cyclization of 3,4-methylenedioxycinnamic acid (XIV). Oxidation of compound XIX would then give hydrastic



XIX

acid. The cyclization was attempted by using an excess of stannic chloride as the Friedel-Craft catalyst on the acid chloride of compound XIV. Although a tin complex was formed, as evidenced by the formation of a yellow-brown precipitate, only 3,4-methylenedioxycinnamic acid was isolated upon its decomposition. If compound XIV had a cis configuration, or if it had a trans configuration and the double bond was suffiently polarized by the excess stannic chloride, the cyclization should have been sucessful. Its failure indicates that like cinnamic acid, the methylenedioxy analogue has a trans structure of comparable stability.

Of the methods previously used for the preparation of hydrastic acid, the four step scheme of Perkin and Robinson[13] appeared to be the most promising for improvement even though the overall yield from piperonal (XIII) was 2 to 3 percent.

In the present work, these steps have been carried out by different means with the result that hydrastic acid has been obtained in an overall yield of 25 to 27 percent and hydrastic anhydride in an overall yield of 20 to 23 percent. The greatest improvement has been realized in the oxidation step which was effected in 45 percent yield in contrast with less than 10 percent by the old method.

The 3,4-methylenedioxycinnamic acid (XIV) was better prepared by the Doebner modification of the Perkin reaction than by the Claisen condensation of piperonal (XIII) and ethyl acetate[15] as used by Perkin. This procedure gave less than 50 percent yield, while the reaction as carried out by the method of Haworth et al[7] proceeded smoothly to give the desired product in yields of 85 percent and of sufficient purity for use in the next step.

Perkin's reduction of the cinnamic acid by sodium amalgam which was adequate only when carried out in small amounts has been replaced by the catalytic reduction of an aqueous slurry of its sodium salt over Raney nickel. At a temperature of 130°, and with an initial hydrogen pressure of 200 atmospheres, the theoretical amount of hydrogen was taken up in one hour, resulting in a 90 percent yield of 3,4-methylenedioxydihydrocinnamic acid (XV). Longer reaction times, were found to slightly decrease the yield.

The preparation of 5,6-methylenedioxyhydrindone-1 (XVI) had been previously effected by Perkin and Robinson[13] by the cyclization of the dihydrocinnamic acid with phosphorus

pentoxide, and in small yields, through the acid chloride using phosphorus oxychloride and aluminum chloride. It was also prepared on a small scale by Borsche and Eberlein[1] by distillation of the acid chloride under 25 mm. pressure. Of these methods, none proved to be satisfactory. On following the phosphorus pentoxide procedure, a yellowish material was obtained in yields of less than 40 percent of the theoretical amount. Repeated recrystallizations of this crude material from ethanol using activated carbon failed to produce a white product. The yield of crude hydrindone was increased to approximately 50 percent by using an inverse procedure, i.e., adding a benzene solution of the acid to a mechanically stirred and refluxing slurry of phosphorus pentoxide in benzene. However, the crude material was still incapable of of decoloration.

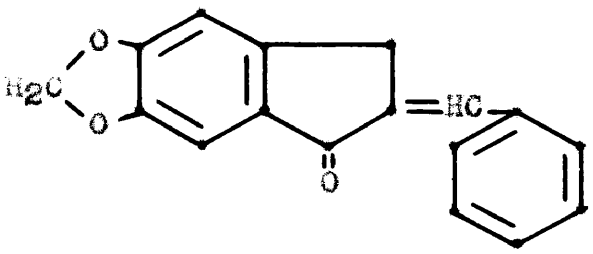
The hydrindone was finally prepared satisfactorily by converting the cinnamic acid to the acid chloride with phosphorus pentachloride and then closing the ring with anhydrous stannic chloride utilizing an inverse addition. In this manner, a tan material was obtained in yields of 85 to 95 percent, depending on the magnitude of the experiment. This material was decolorized to a snow white product through one recrystallization from ethanol with activated carbon.

The various phases of the reaction; the formation of the acid chloride, the formation of the tin complex, and the decomposition of the complex were separately investigated. The acid chloride was formed using both phosphorus pentachloride

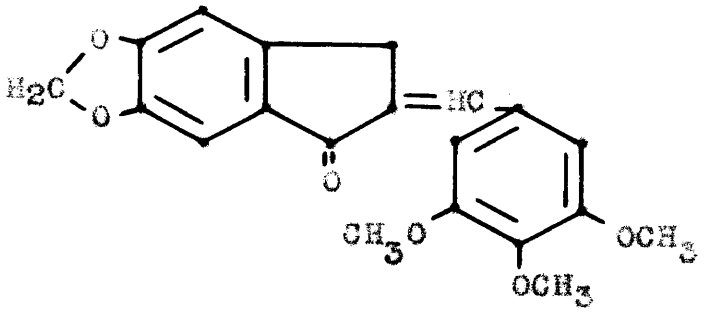
and thionyl chloride. Equivalent results were obtained when an equimolar ratio of phosphorus pentachloride and acid were reacted, however, it was found that increasing the quantity of phosphorus pentachloride decreased the yield of hydrindone; the excess phosphorus pentachloride attacking the methylenedioxy bridge[5]. The tin complex was formed by using both a direct and inverse addition of anhydrous stannic chloride in benzene. The latter method proved to be more satisfactory giving a 10 percent better yield. The usual method of decomposing the tin complex with ice and concentrated hydrochloric acid gave in low yields a slightly tarry product with a heavy hydrochloric acid odor that required a recrystallization from ethanol before obtaining material suitable for the next step. A far superior decomposition was effected by adding the reaction mixture to a mixture of ice and aquous sodium hydroxide; the tin dissolving in the aquous alkaline layer as sodium stannate. However, due to the limited solubility of the hydrindone in benzene, a three phase system resulted with the undissolved hydrindone containing a varying quantity of mechanically occluded salt. This inorganic material was eleminated by dissolving the hydrindone in boiling benzene after isolation by filtration.

The 5,6-methylenedioxy-2-benzylidenehydrindone-1 (XX) was prepared as a derivitive according to the directions of Perkins and Robinson[13] by condensing the hydrindone with benzaldehyde in the presence of concentrated aquous potassium hydroxide as a catalyst. The melting point obtained agreed

with that given by Perkins. A similiar derivitive, 5,6-methylenedioxy-2-(trimethylgallylidene)hydrindone-1 (XXI) was attempted using trimethylgallaldehyde, however, only a small amount of material, insufficient for analysis was obtained.



XX



XXI

The preparation of hydrastic acid (I) from 5,6-methylenedioxyhydrindone-1 (XVI) involved a two-fold problem; that of finding a suitable oxidizing agent with appropriate conditions and secondly, determining a satisfactory isolation and purification procedure. The initial oxidation was carried out with nitric acid in the manner originally prescribed by Perkins and Robinson[13]. A yellow tarry material was obtained that yielded crude crystalline hydrastic acid (I) after recrystallizing from water. However, the quantity was less than 10

percent of the theoretical yield thereby making the step infeasible for our use.

As a result, other oxidizing agents were investigated. Ingold and Piggott[3] had moderate success in oxidizing the 6-nitrohydrindone-1 to the corresponding phthalic acid with potassium permanganate at room temperature. With 5,6-methylenedioxyhydrindone-1 (XVI), under the same conditions, the theoretical amount of potassium permanganate was consumed but no acid whatsoever was isolated. Apparently, this type of oxidation ruptured the bridge before the five-membered ring and hence destroyed the molecule or formed non-acidic materials.

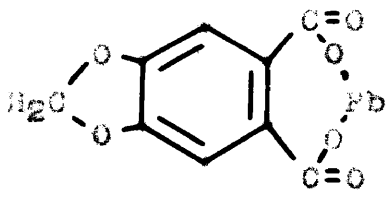
Negative results were also obtained using nitric acid with osmium tetraoxide as catalyst. Here a small amount of acidic material, difficultly soluble in boiling water and melting over 235° was isolated. It may have been homohydrastic acid which is reported to melt at 235°, but because the properties were so unlike hydrastic acid, the compound held no interest and it was not investigated.

The addition of vanadium pentoxide to the nitric acid oxidation procedure increased the yield of crude material from less than 10 percent to over 55 percent, thus giving a satisfactory procedure for the preparation of hydrastic acid (I). During the course of investigating the reaction, the nitric acid concentration was kept constant at 5 percent, but the molar ratio of hydrindone to vanadium pentoxide was varied from 3 to 1 to 30 to 1 and the reaction time from 20 minutes to 4 hours. It was found that with the 5 percent nitric acid

concentration, the best conditions were a 30 to 1 mole ratio of hydrindone to vanadium pentoxide and a 2 hour reaction time. The use of more vanadium pentoxide than that was superfluous. Other reaction periods resulted in decreased yields.

Perkins' isolation procedure consisted in neutralizing the reaction mixture, then acidifying it to congo red paper and evaporating to dryness. Extraction of the residue with ether in a Soxlet apparatus for 48 hours followed by evaporation of the solvent yielded the crude acid. This procedure proved to be unwieldy. It required the use of a highly volatile and flammable solvent over an extended period of time. In its place, a comparatively short and simple means of isolation was developed. The acid was removed from the reaction mixture by precipitation as the lead salt; the free acid being recovered as yellow crystals by dissolving the precipitate in hot 10 percent nitric acid and cooling in an ice-bath. The removal is almost quantitative. Experiments have shown that precipitation occurs with as little as 0.3 mg. of acid per ml. Nevertheless, it was not possible to use the weight of the precipitate as a means of quantitatively estimating the amount of hydrastic acid obtained, for experiments carried out on the composition of the lead hydrastate indicated that it was not precipitated in a constant manner and carried a varying amount of coprecipitated vanadium. The structure of lead hydrastate is given as in figure XXII. Lead analysis of samples precipitated from pure hydrastic acid with an excess of lead acetate showed a varying composition of

around a 4 to 1 ratio of lead hydrastate to lead acetate. However, by dissolving the lead hydrastate in hot dilute nitric



XXII

acid and reprecipitating by the dropwise addition of alkali, an analysis closely approximating the theoretical for figure XXII was obtained indicating the assumed structure to be correct.

The purification of hydrastic acid to a pure white solid was accomplished through three precipitations as the lead salt followed by two recrystallizations from water in the presence of activated carbon. However, to obtain material suitable for use in the next step, only one recrystallization from water was necessary.

Various melting points ranging from 172 to 185° [5,13], have been reported for hydrastic acid. It has been noted that hydrastic acid loses water so rapidly that the reported melting points represented nothing more than the melting point of the particular anhydride-acid mixture at the time of melting. The melting point is greatly dependent upon the rate at which the temperature of the melting point bath is raised, since this determines the composition of the mixture. If hydrastic acid can be said to have a true melting point,

it is approximately 225° as shown in Graph I where the time necessary for a sample to melt at any fixed temperature is plotted against the temperature (Table I). These data were determined by placing samples of pure hydrastic acid in the usual melting point tubes and placing these in a melting point bath held at a fixed temperature. The straight line AB represents the time required for the heat to flow through the glass capillary tube and the sample. If allowance is made for this, then the temperature where immediate melting occurs is about 225°.

In view of the unsuitability of the melting point as a measure of purity, the solubility temperature was determined[14] and found to be 86.5° at a 12:1 ratio of water to hydrastic acid. The solubility temperature at other ratios were also determined (Table II) giving rise to the curve in Graph II where the logarithm of the solubility in grams per liter is plotted against the temperature. From this data the following solubility equation was calculated.

$$\log S = 0.2879 + 0.01384t + 5.82 \times 10^{-5}t^2$$

S = solubility in grams per liter of water

t = temperature in degrees centigrade

The maximum deviation between the observed and the calculated solubility data using the above equation is 2 percent. By differentiation of the above equation and substitution of the appropriate constants, it can be shown that the solubility

temperature is lowered 0.18° for each percent of soluble impurity. Some solubility data calculated from the equation are given in Table III.

The conversion of hydrastic acid to the anhydride (II), was effected by heating to a temperature of 190°. The small amount of acid that remained was separated by recrystallizing the anhydride from benzene in which the acid has a limited solubility. In this manner, hydrastic anhydride was obtained as pure white needles.

TABLE I

The Melting Time of Hydrastic Acid
with Varying Temperatures

Temperature °C.	Time (seconds)
189.5	124
193.5	80
197.5	55
204.5	34
213.0	23
215.5	15
220.0	13
223.5	7
226.5	6
231.0	6
240.5	5
251.0	2

TABLE II

Experimentally Determined Solubilities

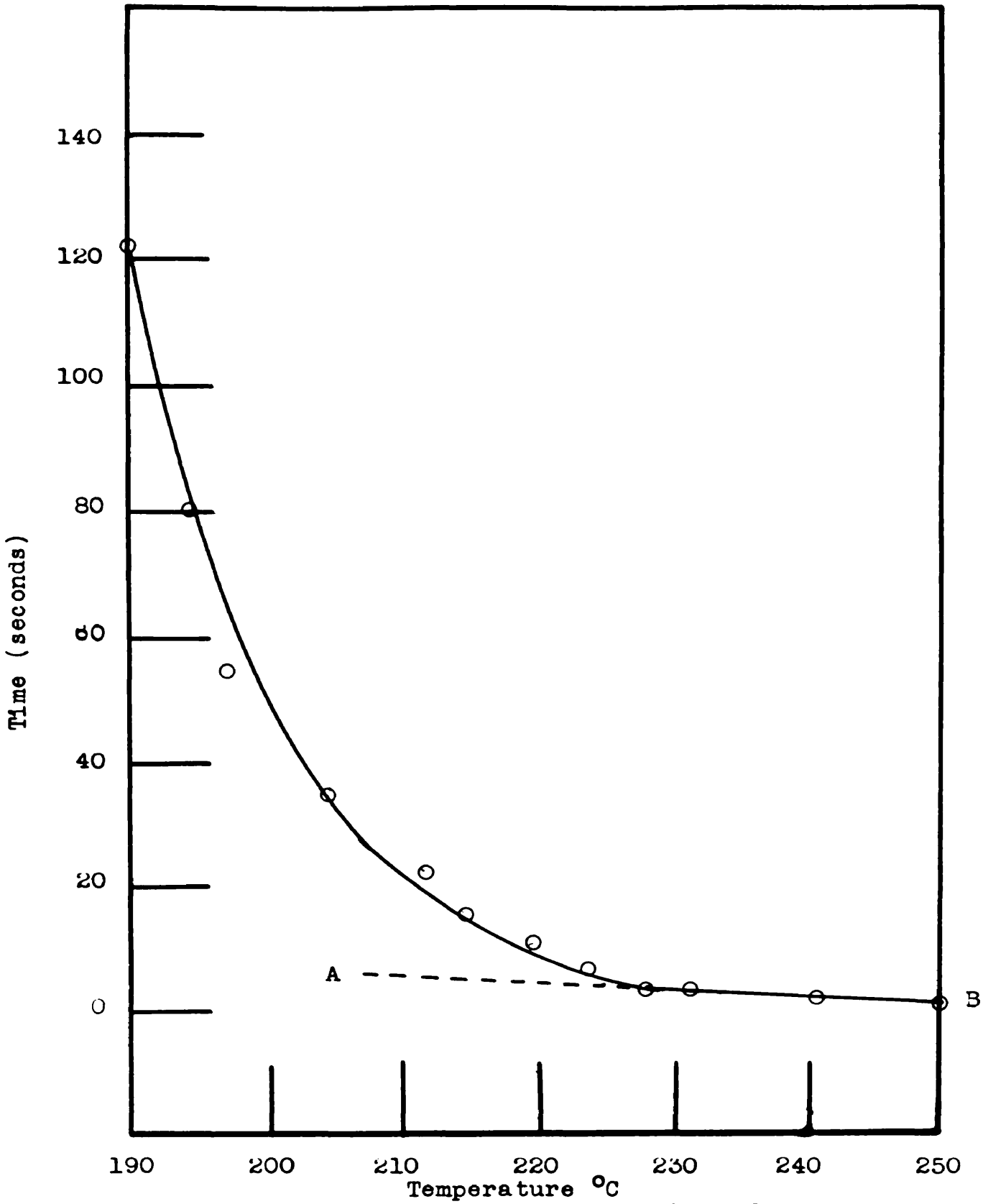
of Hydrastic Acid in Water

Grams Solid per 1000 Grams Water	Temperature °C
83.3	86.5
40.0	73.8
20.0	58.7
10.0	42.8
6.67	33.9

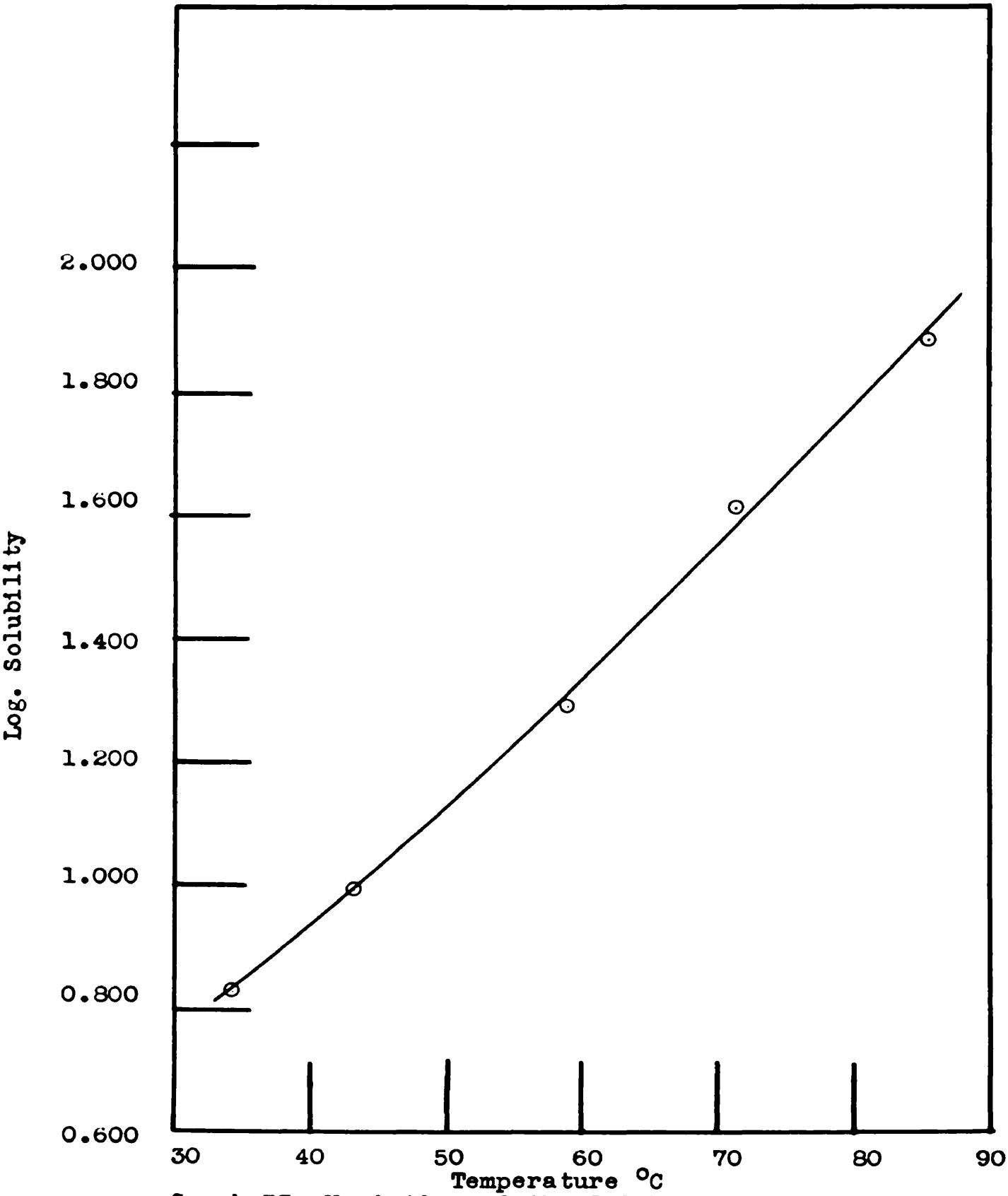
TABLE III

Solubility of Hydrastic Acid in Water

Temperature	g./l. water
20°	3.9
40°	8.6
60°	21.3
80°	57.0
100°	180.0



Graph I; Variation of the Melting Time of Hydrastic Acid with Temperature



Graph II: Variation of the Solubility of Hydrastic Acid in Water with Temperature

EXPERIMENTAL

All melting points are corrected.

Preparation of 3,4-Methylenedioxycinnamic Acid (XIV). This procedure is the same as that outlined by Haworth[7] but on a larger scale. Six hundred and seventy-five grams (4.5 mole) piperonal, 900 grams (8.65 mole) malonic acid, 1700 ml. dry pyridine and 45 ml. piperidine were used giving a yield of 728 grams (84 per cent of the theoretical amount) of a light yellow product melting at 238.5-240°. The recorded melting point is variously reported to be from 233-242°.[9,7]

Attempted Cyclization of 3,4-Methylenedioxycinnamic Acid (XIV). In preparing the acid chloride of 3,4-methylenedioxycinnamic acid (XIV), 7.3 grams (0.037 mole) of phosphorus pentachloride was added in increments to a mechanically stirred and chilled solution of 5 grams (0.023 mole) of acid in 200 ml. anhydrous benzene. The reaction mixture was then stirred at room temperature for 3 hours in attempt to drive it to completion. However a small quantity of material failed to dissolve. This was filtered off, and on recrystallizing from ethyl alcohol, a white material, m.p. 234-238° was obtained. The residue was shown to be the cinnamic acid since the material showed no depression in the melting point when mixed with an authentic sample.

The cyclization was attempted by adding 22 grams

(0.085 mole) of anhydrous stannic chloride in 50 ml. dry benzene to the chilled filtrate and mechanically stirred for 15 minutes. A brown tin complex was formed almost immediately. The complex was decomposed with ice and 75 ml. of concentrated hydrochloric acid to give a three phase system; an acidic aqueous layer, a benzene phase and an insoluble residue. The residue proved to be a tin salt of the cinnamic acid for after treating it with sodium hydroxide, acidifying and recrystallizing the precipitate from ethyl alcohol, no depression in the melting point was obtained when mixed with an authentic sample. The benzene was separated from the aqueous layer and washed with 10 per cent aqueous sodium hydroxide. Acidification of the alkaline washings produced a material that again gave no depression in the melting point when mixed with a pure sample of the starting material. Evaporation of the benzene layer after washing with water gave only a slight tarry residue from which nothing was obtained. In summary, there was no evidence that any 5,6-methylenedioxyhydrindenone-1 (XIX) was formed.

Preparation of 3,4-Methylenedioxydihydrocinnamic Acid (XV). A slurry of the sodium salt of 3,4-methylenedioxy-cinnamic acid (XIV), prepared by adding 380 grams (1.98 mole) of the acid to 1300 ml. distilled water containing 80 grams (2.00 mole) sodium hydroxide, was placed in a steel hydrogenation vessel along with 50 grams of Raney nickel catalyst. Hydrogen was admitted until a pressure

of 2000 pounds per square inch was reached. The vessel was shaken and heated to 130°. The reaction started at 100°, and the theoretical amount of hydrogen was absorbed in 90 minutes. The vessel was washed out with distilled water and the catalyst filtered off. The filtrate was acidified with concentrated hydrochloric acid to congo red paper. Water was added as necessary to thin the thick slurry of the insoluble acid which precipitated. After filtering and air-drying, 345 grams (90 per cent of the theoretical amount) was obtained melting at 83-84°. The literature reports a m.p. of 87-88°.[13] This light tan colored material was satisfactory for the next step. A pure white product, m.p. 87-88°, was obtained after two recrystallizations from water using 2 grams acid and 0.4 grams Darco G-60 per 100 ml. water. The yield after two recrystallizations was 1.4 grams.

Preparation of 5,6-Methylenedioxyhydrindone-1 (XVI).

1) Cyclization with Anhydrous Stannic Chloride.

The acid chloride of the dihydrocinnamic acid (XV) was prepared by adding 208 grams (1 mole) of phosphorus pentachloride in small increments to a chilled and mechanically stirred solution of 194 grams (1 mole) of the crude 3,4-methylenedioxydihydrocinnamic acid, dissolved in 1500 ml. of dry, thiophene free benzene. After the addition was complete, the reaction mixture was stirred for 1.5 hours at room temperature.

The cyclization was carried out by the dropwise addition over a 3 hour period of the above benzene solution

to a chilled and stirred solution of 521 grams (2.0 mole) of anhydrous stannic chloride dissolved in 300 ml. of dry thiophene free benzene. A brown insoluble tin complex formed almost immediately. After the addition was complete, the stirring was continued for 15 minutes, and then 300 ml. of ordinary ether was added. This changed the solid tin complex to a liquid, although it was still insoluble in benzene. The complex was decomposed by pouring this reaction mixture into a mixture of 4 l. of 6 N sodium hydroxide and 2 to 3 liters of chopped ice and stirring for 15 minutes. This gave a three phase system consisting of a benzene solution of the hydrindone and a suspension of the hydrindone in the alkaline aqueous layer. The benzene layer was separated, and the hydrindone suspended in the alkaline solution allowed to stand overnight. It was then filtered, washed with tap water, thoroughly air-dried, dissolved in 5 liters of boiling benzene to remove the inorganic salts, and filtered while hot. The benzene solutions were combined and steam distilled to remove the benzene, and the resulting insoluble hydrindone filtered and washed with water. A light yellow material of 148 grams (83 per cent of the theoretical amount) melting at 159-164° was obtained which was pure enough for the next step. A pure white product was obtained by crystallizing 10 grams of the hydrindone from 150 ml. of a 2:1 ethanol-water mixture in the presence of 3.5 grams of activated carbon. The

was obtained 8.2 grams, m.p. 163-164°. The previously reported values were 160°[5] and 161°[1].

The above inverse addition procedure when applied on a 20 gram (0.11 mole) scale gave a yield of 94.5 per cent of the theoretical amount. A slightly lower yield of 88.5 per cent was obtained when the direct addition of anhydrous stannic chloride in benzene to the benzene solution of the acid chloride was used on a comparative scale.

The acid chloride of 3,4-methylenedioxydihydrocinnamic acid was also prepared using thionyl chloride. To a chilled solution of 50 ml. of dry ether containing 3 drops pyridine, 10 ml. (0.125 mole) commercial thionyl chloride and 20.0 grams (0.11 mole) of the acid was added. The reaction was allowed to proceed with periodic swirling until the solid had gone into solution and then swirled occasionally at room temperature for 15 minutes. The ether was removed under vacuum leaving a slightly viscous yellow liquid that was dissolved in 100 ml. of anhydrous thiophene free benzene and chilled. Cyclization was effected by adding to this solution, at one time, 65 grams (0.25 mole) anhydrous stannic chloride in 50 ml. dry benzene with mechanical stirring. The remainder of the reaction and isolation procedure was carried out as previously described yielding 18.1 gram (38 per cent of the theoretical amount) of a pale yellow product melting at 158-161°.

2) Cyclization with Phosphorus Pentoxide. The

procedure of Perkin and Robinson[13] was used in this preparation. One hundred grams (0.7 mole) of phosphorus pentoxide was added over a one hour period to a mechanically stirred and refluxing solution of 20 grams (0.11 mole) of 3,4-methylenedioxydihydrocinnamic acid (XV) in 100 ml. of anhydrous benzene. The solution was refluxed for 3 hours during which time a red purple complex was formed. Decomposition of this complex was effected by the addition of ice and resulted in a 3 phase system, an acidic aqueous layer, a benzene layer and a brown insoluble material. The precipitate was filtered off and the aqueous and benzene layers separated. By washing the benzene layer with 500 ml. of 5 per cent potassium hydroxide followed by 250 ml. of water and evaporating to dryness, 2.5 grams of a yellow material melting at 158-161° was obtained. An additional 3.5 grams of a brown product melting at 156-160° was isolated from the insoluble material by washing with 5 per cent potassium hydroxide until the washings were colorless, followed by washing with water and air-drying. This gave a total of 6.5 grams (31.3 per cent of the theoretical amount) of the crude hydrindone-1.

By using an inverse procedure, i. e., adding a benzene solution of the acid to a refluxing and mechanically stirred suspension of the phosphorus pentoxide in benzene, a yield of 48.5 per cent of the theoretical amount was obtained.

Condensation of 5,6-Methylenedioxyhydrindone-1 (XVI).

1) With Benzaldehyde. The directions of Perkins and Robinson[13] were followed in this preparation. Two grams (0.0114 mole) of 5,6-methylenedioxyhydrindone-1 (XVI) and 3 grams (0.028 mole) of freshly distilled benzaldehyde were dissolved in 25 ml. of ethyl alcohol with gentle heating. Three drops of concentrated potassium hydroxide was added and the mixture warmed for 5 minutes. A yellow solid crystallized out almost immediately. This was filtered off, washed with 50 per cent ethyl alcohol and air-dried giving 2.8 grams (96.5 per cent of the theoretical amount) of crude 5,6-methylenedioxy-2-benzylidenehydrindone-1 (XX) m.p. 199-201°. Crystallization of the crude material gave a pale yellow product, m.p. 201-202°.

2) With Trimethylgallaldehyde. In a similar manner 1 gram (0.006 mole) hydrindone-1 (XVII) and 2.9 gram (0.075 mole) trimethylgallaldehyde was reacted in the presence of 3 drops concentrated potassium hydroxide. However, only 0.1 gram (5.1 per cent of the theoretical amount) of crude 5,6-methylenedioxy 2-trimethylgallylidene hydrindone (XXI), melting at 214-220° was obtained. Crystallization from benzene gave a yellow powder, m.p. 218-220°.

Preparation of Hydrastic Acid (I).

1) Oxidation with Nitric Acid and Vanadium Pentoxide. A mixture of 100 grams (0.57 mole) of 5,6-

methylenedioxyhydrindone-1 (XVI), 6 grams (0.03 mole) vanadium pentoxide, 1200 ml. distilled water, and 300 ml. concentrated nitric acid was heated in a 5 liter flask under reflux until the reaction started, and then for 1.5 hours with occasional swirling, after the violent phase of the reaction had subsided. The hot reaction mixture was quickly filtered through asbestos to remove the vanadium pentoxide before some of the hydrastic acid crystallized. The filtrate was neutralized to litmus paper with 15 per cent sodium hydroxide solution, acidified with glacial acetic acid to a congo red end-point, and then heated to boiling. Lead acetate solution (500 ml. of a 10 per cent solution) was added until the lead salt of hydrastic acid was completely precipitated, the mixture allowed to cool, filtered and the yellowish-white precipitate washed with three 100 ml. portions of 3 per cent acetic acid.

The lead salt was converted to crude hydrastic acid by making a slurry with 900 ml. of tap water, heating to boiling, and adding concentrated nitric acid (75 ml.) dropwise from a burette until solution was effected. The hot solution was filtered by gravity through a heated funnel, and the filtrate cooled slowly to ice-bath temperature. The resulting crystals were filtered, washed with two 75 ml. portions of ice-water, and air-dried giving 66 grams of a yellow product. An additional 17 grams of crude hydrastic acid was obtained from the filtrate and

washings by neutralizing as before to precipitate the hydrastic acid as lead hydrastate, filtering, washing, and converting this to the crude hydrastic acid as above. The crude materials obtained in this manner were often contaminated with small amounts of lead hydrastate.

The combined crude fractions of hydrastic acid were purified by two precipitations as the lead salt and a recrystallization from water. This was done by dissolving the material in 300 ml. boiling tap water (adding a little nitric acid if an appreciable amount of the insoluble lead hydrastate was present) and precipitating the lead hydrastate with 500 ml. ten per cent lead acetate solution. The lead salt was filtered, dissolved again in dilute nitric acid, filtered, neutralized to litmus paper with sodium hydroxide solution, acidified to congo red paper with acetic acid as before, and the lead hydrastate then converted to hydrastic acid as before. The yield was 57 grams of a pale yellow product with a solubility temperature of 86.1° at 12:1 ratio (corresponding to a purity of 98 per cent). An additional 5 grams was recovered from the filtrate by precipitating the lead salt, etc., as previously described. The combined 62 grams of hydrastic acid was recrystallized from 620 ml. of distilled water, using 6 grams of Darco G-60 to remove the color. In this manner, 53 grams (44.5 per cent of the theoretical amount) of an almost white product with a solubility temperature of 86.5° at

12:1 was obtained. This material was used to prepare the anhydride.

A pure white product, having the same solubility temperature, was obtained after three more recrystallizations from water with activated carbon.

Analyses	Calculated for $C_9H_6O_6$	Found
Carbon	51.44%	51.36% 51.40%
Hydrogen	2.88%	2.94% 2.88%

Neutralization equivalents of 104.7 and 104.8 (Theory, 105.0) were obtained by dissolving the hydrastic acid in hot distilled water and titrating while hot with a standard solution of sodium hydroxide using the usual precautionary measures to eleminate the interference of carbon dioxide.

2) Oxidation With Nitric Acid with no Catalyst. The method of Perkin and Robinson[13] was used in this procedure. Five grams (0.023 mole) of 5,6-methylenedioxyhydrindone-1 (XVI) was digested with 15 ml. of concentrated nitric acid in 60 ml. of distilled water for four hours during which time the solid completely dissolved giving a yellow solution. The reaction mixture was cooled, neutralized with 10 per cent sodium hydroxide, acidified to congo red paper with 5 per cent hydrochloric acid and evaporated to dryness. The resulting residue was

extracted in a Soxlet apparatus for four days with diethyl ether. Evaporation of the ether on a steam bath yielded 2.5 grams of brown tarry residue. Three recrystallizations of this residue from water in the presence of Darco G-60 resulted in 0.5 grams (8.4 per cent of the theoretical amount) of a white product melting at 171.5-172.0°. Various melting points ranging from 172° to 188°[5,13] were previously reported for hydrastic acid.

3) Oxidation With Potassium Permanganate. Six hundred milliliters of a 3 per cent potassium permanganate solution was added dropwise to a mechanically stirred suspension of 5 grams (0.023 mole) of 5,6-methylenedioxyhydrindone-1 (XVI) in 50 ml. of 2 N sodium hydroxide at room temperature. The voluminous quantity of manganese dioxide formed was filtered off and washed with hot water. The combined filtrate and washings was acidified to congo red with dilute hydrochloric acid and evaporated to dryness. A two day ether extraction of this residue employing a Soxlet apparatus gave only a slight yellow residue on evaporation of the solvent.

4) Oxidation with Nitric Acid and Osmium Tetroxide. Five grams (0.023 mole) of 5,6-methylenedioxyhydrindone-1 (XVI) was digested for four hours with 15 ml. concentrated nitric acid in 50 ml. distilled water and 0.1 g. osmium tetroxide in 10 ml. distilled water. The reaction solution was chilled, neutralized with 10 per cent sodium hydroxide, acidified to congo red paper with

5 per cent hydrochloric acid and then evaporated to dryness. The residue was digested with four 25 ml. portions of boiling dioxane to separate the organic from the inorganic material. On evaporation of the dioxane to dryness, 0.65 grams of material with a melting point greater than 250° and only slightly soluble in water was obtained. These properties did not correspond to hydrastic acid.

Preparation of Hydrastic Anhydride (II). Fifty grams (0.24 mole) of hydrastic acid (I) was placed in a 1 liter beaker and heated with hand stirring in an oil bath maintained at 190° for five minutes after the entire mass had melted. The beaker was then removed and the melt was allowed to slowly solidify with constant stirring by hand. In this manner, 44.1 grams (94.6 per cent of the theoretical amount) of a light tan product, m.p. 178.5-181° was obtained. The literature reports the melting point as 175°[1].

The crude hydrastic anhydride was purified by recrystallizating from 1 liter of benzene in the presence of 9 grams of Darco G-60. This yielded 29.2 grams of a pure white product, with a melting point 179-180°. An additional 9 grams of white material, m.p. 177.5-179.5° was isolated by concentrating the filtrate to 200 ml.

Analysis	Calculated for $C_9H_4O_5$	Found
Carbon	56.59%	56.54% 56.42%
Hydrogen	2.11%	2.31% 2.23%

Due to the sublimation of the hydrastic anhydride at temperatures above 150°, a sealed tube was used in obtaining the melting points.

BIBLIOGRAPHY

1. Borsche, W., and W. Eberlein, Ber., 47, 1469 (1914).

2. Danckwortt, H., Arch. Pharm., 250, 643 (1930).

3. Dareckson, W. M. III., "The Synthesis of Compounds Related to Podophyllotoxin", Thesis, University of Maryland, College Park, Maryland. (1950).

4. Freund, M., and S. Lachman, Ber., 22, 2325 (1889).

5. Freund, M., Ann., 271, 343 (1892).

6. Goldschmiedt, G., Monatsch., 9, 779 (1888).

7. Haworth, R. D., W. H. Perkins, Jr., and J. Rankin, J. Chem. Soc., 125, 1693 (1924).

8. Ingold, C. K., and H. A. Piggott, J. Chem. Soc., 123, 1499 (1923).

9. Johnson, J. R. "Organic Reactions", John Wiley and Sons, Inc. New York, New York, Vol. 1, p. 249 (1942).

10. Konek, S., and R. Janovics, Math. naturw. Anz. unger. Akad. Wiss., 42, 210-2 (1920).

11. Oertly, E., and A. Pictet, Ber., 43, 1333 (1910).

12. Perkin, W. H., Jr., J. Chem. Soc., 57, 1095 (1890).

13. Perkin, W. H., Jr., and R. Robinson, J. Chem. Soc., 91, 1080 (1907).

14. Reeve, W., and R. K. Adams, Anal. Chem., 22, 755 (1950).

15. Reychler, S., Bull. soc. chim. France, (s) 17, 515 (1897).

16. Rossin, L., Monatsch., 12, 493 (1891).

17. Spath, E., F. Wessely, and E. Nadler, Ber., 65, 1773 (1932).

18. Spath, E., and L. Kahovec, Ber., 67B, 1501-6 (1934).

19. Sterling, J. D. Jr., "The Synthesis of Intermediates Leading to Anti-carcinogenic Compounds", Thesis University of Maryland, College Park, Maryland. (1949).

20. Stevens, T. S., and M. C. Robertson, J. Chem. Soc., 2790-2 (1927).

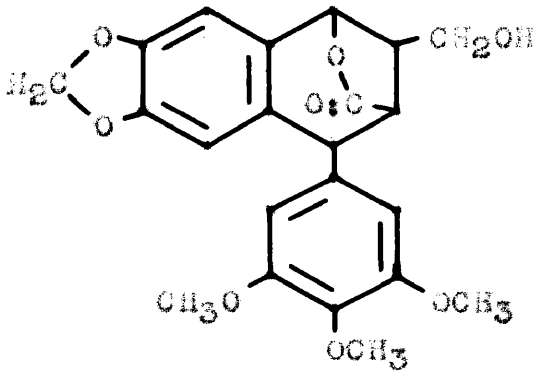
21. Wegscheider, R., Monatsch., 3, 367 (1882).

Part II

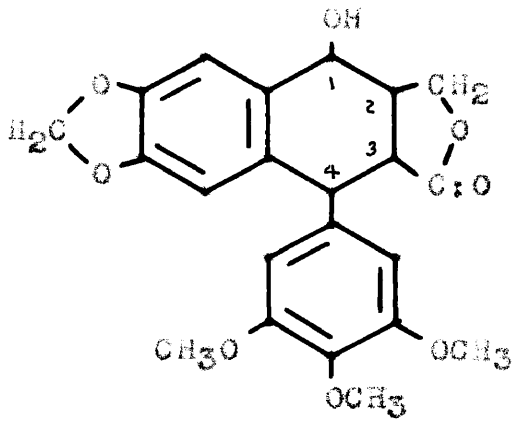
Studies on the Degradation of Picropodophyllin

INTRODUCTION

The early work on podophyllotoxin, its chemistry and its relationship to picropodophyllin that have led to the postulated structures given in figures I and IA has been reviewed in the British Annual Reports on the Progress of Chemistry[8,9] and more recently by Sterling[17]. Consequently



I



IA

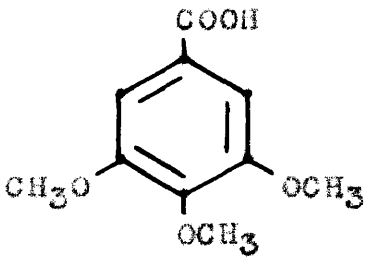
no expansive review on this early work will be attempted in this thesis.

Recently, podophyllotoxin was found to possess anti-carcinogenic activity[4,5,10] with the result that interest in its structure was revived. Considerable doubt has now been cast upon the position of the lactone ring as pictured in figure I for podophyllotoxin. Price[11], at this institution, has succeeded in obtaining two different trihydroxy compounds by the lithium aluminum hydride reduction of podophyllotoxin and picropodophyllin. If the structures as presented were correct, assuming both podophyllotoxin and picropodophyllin to have the same steric configuration at carbon 3, then the

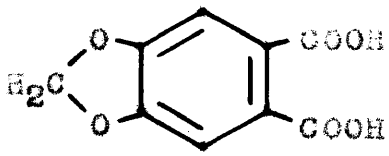
same trihydroxy compound should have been obtained from both sources. In addition, Hartwell and Schrecker[7] have obtained acetylpicropodophyllin and benzoylpicropodophyllin in good yields by refluxing the corresponding derivatives of podophyllotoxin with sodium acetate in various solvents. These reactions can only be satisfactorily explained by inversion of the carboxyl group through enolization. It is now believed that podophyllotoxin and picropodophyllin are not structural isomers but diastereoisomers represented by IA and differing only in the configuration around carbon 3.

DISCUSSION

The structure of podophyllotoxin, as it is believed to be at this time (IA), contains a free hydroxyl group at the C_1 position. It has been placed there by virtue of eliminating the other possible positions rather than by direct evidence. Attempts to date to oxidize or dehydrogenate the hydroxyl group to a ketone have failed. Podophyllotoxin and picropodophyllin were both oxidized to trimethylgallic acid (II) with hot potassium permanganate solution[2,14]. Hydrastic



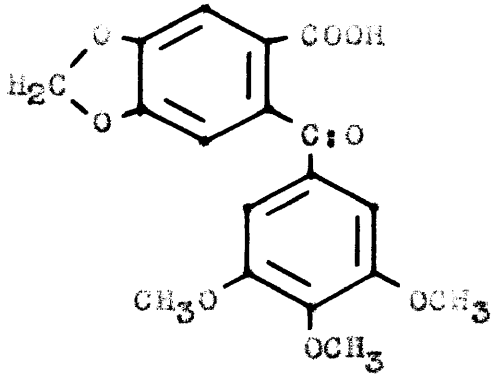
II



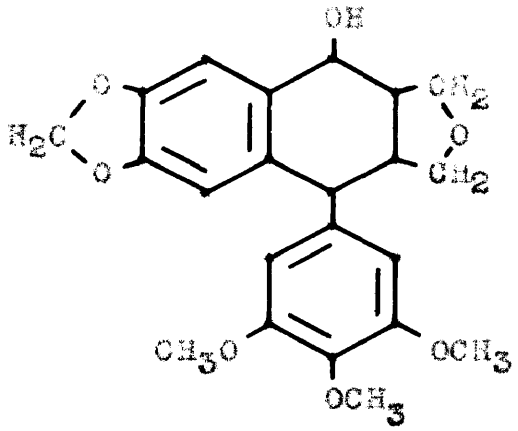
III

acid (III) was obtained by the oxidation of podophyllotoxin with alkaline potassium permanganate solution[15]. By more careful oxidation of picropodophyllin with alkaline potassium permangate at 50°, Späth[16] was able to isolate the keto acid IV. This showed that the hydroxyl group was not on carbon 4. Numerous attempts were also made by Price[7] to oxidize picropodophyllin and the anhydro compound V by using potassium dichromate and chromic oxide in glacial acetic acid and in acetone, by using aluminum tertiary butoxide and cyclohexanone, and with benzoquinone under ultra-violet irradiation. In

most cases, a theoretical amount of the oxidizing agent was consumed, however, as much as 60 percent of the starting material was recovered after all the oxidizing agent was con-

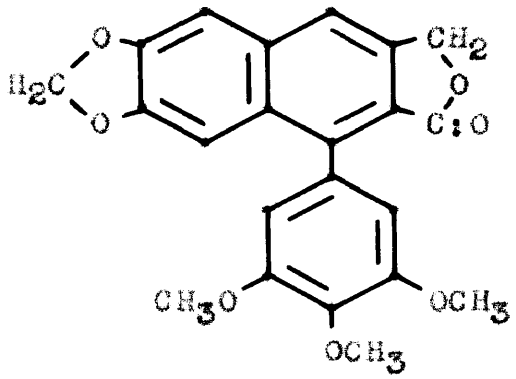


IV



V

sumed. This fact indicated extensive degradation, and that the tetralone first formed (assuming a secondary alcohol) was more easily oxidized than the alcohol itself. Dehydrogenation attempts by Späth[14] using both podophyllotoxin and picropodophyllin with palladium at 230° yielded only the lactone VI.



VI

On the other hand, attempts to prove the existance of a

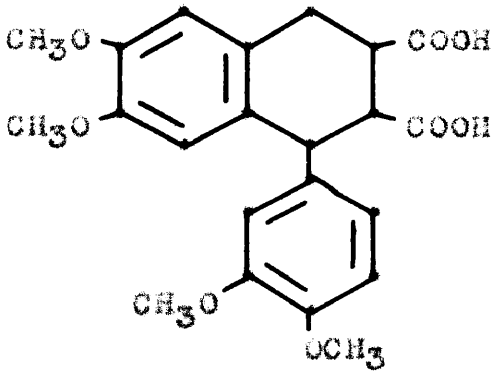
tertiary alcohol (i.e., the hydroxyl group at the C_2 or C_3 position) have also failed. Hartwell and Schrecker[6] attempted to oxidize picropodophyllin with periodic acid. If a tertiary alcohol were present, then formaldehyde would have been liberated by the reaction. None was detected.

This phase of the research represents another attempt to obtain direct evidence as to the position of the free hydroxyl group. The picropodophyllin used in this work was prepared from podophyllotoxin in three ways: Treatment with sodium acetate[1] giving a 55 percent yield of crude picropodophyllin; treatment with sodium carbonate[1] giving a 91 percent yield of crude material and treatment with piperidine[13] giving a 20 percent yield of crude material. Of the three methods, the use of sodium acetate proved to be best for the product obtained had a higher melting point and was more easily purified than those obtained by the other methods. Considerable difficulty was encountered in obtaining a method for satisfactorily purifying the picropodophyllin. A white product melting at 229° was finally isolated by recrystallizing the crude material from 60 percent ethanol followed by a slow recrystallization from ethyl acetate. This melting point was slightly higher than those reported by other investigators[11,1,13].

The purified picropodophyllin was shaken with a copper chromite catalyst in an ethylene atmosphere of 150 psi. at 280° for two hours (the ethylene acting as a hydrogen acceptor) in an attempt to dehydrogenate the compound to a

ketone. Reeve and Adkins[12] have successfully used this method in preparing aliphatic aldehydes and ketones from the corresponding primary and secondary alcohols. In this case, the crude material obtained from the reaction appeared to be a mixture of two products. By utilizing a fractional crystallization from aqueous ethanol, a white solid melting at 270-271° was isolated. This corresponded fairly closely to the melting point of dehydroanhydropicropodophyllin (VI) previously obtained by Spath[14] by the dehydrogenation of picropodophyllin with palladium. The carbon, hydrogen and methoxyl analysis obtained differed slightly from the theoretical values for compound VI but were close enough to enable one to assume the two to be identical. A small amount of a second substance melting at 254-255° with decomposition was also isolated. However its purity was unknown, and there was not enough material for an analysis. Qualitatively, it appeared to form a 2,4-dinitrophenylhydrazone that melted with decomposition at 234°. But again there was no absolute proof for the hydrazone formation. In short, a ketone might have been formed in the reaction. If it was formed, it was isolated in too small an amount to permit obtaining any conclusive evidence. A further investigation into this reaction would necessitate the use of a larger quantity of starting material and the development of a more satisfactory means of isolation and purification.

The remainder of the degradation work comprised the oxidation of picropodophyllin in different solvents using lead tetraacetate. This was another attempt to break off formaldehyde if a tertiary alcohol was present. There was also the possibility that with a secondary alcohol, a dehydrogenation might occur to give a naphthol-1 derivative. Erdtmann[3] discovered that the tetralin derivative VII was smoothly dehydrogenated into a naphthalene derivative.



VII

Picropodophyllin was treated with lead tetraacetate using both benzene and glacial acetic acid as solvents. In both cases, more than 50 per cent of the starting material was recovered. In addition, no trace of formaldehyde was detected using dimedone and 2,4-dinitrophenylhydrazine as reagents. Similar results were obtained when sodium podophyllate was subjected to the lead tetraacetate treatment in dilute acetic acid. These negative results are inconclusive in respect that they do not entirely eliminate the possibility of a tertiary alcohol. Under the conditions

imposed, the C_2 methylol group may have been in the lactone form making oxidation to the formaldehyde difficult. A much more satisfactory compound for use would have been the trihydroxy compound from which the anhydro compound V was derived. Again further investigation into this possibility was left for the future.

EXPERIMENTAL

All melting points are corrected.

Preparation of Picropodophyllin (IA).

1) With Sodium Acetate. The method of Borsche and Niemann[1] was followed in this preparation. Fifteen grams (0.0363 mole) of podophyllotoxin (IA) as obtained from S. B. Penick Company, 335 ml. 95 per cent ethanol and 170 ml. 5 per cent sodium acetate solution were refluxed for 23 hours. At the end of this period, 200 ml. of solvent was distilled off, and remainder being filtered and chilled in an ice-bath. In this manner, 8.05 grams (54 per cent of the theoretical amount) of crude material was obtained. The product had a melting point of 219-223° after drying under 1 mm. pressure at 116°.

2) With Sodium Carbonate. In the manner of Borsche and Niemann[1], 10 grams (0.0145 mole) podophyllotoxin (IA) that had been dried at 100° for 3 hours, 250 ml. methanol and 100 ml. 2N sodium carbonate were refluxed for 3 hours. The reaction mixture was then acidified to congo red paper with 2N hydrochloric acid and 100 ml. of solvent distilled off. The remainder was filtered and chilled in an ice-bath giving 6.9 grams of product after drying at 115° for 3 hours under 1 mm. pressure. This melted at 218-227°. An additional 2.20 grams melting at 202-205° was obtained by concentrating the filtrate to 100 ml. A total of 9.1 grams (91 per cent of the theoretical

amount) of crude material thus was obtained.

3) With Piperidine. Ten grams (0.0145 mole) of podophyllotoxin (IA) that had previously been recrystallized from 50 per cent ethanol and dried under 1 mm. pressure at 116° was refluxed with 600 ml. 60 per cent ethanol and 2 ml. piperidine as prescribed by Robertson and Waters.[13] The reaction mixture was then filtered and chilled in an ice-bath. The resulting crystals were filtered off and dried yielding 2.0 grams (20 per cent of the theoretical amount) melting at 220-223°.

The crude material in all cases was purified by recrystallizing from 50 per cent ethanol followed by a slow recrystallization from ethyl acetate. A 60 per cent overall recrystallization yield was obtained with the crude material from the sodium acetate and piperidine conversions but less than 30 per cent was obtained from the sodium carbonate conversion. The material obtained thusly, melted at 228.5-229°. Previously reported values ranged from 223-227°.[11,1,13]

Due to the tendency to decompose at the melting point, a sealed evacuated tube was used to determine this physical constant for picropodophyllin.

$$\alpha\Big]_0^{25} = 0$$

c = 1.038 g. / 100 ml. chloroform

Preparation of Sodium Podophyllate. The method of Borsche and Niemann[1] was followed. Three grams (0.0072 mole) of picropodophyllin (IA) melting at 228-229°, 45 ml. absolute ethanol and 9 ml. saturated alcoholic sodium hydroxide solution were heated on a water bath for 15 minutes. A white solid precipitated almost immediately. At the end of the reaction period, the mixture was cooled to room temperature and 10 ml. ether added to decrease the solubility of the sodium podophyllate. The resulting white precipitate was filteredd off, washed with ether and dried in vacuo at room temperature. In this manner, 3.1 grams (99 per cent of the theoretical amount) was obtained.

Dehydrogenation of Picropodophyllin (IA). Three grams (0.0072 mole) of picropodophyllin (IA) melting at 228-229° and 0.2 gram copper chromite catalyst was placed in a 100 ml. steel reaction vessel. Ethylene was introduced to a pressure of 150 psi. The vessel was then shaken at 230° for 2 hours. At the end of this time, the vessel was cooled to room temperature, the contents washed out with five 50 ml. portions of chloroform and the catalyst filtered off. Addition of an equal volume of petroleum ether to the filtrate precipitated a tan colored material. This material was purified by dissolving in 100 ml. absolute ethanol and then adding enough distilled water to make a 70 per cent ethanol solution. Upon refrigeration, a white material crystallized out that was separated with a centrifuge. The supernatent liquid

was further diluted to 40 per cent ethanol with additional water and another fraction was obtained. This purification procedure was repeated twice more on each fraction. As a result, a white material was isolated from the 70 per cent alcohol that melted at 271-272°. Spath[14] reported 266° for dehydroanhydropicropodophyllin (VI).

Analysis	Calculated for $C_{22}H_{18}O_7$	Found
Carbon	67.00%	66.39% 66.35%
Hydrogen	4.60%	4.79% 4.68%
Methoxyl	22.08%	23.14% 22.34%

A small quantity of white material was also isolated from the 40 per cent ethanol fraction that melted at 255-256° with decomposition. The amount, however, was too small for an element analysis but it did appear to give a qualitative 2,4-dinitrophenylhydrazine test. Here again, the results were inconclusive.

Oxidation of Picropodophyllin (IA) with Lead Tetraacetate.

1) In Benzene. One gram (0.0034 mole) picropodophyllin (IA) and 2.5 grams (0.006 mole) lead tetraacetate were dissolved in 600 ml. hot anhydrous benzene. The solution was refluxed for ½ hour, at which time, it was cooled to room temperature by standing overnight. During this period, an orange-brown solid appeared that was filtered off. The residue appeared to be entirely inorganic for it gave no carbonization on ignition. The benzene filtrate was

washed with five 100 ml. portions of water and evaporated to dryness giving 0.7 gram of a solid yellow residue. The aqueous layer gave a positive test for lead, a negative test for the phenolic group using ferric chloride and a negative test for formaldehyde using both dimedone and 2,4-dinitrophenylhydrazine. The residue obtained by the evaporation of the benzene was dissolved in 25 ml. 95 per cent ethanol. On chilling, a white material crystallized out that weighed 0.4 gram and melted at 205-210° after filtering and drying in vacuo. Dilution of the filtrate with an equal volume of water gave an additional 0.2 grams of material melting at 198-202°. Both solids gave no depression in the melting point when mixed with a pure sample of picropodophyllin.

2) <u>In glacial Acetic Acid.</u> To 1 gram (0.0024 mole) picropodophyllin dissolved in 60 ml. of glacial acetic acid, 2.6 gram (0.0055 mole) lead tetraacetate in 70 ml. glacial acetic acid was added and mechanically stirred for 20 minutes at 70-75°. At the end of this time, the reaction mixture was poured into 400 ml. distilled water and allowed to stand overnight. During this time, a tan insoluble material appeared that was filtered off. On drying in vacuo, 0.6 gram of material was obtained that melted at 204-212°. A mixture of this product with pure picropodophyllin melted at 219-223°. The filtrate gave a negative test for the phenolic group using ferric chloride, and also gave a negative test for formaldehyde using dimedone

and 2,4-dinitrophenylhydrazine.

Treatment of Sodium Podophyllate with Lead Tetraacetate.

A solution of 3.5 gram (0.009 mole) lead tetraacetate in 50 ml. warm glacial acetic acid was added dropwise over a one hour period to a mechanically stirred solution of 3.3 grams (0.0072 mole) of sodium podophyllate in 100 ml. water maintained at 30-35°. An orange-red color developed. On standing overnight, a white solid appeared. After filtering and drying in vacuo, the material weighed 0.3 grams and melted at 216-219°. The filtrate was diluted with 300 ml. of water producing an additional quantity of a light tan solid that weighed 2.2 grams and melted at 200-204°. Both solids isolated gave no depression in the melting point when mixed with pure picropodophyllin. Again no trace of formaldehyde was detected.

BIBLIOGRAPHY

1. Borsche, W., and J. Niemann, Ann., 494, 126 (1932).

2. Borsche, W., and J. Niemann, Ann., 499, 59 (1932).

3. Erdtman, H., Ann., 513, 229 (1934).

4. Hartwell, J. L., and M. J. Shear, Cancer Research, 7, 716 (1947).

5. Hartwell, J. L., J. Am. Chem. Soc., 69, 2918 (1947).

6. Hartwell, J. L., and A. W. Schrecker, Paper presented at the Philadelphia meeting of the American Chemical Society, April 10, 1950.

7. Hartwell, J. L., and A. W. Schrecker, J. Am. Chem. Soc., 72, 3320 (1950).

8. Haworth, R. D., Ann. Repts. Progress Chem. (Chem. Soc. London) 33, 275 (1936).

9. Kon, C. A., Ann. Repts. Progress Chem. (Chem. Soc. London) 30, 191 (1933).

10. Ormsbee, R. A., I. Cornman, and R. E. Berger, Proc. Soc. Exp. Biol. and Med., 66, 586 (1947).

11. Price, E. M., "The Chemistry of Podophyllotoxin", Thesis, University of Maryland, College Park, Maryland (1949).

12. Reeve, E. W., and H. Adkins, J. Am. Chem. Soc., 62, 2874-6 (1940).

13. Robertson, A., and R. B. Waters, J. Chem. Soc., 143, 85 (1933).

14. Spath, E., F. Wessely, and L. Kornfeld, Ber., 65, 1536 (1932).

15. Spath, E., F. Wessely, and E. Nadler, Ber., 65, 1773 (1932).

16. Spath, E., F. Wessely, and E. Nadler, Ber., 66, 125 (1933).

17. Sterling, J. D. Jr., "The Synthesis of Intermediates Leading to Anti-carcinogenic Compounds", Thesis, University of Maryland, College Park, Maryland (1949).

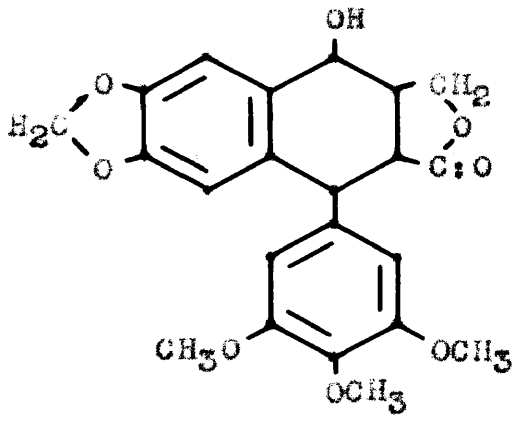
Part III

Studies on the Synthesis of Podophyllotoxin

DISCUSSION

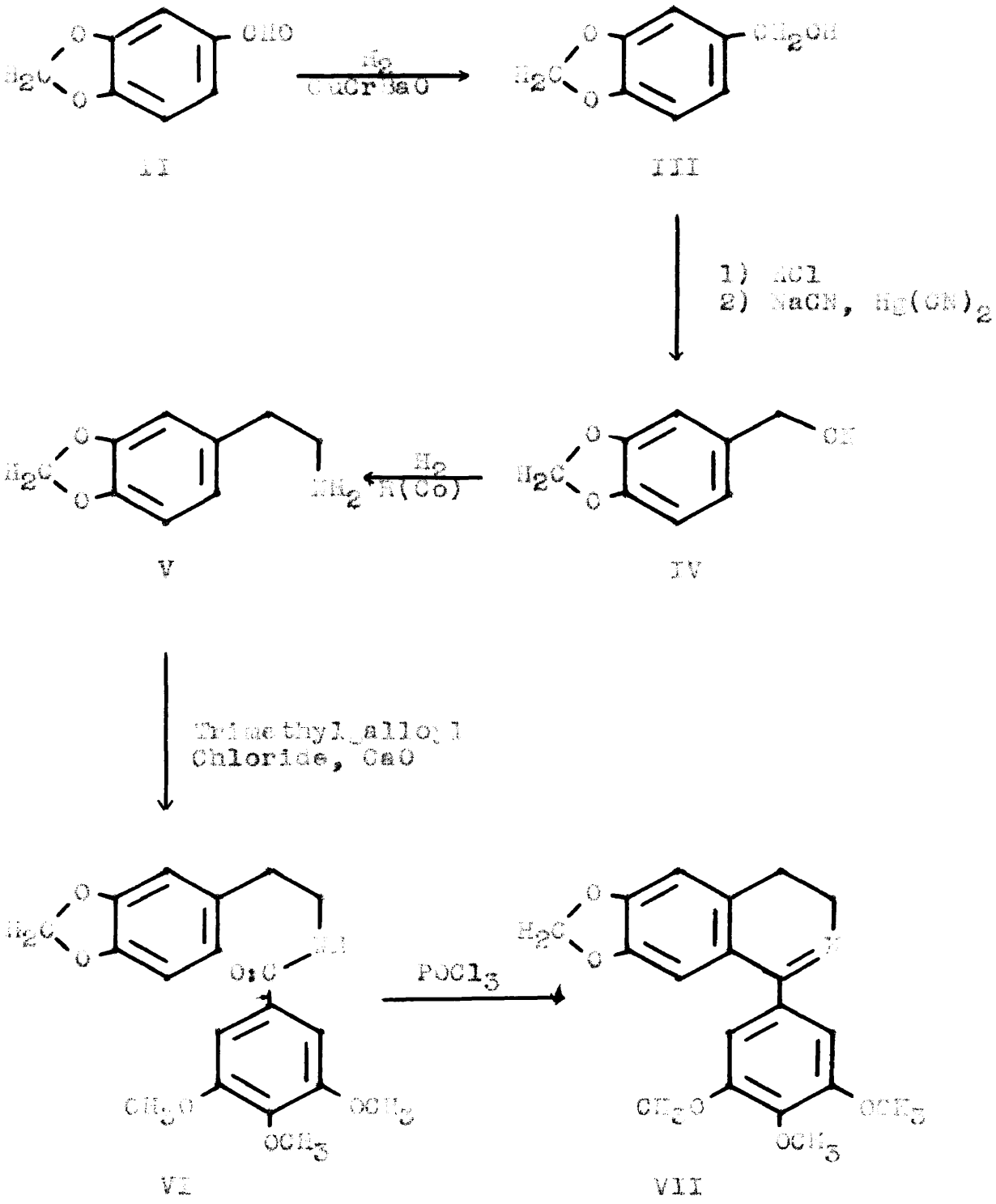
The attempted synthesis of podophyllotoxin (I) was carried on with two purposes in mind: a proof of structure through synthesis and a development of a general method for obtaining compounds of similar structure. Podophyllotoxin (I) has been shown to have tumor damaging activity[8,9,17] but has proved to be too toxic for the human system. Consequently, it would be of interest to synthesize similar structures with the hope of obtaining one with the same activity but with none of the toxicity of podophyllotoxin (I).

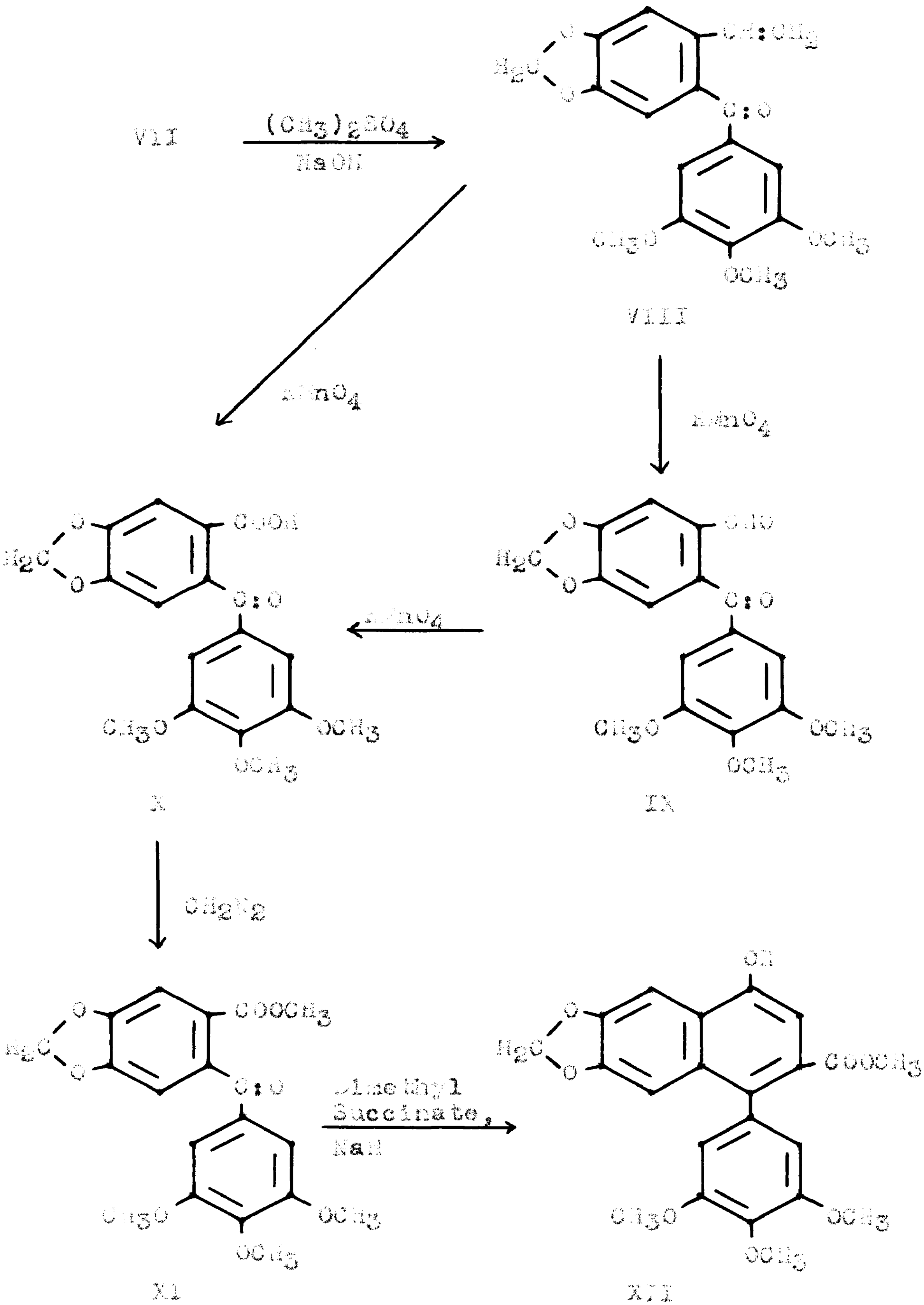
Despite the fact that the exact position of the hydroxyl group in ring 2 has never been definitely established, the evidence accumulated so strongly indicates the linkage to be at carbon 1 that the synthesis has been developed along lines leading to figure I.

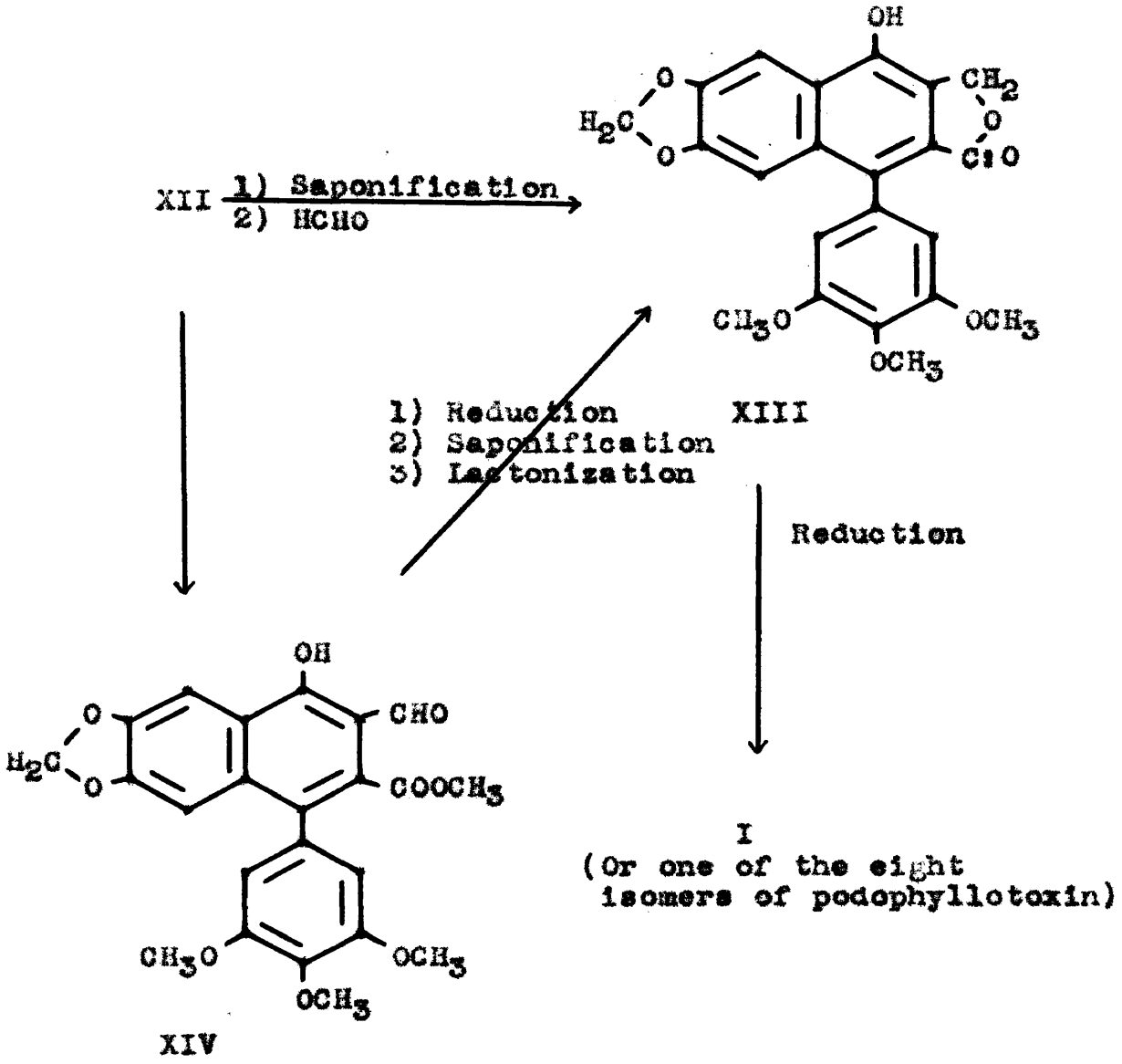


I

The following is the proposed synthesis.


1) HCl
2) NaCN, Hg(CN)2
Trimethyl_allo[illegible]
Chloride, CaO
POCl3

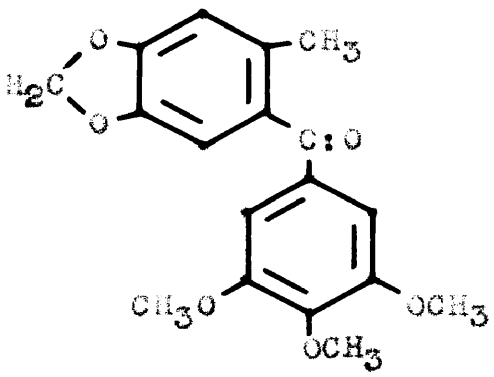

VII
$(CH_3)_2SO_4$
NaOH
$CH:CH_2$
C:O
H_2C
CH_3O
OCH_3
VIII
$KMnO_4$
CHO
IX
COOH
X
CH_2N_2
$COOCH_3$
XI
Dimethyl
Succinate,
NaH
OH
XII



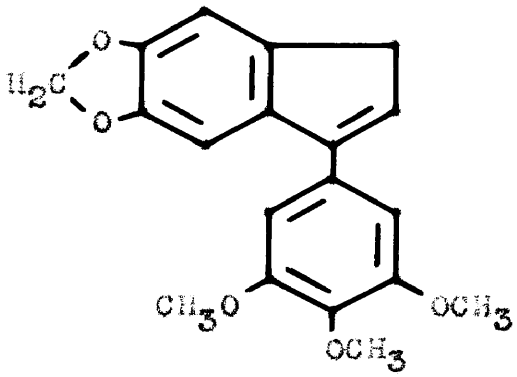
Compound XII has been prepared and attempts have been made toward both compounds XIII and XIV.

Until very recently, the attempts to develop a satisfactory procedure for the preparation of the keto acid (X) had been only partially successful. Earlier attempts to prepare this keto acid by the oxidation of XV, prepared by a Friedel-Crafts condensation of 3,4-methylenedioxytoluene and 3,4,5-trimethoxybenzoyl chloride were failures[18]. Attempts

to prepare compounds similar to XV but with a CH_2OCOCH_3, $COOC_2H_5$, $CH(OC_2H_5)_2$, CHO and $CH_2CH{:}CH_2$ group in the one



XV

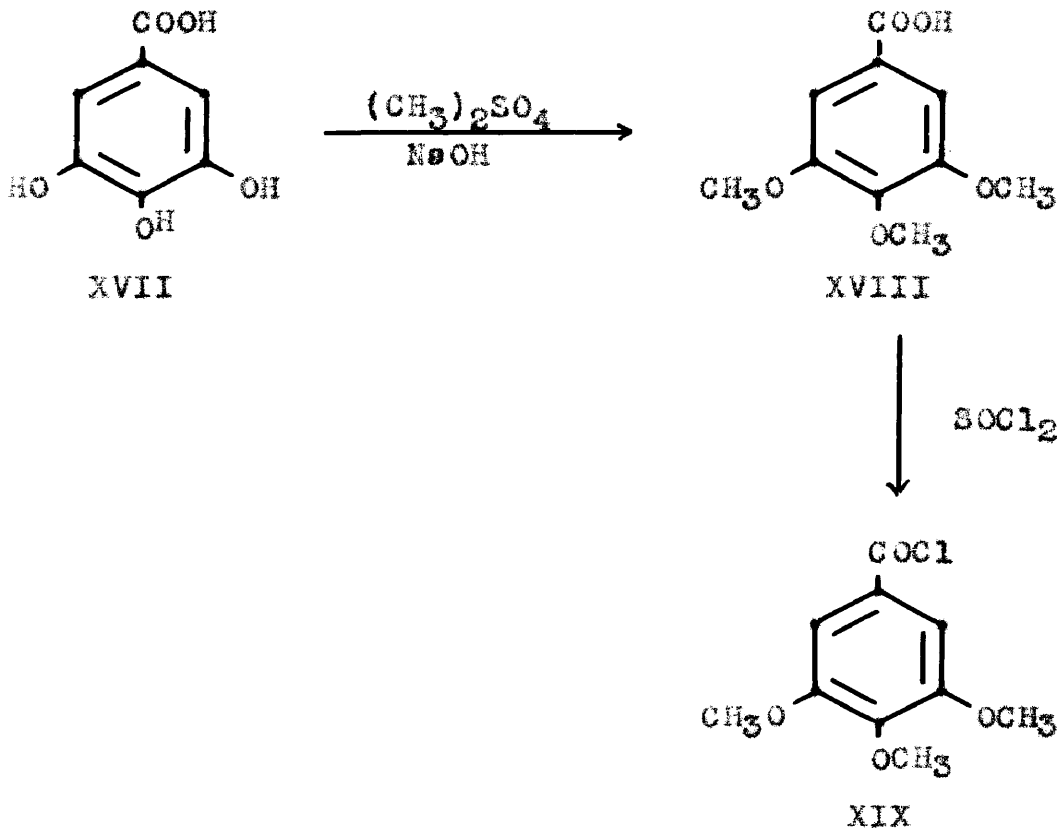


XVI

position in a similar manner gave no condensation product[18]. When this approach failed, other means were attempted by Bareckson[5]. He tried to prepare compound XVI without success. Oxidation of this compound would give the keto acid X. He succeeded in obtaining small amounts of X by the oxidative degradation of compound VII and a series of derivatives of this basic structure. Recently, Gensler[7] prepared the keto acid in good yields by a Hoffman degradation of VII followed by an oxidation of the resulting unsaturated ketone (VIII).

The keto acid (X) prepared in this phase of the research has involved the work of Bareckson[5] in obtaining the substituted dihydroisoquinoline (VII) and a variation of the Gensler[7] reaction to reach the keto acid (X). Piperonal (II) was hydrogenated to piperonyl alcohol (III) using copper chromite as catalyst and dioxane as solvent. Treatment of

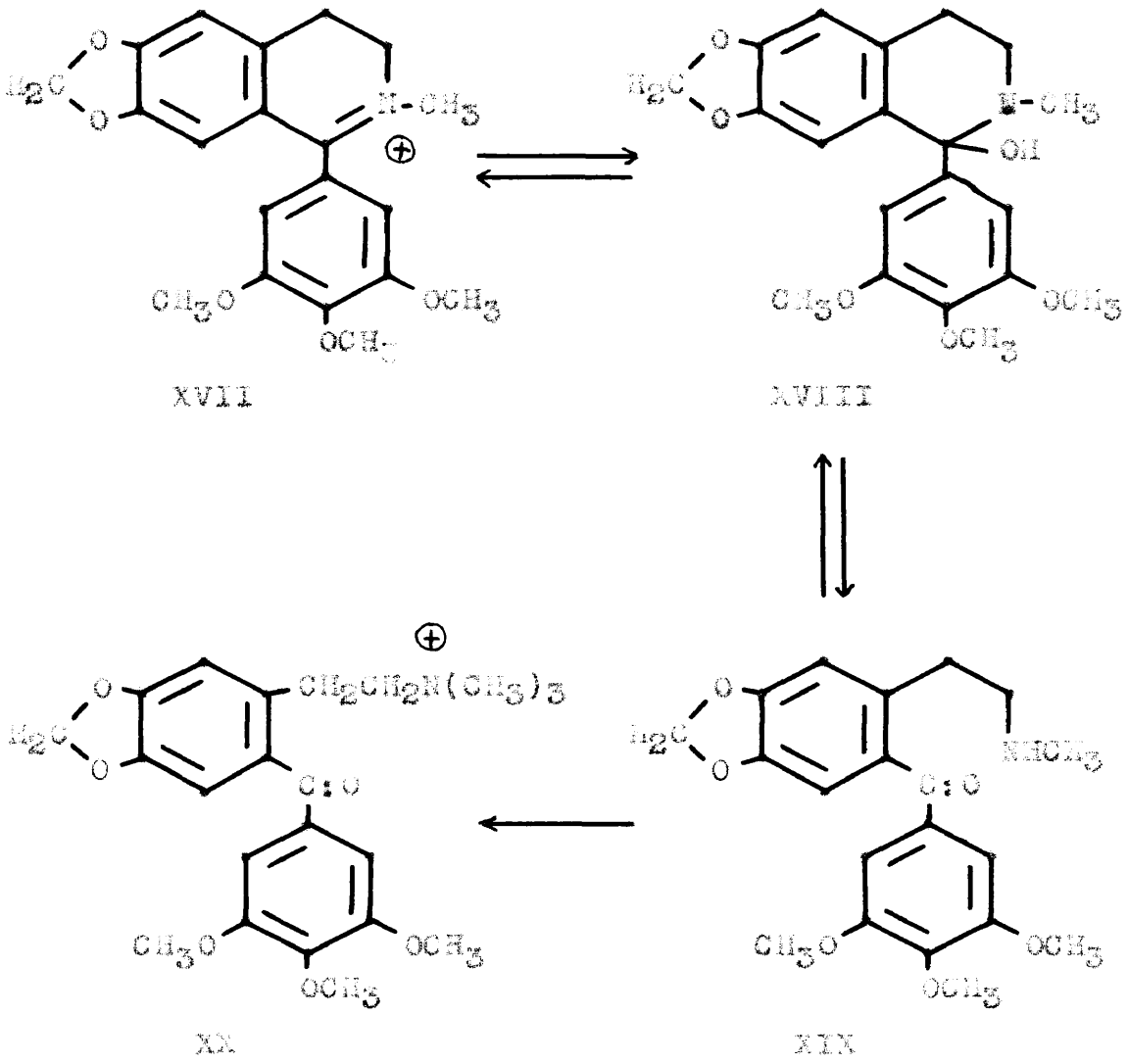
the alcohol with concentrated hydrochloric acid produced the chloride which was reacted without isolation with sodium cyanide in the presence of mercuric cyanide to yield the nitrile (IV). Reduction of the nitrile to the amine (V) by hydrogenation over Raney cobalt followed by a modified Schotten-Baumen reaction with trimethylgalloyl chloride (XIX) in the presence of calcium oxide gave the amide (VI). The trimethylgalloyl chloride (XIX) was prepared by methylating gallic acid (XVII) with dimethyl sulfate and sodium hydroxide and converting to the acid chloride with thionyl chloride.



XVII XVIII XIX

Cyclization of the amide (VI) to the dihydroisoquinoline (VII) was effected with phosphorus oxychloride in toluene.

On treating the dihydroisoquinoline (VII) with dimethyl sulfate and sodium hydroxide, a Hoffman type degradation occurs yielding the keto styrene (VIII). This novel one step conversion is postulated to proceed through several intermediate stages. It is likely that the first step involves the formation of a dihydroisoquinolinium cation (XVII).



On treatment with alkali, such compounds are known to yield the corresponding carbinol amine (XVIII) which is in equil-

ibrium with the amino ketone (XIX). In the presence of dimethyl sulfate and alkali, the quaternary ammonium compound (XX) would be formed and then decomposed to the vinyl compound (VIII) in a normal Hoffman exhaustive methylation process.

Gensler[7] used ethyl alcohol as the solvent and obtained the keto styrene (VIII) in 80-85 per cent yield. By changing the solvent to dioxane, the yield was increased to 97-98 per cent. This increased yield is probably the result of eliminating the side reaction between dimethyl sulfate and ethanol and increasing the reaction temperature.

The keto acid (X) was obtained by oxidizing the keto styrene (VIII) in 1:1 tertiary butyl alcohol-water with potassium permanganate. When the theoretical amount of potassium permanganate was used, an equal quantity of the keto aldehyde (IX) was also formed. Increasing the mole ratio of potassium permanganate to keto styrene decreased the amount of aldehyde formed but also decreased the yield of the keto acid. At a 10:1 ratio, the quantity of aldehyde recovered was small but the yield of keto acid was only 29 per cent. It is apparent that the potassium permanganate attacks some other part of the molecule causing extensive degradation along with rupturing the double bond. A compromise 5:1 mole ratio was finally used with the recovered aldehyde being reoxidized in a similar manner. In this way, an overall conversion of 60 per cent was obtained.

The acid (X) was converted to the methyl ester (XI) with diazomethane in methylene dichloride. The reaction

proceeded smoothly to give an almost quantitative yield of the ester. The use of methylene dichloride as solvent had two advantages over diethyl ether. Both the acid and the ester were soluble in methylene dichloride but neither showed much solubility in ether. As a result, the reaction proceeded more rapidly in the first case. In addition, the hazard of fire, which too often occurs in the preparation of diazomethane when ether is used as the solvent, was eliminated.

As a result of this series of reactions, the keto ester (XI) was obtained in an overall yield of 24 per cent.

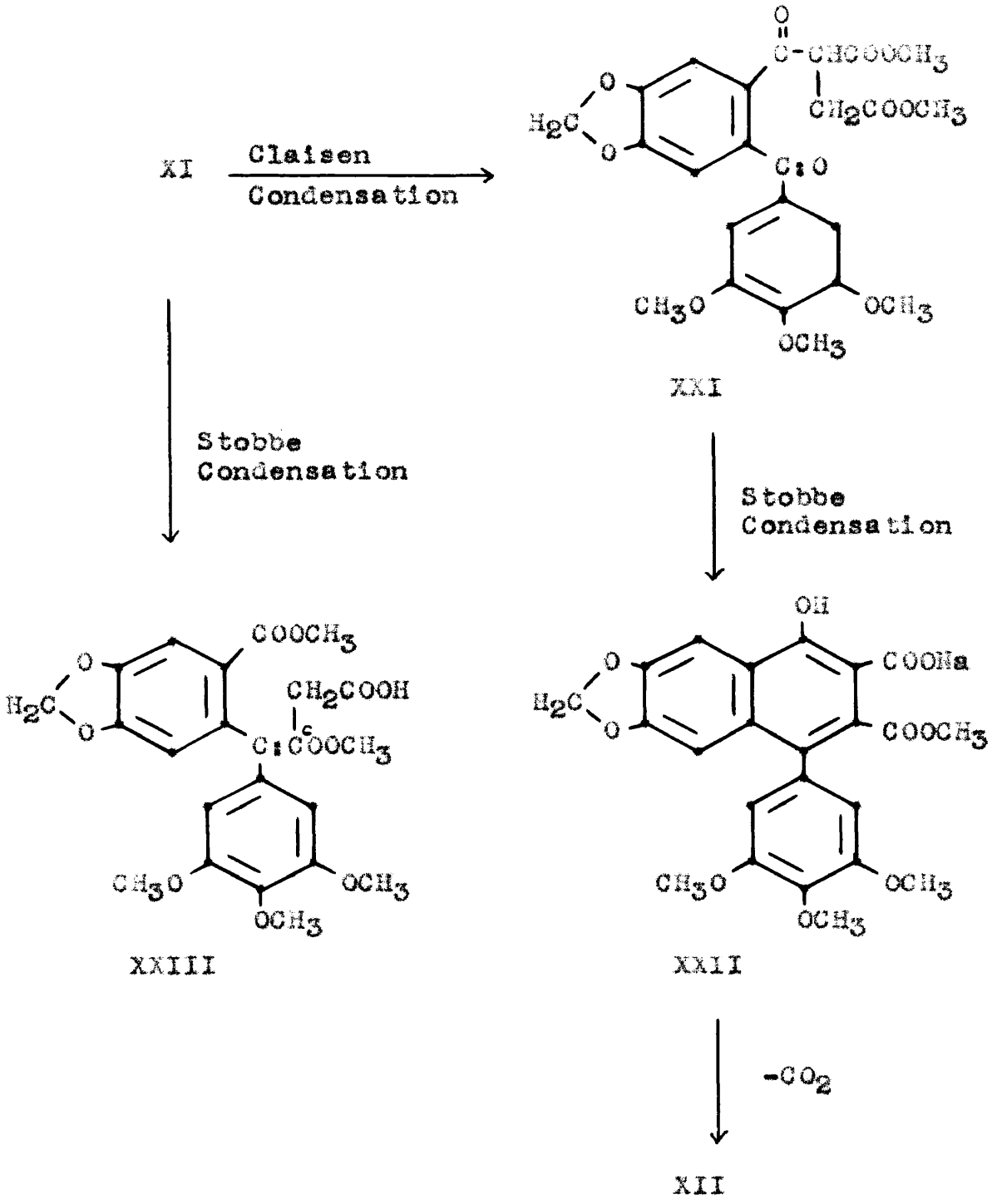
The naphthol-1 ester (XII) was obtained by means of a modified Stobbe condensation of the keto ester (XI) and dimethyl succinate with sodium hydride as the basic catalyst. The compound was white when pure and melted at 204-205°. It was only difficultly soluble in hot 95 per cent ethanol, ethyl acetate, and benzene but quite soluble in chloroform, hot dioxane and glacial acetic acid. The best recrystallization medium was found to be the latter. It was also noted that its alkali salts were rather insoluble in water. The free carbon 2 position was demonstrated qualitatively by its coupling reaction with diazotized p-toluidine producing an orange colored dye.

The alkoxide catalyzed aldol condensation of succinic ester with a ketone (or aldehyde) was discovered by Stobbe in 1893. Originally sodium ethoxide was used as the catalyst, but Johnson has found that the use of potassium tertiary butoxide[11] or sodium hydride[3] generally gave higher yields

and purer products during shorter reaction periods. Johnson[12,4] was able to condense a keto-nitrile with diethyl succinate to form a six membered ring. In the reaction carried out in this research, both a Stobbe and a Claisen condensation have occurred to yield a naphthol-1 derivative (XII). Only one other such condensation has been reported. Turner[20] has isolated a cyclicized half-ester from the Stobbe condensation with methyl γ-anisoyl butyrate in yields of about 15 per cent.

In studying this reaction, both potassium tertiary butoxide and sodium hydride were used as catalysts with benzene and toluene as solvents. Potassium tertiary butoxide yielded none of the desired compound while the higher reaction temperature obtained with toluene gave a slightly higher yield in one-tenth of the reaction time required when benzene was used.

Two routes are possible in this reaction. Either a Stobbe condensation occurs first followed by a Claisen or the Claisen condensation occurs first followed by a Stobbe. It appears from the evidence that both of these possibilities are in competition with each other in the initial stages, with the velocity of the Stobbe condensation being somewhat greater than that of the Claisen. As evidence for the Claisen condensation occurring first, we have the isolation of the naphthol-1 derivative (XII). This could only have occurred if compound XXI was formed initially. Cyclization of this via the Stobbe condensation would then give

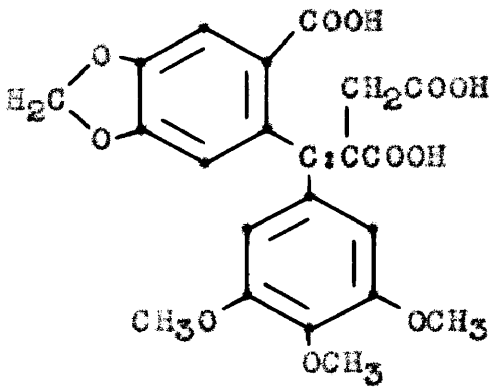


compound XXII which would decarboxylate to the compound XII that was obtained. The decarboxylation probably occurs during the reaction, for when the reaction mixture was acidified in the isolation procedure, no evolution ofcarbon

dioxide was noted.

If the Stobbe had occurred first, then the end product would not have been the naphthol-1 derivative (XII). Instead compound XXIII would have formed initially and the reaction would have stopped at this point.

Accordingly, there is evidence for the Stobbe condensation occurring first. The itaconic acid derivative (XXV) was isolated from the sodium bicarbonate fraction. This would have been formed, if after the initial Stobbe condensation, saponification had occurred. The evidence obtained for this

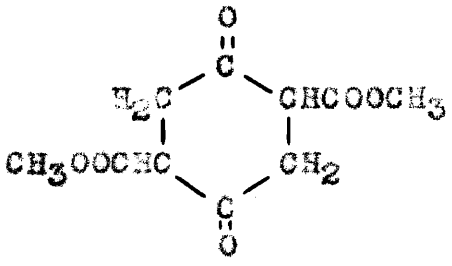


XXV

structure is as follows: a carbon, hydrogen and methoxyl analysis that checks the theoretical fairly well, a positive qualitative test for the methylenedioxy bridge and the piperonylic acid group, a positive potassium permanganate test for the double bond, an effervescence at the melting point that would be accounted for by anhydride formation and a neutral equivalent indicative of a tribasic acid.

A statement was made in a previous paragraph that the velocity of the Stobbe condensation was believed to be greater than that of the Claisen reaction. This was based on the fact that in all cases, the weight of the material obtained from the sodium bicarbonate fraction was twice that of the naphthol-1 (XII) isolated. It is believed that the greater part of this weight was due to compound XXV. Only one other product has been isolated from this fraction and that in only small amounts. There was a good possibility that others remained unisolated. This other product was a white solid; melting above 275°, giving a brilliant emerald green color with ferric chloride and a negative methylenedioxy bridge test and methoxyl test. Its element analysis fitted that of a composition of $C_4H_3O_3$. This did not agree with any of the possible degradation products that could be visualized.

A self-condensation product (XXVI) of dimethyl succinate was also isolated. The ethyl ester of this compound had been previously reported by Johnson.[13]



XXVI

The remainder of this research has been directed toward

putting a methylol or aldehyde group into the two position of the naphthol-1 derivative (XII). The first attempt was th the base catalyzed formaldehyde condensation with the free acid of XII, prepared by the saponification with potassium hydroxide in 1:1 ethylene glycol - water. In the second attempt, N-methylformanilide and phosphorus oxychloride were used in an effort to introduce an aldehyde group.

Two variations of the formaldehyde reaction were carried out. In the first case, the free acid of XII, base and formaldehyde were mixed together and allowed to stand at room temperature for 14 days. It was with this type of a procedure that Manasse[15] prepared saligenin. The same green coloration that Manasse observed also occurred here and a neutral compound with a melting point of 275° was isolated. However, the analysis did not correspond with the theoretical for compound XIII. In the second case, the same mixture was heated under reflux for 24 hours in a nitrogen atmosphere. No coloration occurred, but a white solid precipitated. The reaction mixture was also found to be slightly acid. Apparently, the formaldehyde had undergone a Canizarro reaction at the elevated temperature. On filtering off the precipitate, it was found to be in the same high melting solid previously obtained. It was noted that when this solid was allowed to stand in base, the surface turned green and when left in acid, it turned pink. Evidently the green coloration observed by both Manasse and us was due to some air-oxidation product that was acting as an acid-base

indicator. None of the usual formaldehyde-phenol condensation products fitted the analysis obtained. The methoxyl determination placed the molecular weight in the range of 345 which would mean that more than one group had been added. Consequently, the structure appears to be one that involves the free acid group in some complex manner.

The use of N-methylformanilide and phosphorus oxychloride for the introduction of an aldehyde group has been successfully used in the past[2,6,14] The reaction was run with and without o-dichlorobenzene as solvent, and although a color change was noted and acidic vapor evolved, only starting material was recovered.

Although the work to date has not resulted in the synthesis of podophyllotoxin (I), plans call for a continued effort along the same lines. Further reactions will be tried in order to place a group in the two position of compound XII. If compound XIII is obtained, a selective reduction of the B ring will give the desired product. If compound XIV is obtained, a reduction to the alcohol followed by a saponification and lactonization would be required before the selective hydrogenation.

The natural product podophyllotoxin is an optically active compound with the structure assigned having four centers of asymmetry. The synthetic product will of course be optically inactive. Consequently some other means than physical properties will have to be used to compare the two.

Infra-red studies do not as a rule show any difference between optical isomers, and it is planned to use this means for comparing the two products.

EXPERIMENTAL

All melting points are corrected.

Preparation of Piperonyl Alcohol (III). The procedure of Reeve and Sterling[18] was followed employing 720 grams (4.83 mole) piperonal (II) that had been purified by distillation under a nitrogen atmosphere. Along with an 80 per cent yield of the desired piperonyl alcohol, an additional fraction, in the boiling range 72-92° at 2 millimeters and amounting to 15 per cent by weight was also obtained. This boiling point corresponded to the hydrogenolosis product 4,5-methylenedioxytoluene, previously obtained by the hydrogenation of piperonal using the same catalyst but at a temperature of 280°. There is no explanation for the considerable quantity formed at the lower temperature used in this preparation.

Preparation of Piperonyl Cyanide (IV). The directions of Bareckson[5] were employed but on a larger scale. Four hundred and ten grams (2.7 mole) piperonyl alcohol (III) was converted to the chloride with 675 ml. concentrated hydrochloric acid and then added dropwise, without isolating, to a solution of 475 grams (9.7 mole) of sodium cyanide and 16.7 grams mercuric cyanide in 715 ml. of distilled water. After maintaining the reaction for 7 hours at 80°, the layers were separated, washed and the organic solvent removed at water pump pressure. The nitrile distilled at 140-150°

under 2 millimeters pressure to give 360 grams (83.5 per cent of the theoretical amount) of a yellow liquid that slowly solidified. This product contained a small amount of chloride. As a result, a crystallization from ethanol and a redistillation was required, before it was pure enough for use in the next step. In this manner, a white solid melting at 42-43° was obtained. The previously reported values were 38-40° [5] and 42° [16].

Preparation of Homopiperonylamine (V).

1) Dioxane-Ammonia Solvent. Forty-two grams (0.26 mole) of piperonyl cyanide (IV), 100 ml. dioxane saturated with ammonia and two grams of Raney cobalt catalyst were placed in a steel reaction vessel. Hydrogen was admitted at room temperature until a pressure of 3025 pounds per square inch was reached. The vessel was then rocked and heated. At 100°, the pressure started to drop rapidly with the theoretical amount of hydrogen being absorbed in 15 minutes. During this time, the temperature rose to 150° and was maintained there until no further drop in pressure was noted. The vessel was cooled to room temperature, washed out with two 20 ml. portions of dioxane and the catalyst filtered off. The solvent was removed at water pump pressure, and the amine was distilled at 122° under 5 millimeters pressure in a nitrogen atmosphere giving 39 grams (91 per cent of the theoretical amount) of a colorless liquid.

2) Dioxane Solvent. In a similar manner, 42 grams (0.262 mole) of piperonyl cyanide (IV) was hydrogenated using

2 grams Raney cobalt catalyst and 100 ml. dioxane as solvent. The reaction started at 100° and was complete in 20 minutes. After washing out the vessel with dioxane, filtering off the catalyst and removing the solvent at water pump pressure, the homopiperonyl amine was distilled to yield 39.6 grams (92 per cent of the theoretical amount) of a water white liquid. As can be noted, there appeared to be no advantage in using a dioxane-ammonia solvent.

Preparation of Trimethylgallic Acid (XVIII). This procedure is the same as that outlined by Bareckson[5] but on a larger scale. Seven hundred and fifty grams (4.41 mole) of gallic acid (XVII) was dissolved in a solution of 1200 grams of sodium hydroxide in 7 liters of water. Two liters of dimethyl sulfate were added dropwise over a 3 hour period with mechanical stirring. The temperature was maintained below 35° for the first two hours but allowed to rise to 50° for the remainder of the addition. The mixture was then heated under reflux for 2 hours, at which time, an additional 300 grams of sodium hydroxide in 600 ml. water was added. The refluxing was then continued for another hour. After acidifying the alkaline reaction mixture with 1:1 hydrochloric acid, the precipitated acid was filtered off and washed with 5 liters of water. On air-drying, 720 grams (86.2 per cent of the theoretical amount) of a light tan product melting at 161-164° was obtained. Recrystallization from water in the presence of activated carbon yielded a white solid melting at 167-168.5°.

Preparation of Trimethylgalloyl Chloride (XIX). The method prescribed by Asano and Yamaguti[1] was followed. A solution of 320 grams (1.51 mole) of trimethylgallic acid (XVIII) in 1500 ml. of anhydrous benzene and 460 ml. of thionyl chloride was heated under reflux for 4 hours. The benzene was removed at water pump pressure, and the acid chloride distilled at 155-157° at 8 millimeters pressure yielding a colorless liquid that slowly solidified on standing. Previously reported boiling points were 138-139° at 2 mm. pressure[16] and 150° at 2 mm. pressure.[1]

Preparation of N-3,4,5-Trimethoxybenzoylhomopiperonylamine (VI). This preparation was a variation of the one used by Lareckson[5] and involved an inverse addition. Sixty-six grams (0.236 mole) of trimethylgalloyl chloride (XIX) dissolved in 300 ml. anhydrous benzene was added dropwise over a one hour period to a mechanically agitated slurry of 47.0 grams (0.236 mole) homopiperonylamine (V) dissolved in 300 ml. of anhydrous benzene and 28 grams of 150 mesh calcium oxide. The mixture was heated under reflux for one hour, then filtered by gravity while hot. On cooling the benzene solution, a white solid appeared that was filtered off and dried, yielding 34.6 grams of the amide melting at 136-136.5°. The original residue from the reaction mixture was extracted with four 150 ml. portions of boiling 95 per cent ethanol and filtered. The filtrate on cooling, yielded 32.6 grams of a light tan solid melting at 136.5-137.5°. An additional 8.0 grams melting at 134-136° was obtained by

concentrating the ethanol solution to 50 ml. This gave a total yield of 75.2 grams (73 per cent of the theoretical amount).

An easier method of isolation was effected by dissolving the calcium oxide residue in cold 10 per cent hydrochloric acid instead of extracting it with boiling ethanol. In the experiment in which this isolation procedure was applied, 210 grams (1.28 mole) of homopiperonyl amine (V), 296 grams (1.28 mole) of trimethylgalloyl chloride and 110 grams of calcium oxide were used. The resulting calcium oxide residue, after filtering off the benzene solution, was added to a mixture of 1 liter water and 1 liter ice and acidified to congo red paper with 10 per cent hydrochloric acid. The precipitated amide was filtered off and washed with one liter cold 5 per cent hydrochloric acid, one liter 10 per cent sodium bicarbonate and one liter water. After air-drying, 168 grams of material melting at 134-135° was obtained. An additional 167 grams were isolated from the benzene solution giving a total of 335 grams (73 per cent of the theoretical amount).

Preparation of 1-(3,4,5-Trimethoxyphenyl)-6,7-Methylenedioxy-3,4-dihydroisoquinoline (VII). The preparation as outlined by Bareckson[5] was used. Three hundred and fifty-five grams (0.987 mole) of N-3,4,5-trimethoxybenzoylhomopiperonyl amine (VI) was dissolved in 2 liters of anhydrous toluene and 800 ml. phosphorus oxychloride was added. The solution was heated under reflux for 3 hours, during which

time, a yellow solid precipitated. On cooling to room temperature, more of the yellow solid appeared. The cooled reaction mixture was poured into 5 liters of petroleum ether, and after standing one hour, the supernatent liquid was decanted. The gummy residue that remained was treated with one liter of 95 per cent ethanol with the flask being placed in an ice-bath to keep the resulting vigorous reaction under control. After all the ethanol had been added, the initial reaction had subsided, the mixture was heated to boiling and then cooled in an ice-bath. The hydrochloride that was obtained after filtering, was dissolved in one liter of boiling water and made alkaline with sodium hydroxide. Periodic additions of water were required to thin the resulting slurry. After filtering, washing with 5 per cent sodium hydroxide and water, 315 grams (93.5 per cent of the theoretical amount) of a light tan solid melting at 159-160° was obtained. Lareckson[5] reports a melting point of 159.5-160° for material that had been recrystallized from ethanol-water.

Preparation of 4,5-Methylenedioxy-2-(3,4,5-trimethoxybenzoyl) styrene (VIII).

1) With Ethanol Solvent. This procedure was adopted from the outline published by Gensler.[7] Dimethyl sulfate (11.3 ml.) was added dropwise over a 20 minute period to a mechanically stirred solution of 2.0 grams (0.006 mole) of the dihydroisoquinoline derivative (VII) in 30 ml. of hot 95 per cent ethanol. This was followed by a solution of 5 grams of sodium hydroxide in 20 ml. of distilled water and

the mixture was heated on a steam-bath under reflux and with mechanical stirring for 5 hours. A white solid precipitated during the heating. The mixture was cooled and poured into 300 ml. of water. The white solid was apparently sodium sulfate for it dissolved completely. The precipitate that formed on dilution was filtered off and air-dried giving 1.72 grams (85 per cent of the theoretical amount) of a yellow solid that melted at 139.5-140.5°. Gensler[7] reported a value of 138-139°.

When the quantity of the starting material was increased ten-fold, a gummy solid was obtained as the end-product. Upon recrystallizing this from ethanol, a yellow solid was isolated, m. p. 139-140°, but only in a 10 per cent yield.

2) <u>With Dioxane Solvent.</u> One hundred grams (0.293 mole) of the dihydroisoquinoline derivative (VII) was dissolved in 600 ml. of boiling dioxane, and 200 grams of sodium hydroxide in 400 ml. of water was added. Dimethyl sulfate (272 ml.) was added to this solution dropwise over a 90 minute period with vigorous mechanical agitation and then heated under reflux for 4 hours. During this period, both the styrene derivative and sodium sulfate precipitated giving an accumulation of yellow and white crystals. At the end of the reaction time, 2500 ml. of water were added. The resulting slurry was allowed to stand overnight and then filtered and washed with one liter of hot water. To eliminate the last trace of occluded inorganic salt, the residue was swirled with one liter of boiling water and refiltered.

On drying, 97.5 grams (97.2 per cent of the theoretical amount) of a yellow product melting at 138-139° was obtained.

Preparation of 4,5-Methylenedioxy-2-(3,4,5-trimethoxybenzoyl) benzoic acid (X).

1) With Potassium Permanganate in Glacial Acetic Acid. Fifty milliliters of a 5 per cent potassium permanganate solution was added dropwise to a mechanically stirred solution of 2 grams (0.0058 mole) of the keto styrene (VIII) in 30 ml. of glacial acetic acid maintained at 60-65°. After the final addition, the reaction solution was kept at this temperature for an additional two hours and then refluxed for one hour. During this period, the solution became orange-red in color. The solution was then poured into 300 ml. of water and allowed to stand overnight. The resulting precipitate was filtered off, washed with water and dried giving 1.0 gram of a reddish-brown material with a melting point of less than 120°. This substance was insoluble in sodium bicarbonate and consequently was of no interest.

2) Alkaline Potassium Permanganate in 1:1 tertiary Butyl Alcohol and Water. Fifty grams (0.146 mole) of the keto styrene (VIII) was added to 1500 ml. of tertiary butyl alcohol along with 42.5 grams (1.05 mole) of solid sodium hydroxide and heated with mechanical stirring to 60°. One hundred and fifteen grams (0.730 mole) of potassium permanganate in 1500 ml. of warm water was added through an addition funnel as rapidly as the reaction would allow. After the final addition, the reaction mixture was heated under reflux

for 20 minutes and the manganese dioxide formed in the reaction filtered off and washed with one liter of boiling water. The filtrate was cooled to room temperature and acidified to congo-red paper with concentrated hydrochloric acid. An additional 50 ml. of acid was then added. The resulting precipitate was allowed to stand overnight and then filtered and washed with one liter of water. On air-drying, 37.5 grams of a tan solid was obtained that melted at 198-200°. This residue was triturated thrice with 150 ml. portions of 10 per cent sodium bicarbonate and the insoluble material filtered off and washed with 500 ml. of water. After air-drying, the residue weighed 18.5 grams and melted at 208-210°. The sodium bicarbonate filtrate was acidified with 1:1 hydrochloric acid. Additional water was necessary to thin the slurry of precipitated acid. After filtering, washing with water and air-drying, 18.0 grams of a white product melting at 214-216° was obtained. This material gave no depression in the melting point when mixed with a pure sample of the keto acid (X) obtained by Bareckson[5] from the oxidation of compound VII. Recrystallization from aqueous ethanol gave a fluffy white solid melting at 216-217°. Previously reported values were 215-216° [5], 214-216° [19] and 215-2-215.7°[7].

Analysis showed that the sodium bicarbonate insoluble material was the keto aldehyde (IX). The sample was purified for analysis through 3 recrystallizations from chloroform-ethanol mixture yielding a white solid melting at 222.5-223°.

Analysis	Calculated for $C_{18}H_{16}O_7$	Found
Carbon	62.79%	62.64% 62.71%
Hydrogen	4.69%	4.71% 4.90%
Methoxyl	27.04%	26.41% 26.13%

The keto aldehyde (IX) was further oxidized to the keto acid (X) in an analagous manner using a 2:1 mole ratio of potassium permanganate to aldehyde. In this manner, an additional 13.5 grams of the keto acid melting at 215-216° was obtained. Two and one-half grams of aldehyde was also recovered. This gave a total of 31.5 grams (80 per cent of the theoretical amount based on moles acid recovered and moles of starting material used).

Preparation of Methyl 4,5-Methylenedioxy-2-(3,4,5-trimethoxybenzoyl)benzoate (XI). A solution of diazomethane in methylene dichloride prepared from 20 ml. of N-nitrosomethylurethane was added to a solution of 15 grams (0.0415 mole) keto acid (X) in 250 ml. of methylene dichloride. A rapid evolution of nitrogen occurred with the reaction being complete in a few minutes. A small quantity of insoluble material was filtered off, and the solvent was removed by steam distillation leaving an aqueous suspension of a yellow gummy solid that crystallized on standing overnight. This was filtered off, triturated with 100 ml. of 5 per cent sodium bicarbonate, refiltered and washed with one liter of water. The filtrate gave no precipitate when acidified with

hydrochloric acid. On air-drying, 14.8 grams (95.0 per cent of the theoretical amount) of a pale tan solid melting at 129-130° was obtained. Recrystallization from methanol in the presence of activated carbon yielded pure white needles melting at 131-132°. The literature reports 128-129°[19] and 127-128°[10].

The preparation of 6,7-Methylenedioxy-4-(3,4,5-trimethoxybenzoyl)-3-carbomethoxynaphthol-1 (XII) -- The Stobbe Condensation.

1) With Sodium Hydride as Catalyst. The condensation was carried out in a three-necked, two liter flask equipped with a sealed stirrer, a tube for admitting dry nitrogen and a condenser connected through a calcium chloride tube in a mercury trap. The flask was charged with 25 grams (0.067 mole) of the keto ester (XI), 700 ml. of anhydrous toluene and 100 grams (0.67 mole) of dimethyl succinate. After sweeping the system with dry nitrogen, 35 grams (1.52 mole) of sodium hydride was added at one time and the mixture heated to reflux. One milliliter of methanol was then added to start the reaction which was allowed to proceed at 115° for two hours. At the end of that time, the flow of hydrogen had stopped indicating the reaction to be over. A yellow-orange color appeared in the initial stage that deepened to a red-orange as the reaction proceeded. That reaction mixture was chilled in an ice-bath and the excess sodium hydride decomposed by the cautious addition of water. The mixture was then transferred to a separatory funnel,

acidified with 100 ml. of concentrated hydrochloric acid and shaken with 2 liters of ether. Most of the solid dissolved in the organic phase, the remainder being removed with the acid layer and filtered off. The ether-toluene solution was successively washed with two 150 ml. portions of water to remove the last traces of hydrochloric acid, three 150 ml. portions of 5 per cent sodium bicarbonate and two 150 ml. portions of water; the sodium bicarbonate fraction and its washings being put aside for further treatment. An aqueous suspension of a yellow solid was obtained on removing the organic solvent by steam distillation. This precipitate was filtered off, washed with water, triturated with 400 ml. of 5 per cent sodium hydroxide and refiltered. The residue was washed with water til the washings were colorless, and then with 500 ml. of 5 per cent hydrochloric acid and 500 ml. of water. This gave 5.1 grams of the naphthol-1 derivative (XII) melting at 195-200°. Further purification was effected by swirling the product with 100 ml. of boiling 95 per cent ethanol and filtering. The white solid obtained weighed 4.1 grams (16 per cent of the theoretical amount) and melted at 201-202°. The sample submitted for analysis was recrystallized from 95 per cent ethanol and melted at 204-205°.

Analysis	Calculated for $C_{22}H_{20}O_8$	Found
Carbon	64.07%	64.10% 64.25%
Hydrogen	4.39%	5.01% 5.03%
Methoxyl	30.10%	29.93% 30.20%

On acidifying the alkaline filtrate, 11.7 grams of crude dimethyl 1,4-cyclohexandione-2,5-dicarboxylate melting at 143-149° and giving an intense violet color with ferric chloride was obtained. The material purified by two recrystallizations from aqueous ethanol in the presence of activated carbon was pure white and melted at 155.5-157°. The ether

Analysis	Calculated for $C_{10}H_{12}O_6$	Found
Carbon	52.70%	52.78%
Hydrogen	5.30%	5.34%

insoluble material (3 grams) originally filtered off proved to be this compound. Recrystallization from ethanol gave a white solid that melted at 154-155° and gave no depression in the melting point when mixed with the above sample.

Acidification of the sodium bicarbonate fraction with 1:1 hydrochloric acid, followed by extraction with 300 ml. of ether and evaporation of the solvent, yielded 10.0 grams of an orange gummy solid. Two products were separated by fractional crystallization from aqueous methanol. One melted above 275° and gave the following element analysis: C, 43.54%, H, 3.42%. The analysis and properties of the compound did not fit any of the possible degradation products visualized. The other compound melted at 185° with effervescence and proved to be compound XXV. The material was found to be slightly hydroscopic, and a trace of water in the sample would account for the slight discrepences in the

comparison between calculated and determined values in the analysis.

Analysis	Calculated for $C_{22}H_{21}O_{11}$	Found
Carbon	57.26%	56.86% 56.66%
Hydrogen	4.58%	4.65% 4.66%
Methoxyl	20.17%	20.53% 20.31%

2) With Potassium tertiary-Butoxide as Catalyst.

A 200 ml. flask fitted with a condenser connected through a calcium chloride tube to a mercury trap was charged with a solution of potassium tertiary butoxide prepared from 0.6 grams of potassium in 20 ml. of tertiary butyl alcohol, 4.0 grams (0.027 mole) of dimethyl succinate and 2.0 grams (0.0054 mole) of the keto ester (XI). The flask was swept with nitrogen and heated under reflux for three hours. A color change to a reddish-purple and the formation of an orange precipitate occurred during this time. At the end of this reaction period, the mixture was cooled, acidified with 10 ml. of concentrated hydrochloric acid in 50 ml. of water and transferred to a separatory funnel with the aid of two volumes of water. The aqueous phase was extracted with two 50 ml. portions of ether. The ether, in turn, was washed with two 50 ml. portions of water to remove the last trace of hydrochloric acid, three 50 ml. portions of 5 per cent sodium bicarbonate and two 50 ml. portions of water. On steam distilling off the solvent, a sticky yellow solid was obtained. One hundred

milliliters of 10 per cent sodium hydroxide was added. Some of the material dissolved and the remaining solid filtered off. The residue was recrystallized from 50 per cent ethanol giving 0.5 grams of a cream colored solid melting at 120-140°. This neutral material was probably a mixture of starting material, naphthol and disuccinate. The sodium hydroxide filtrate yielded 0.05 grams of the disuccinate (XXVI) on acidification with hydrochloric acid. The sodium bicarbonate fraction when acidified with hydrochloric acid, extracted with 100 ml. of ether and the ether evaporated, yielded 1.3 grams of a brown gummy substance. This was not further investigated.

Saponification of Compound XII. Four grams (0.0096 mole) of the naphthol-1 ester (XII) was heated with 72 ml. of a .25 N solution of potassium hydroxide in 1:1 ethylene glycol - water until solution was effected and then for five minutes more. One hundred milliliters of water were then added; the solution cooled to room temperature and filtered. The small amount of residue left was discarded. Acidification of the filtrate with 1:1 hydrochloric acid produced a gelatinous precipitate that was filtered off, washed with water and triturated with 300 ml. of a sodium bicarbonate solution. The insoluble material was filtered off, washed and air-dried to give 2.33 grams of grey material that had an indeterminate melting point. On acidifying the filtrate with hydrochloric acid, 1.75 grams of the free acid melting at 238° with effervescence was obtained. Purification of this material for analysis was effected by swirling with 30 ml.

of boiling benzene and filtering followed by two recrystallizations of the residue from aqueous dioxane in the presence of activated carbon. The pure white sample melted at 245° with effervescence.

Analysis	Calculated for $C_{21}H_{18}O_8$	Found
Carbon	63.31%	63.12% 63.05%
Hydrogen	4.55%	4.78% 4.84%
Methoxyl	23.39%	23.34% 23.53%

Crystallization of the sodium bicarbonate insoluble fraction from 30 ml. of glacial acetic acid yielded 1.15 grams of a white material melting at 233° with effervescence. A mixed melting point with a pure sample of the naphthol-1 acid gave no depression. Thus, an overall yield of 3.30 grams (76 per cent of the theoretical amount) was isolated. It is believed that the sodium bicarbonate insoluble material was a mixture of the sodium salt of the acid and unsaponified ester.

Attempted Preparation of Compound XIII.

1) With Formaldehyde at Room Temperature. Seven milliliters of a 37 per cent formaldehyde solution was added to a solution of 1 gram (0.0026 mole) of the naphthol-1 acid in 65 ml. of 0.1 N sodium hydroxide and allowed to stand 14 days with occassional swirling. A green coloration developed during this time. On acidification with hydrochloric acid, a blue-grey precipitate appeared. The slurry

was transferred to a separatory funnel and was dissolved in 100 ml. of chloroform. A small amount of insoluble material was filtered off and discarded. The organic layer was separated, washed with four 25 ml. portions of 10 sodium bicarbonate and two 25 ml. portions of water. On steam-distilling off the solvent, 0.25 grams of a tan material melting at 253-255° (sealed evacuated tube) was obtained. By recrystallizing from glacial acetic acid, a white product melting at 285-287° (sealed evacuated tube) was isolated. The analysis on this material was: C, 67.80%; H, 5.24%; and methoxyl, 20.92%. Further recrystallization did not change the analysis. No possible structure fitting these figures could be visualized. Acidification of the sodium bicarbonate fraction gave 0.62 grams of a tan solid that melted with effervescence at 235°. This corresponded to the starting material.

2) <u>With Formaldehyde under a Nitrogen Atmosphere at 100°.</u> A solution of 0.75 grams (0.0019 mole) of the naphthol-1 acid in 50 ml. of 0.1 N sodium hydroxide and 5.5 ml. of 37 per cent formaldehyde was heated under reflux in a nitrogen atmosphere with mechanical stirring for 24 hours. During this time, no green coloration appeared, but a cream colored solid precipitated. At the end of the reaction period, the solid was filtered off and dried to give 0.4 gram of material that melted at 270-275° (sealed evacuated tube). A mixed melting point with the high melting solid obtained in the previous experiment showed no depression. Acidification of the filtrate produced a gelatinous precipitate

that weighed 0.2 grams after drying. It melted at 235° with effervescence and gave no depression when mixed with a sample of the starting material.

Attempted Preparation of Compound XIV.

1) N-Methylformanilide and Phosphorus Oxychloride with no Solvent. A mixture of 1.0 gram (0.0024 mole) of the naphthol-1 ester (XII), 0.90 grams (0.0032 mole) of phosphorus oxychloride and 0.44 grams (0.0032 mole) of N-methylformanilide was heated in a test tube on a steam-bath for 6 hours. Although the mixture remained as such, the color changed to reddish-purple color and evolved an acidic gas. At the end of this time, the reaction mixture was transferred to a filter with the aid of water and washed with 100 ml. of 6 N hydrochloric acid followed by 100 ml. of water. On recrystallizing this residue from glacial acetic acid in the presence of activated carbon, 0.3 grams of a tan material was obtained that melted at 201-203° and gave no depression in the melting point when mixed with a pure sample of starting material.

2) N-Methylformanilide and Phosphorus Oxychloride with o-Dichlorobenzene as Solvent. A mixture of 1 gram (0.0024 mole) of the naphthol-1 ester (XII), 0.65 grams (0.0043 mole) of N-methylformanilide, 0.65 grams (0.0043 mole) of phosphorus oxychloride and 2 ml. of o-dichlorobenzene were placed in a test tube and heated on a steam-bath along with mechanical stirring for one-half hour. A reddish-purple solution formed and the evolution of an

acidic vapor was noted. The solution was then cooled and neutralized with 5 grams of sodium acetate in 10 ml. of water. A steam distillation was then carried out. The resulting solid was filtered off and washed with 60 ml. of 6 N hydrochloric acid and 100 ml. of water. The product was brown, weighed 0.85 grams and melted at 185-195°. A recrystallization from glacial acetic acid gave a light tan material that melted at 198-201° and gave no depression when mixed with a sample of the naphthol-1 ester.

BIBLIOGRAPHY

1. Asano, M., and K. Yamaguti, J. Pharm Soc. Japan, 60, 105 (1940).

2. Bou-Hoi, Ng. Pr., and Ng. Hoan, J. Am. Chem. Soc., 73, 99, (1951).

3. Daub, G. H., and W. S. Johnson, J. Am. Chem. Soc., 70, 418 (1948).

4. Daub, G. H., and W. S. Johnson, J. Am. Chem. Soc., 72, 502 (1950).

5. Bareckson, W. M. III, "The Synthesis of Compounds Related to Podophyllotoxin", Thesis, University of Maryland, College Park, Maryland (1950).

6. Fieser, L. F., J. L. Hartwell, and J. E. Jones, "Organic Synthesis", John Wiley and Sons, Inc., New York, N. Y., Vol. XX, p. 11 (1940).

7. Gensler, W. J., and C. M. Samour, J. Am. Chem. Soc., 72, 3318 (1950).

8. Hartwell, J. L., and M. J. Shear, Cancer Research, 7, 716 (1947).

9. Hartwell, J. L., J. Am. Chem. Soc., 69, 2913 (1947).

10. Haworth, R. D., and T. Richardson, J. Chem. Soc., 149, 348 (1936).

11. Johnson, W. S., A. Goldman, and W. P. Schneider, J. Am. Chem. Soc., 67, 1357 (1945).

12. Johnson, W. S., J. W. Peterson, and C. D. Rutsche, J. Am. Chem. Soc., 69, 2949 (1947).

13. Johnson, W. S., and M. W. Miller, J. Am. Chem. Soc., 72, 511 (1950).

14. King, H., and F. F. Nord, J. Org. Chem., 13, 639 (1948).

15. Manasse, O., Ber., 27, 2409 (1894).

16. Medinger, P., Monatsch., 27, 240 (1906).

17. Ormsbee, H. A., I. Cornman, and R. E. Berger, Proc. Soc. Exp. Biol. and Med., 66, 586 (1947).

18. Reeve, W., and J. D. Sterling, Jr., J. Am. Chem. Soc., 71, 3557 (1949).

19. Spath, E., F. Wessely, and E. Nadler, Ber., 66, 125 (1933).

20. Turner, D. L., J. Am. Chem. Soc., 73, 1284 (1951).

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Title of Thesis: Part I: The Synthesis of Hydrastic Acid
Part II: Studies on the Degradation of Picropodophyllin
Part III: Studies on the Synthesis of Podophyllotoxin

Thesis directed by Professor Wilkins Reeve
Major: Organic Chemistry, Department of Chemistry
Minors: Inorganic Chemistry, Physical Chemistry

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This research has been carried out in three parts: The Synthesis of Hydrastic Acid; Studies on the Degradation of Picropodophyllin; and Studies on the Synthesis of Podophyllotoxin.

In the first case, it was desired to synthesize hydrastic acid in yields large enough to enable its use as an intermediate in the preparation of 4,5-methylenedioxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid. This was accomplished by applying newer methods and techniques to the procedure developed by Perkin. As a result, hydrastic acid was prepared in an overall yield of 25-27 per cent and hydrastic anhydride in an overall yield of 20-23 per cent. The 4,5-methylenedioxycinnamic acid was better prepared by the Doebner modification of the Perkin reaction rather than by the Claisen condensation of piperonal and ethyl acetate as used by Perkin. Perkin's reduction of the cinnamic acid by sodium amalgam has been replaced by the catalytic reduction of an aqueous solution of its sodium salt over Raney nickel. The cyclization of the dihydrocinnamic acid to the hydrindone was

better effected by preparing the acid chloride and then closing the ring with stannic chloride rather than using phosphorus pentoxide on the acid. The nitric acid oxidation step was improved by using vanadium pentoxide as a catalyst and a different isolation procedure, so that a 45 per cent yield of hydrastic acid was obtained in this step. The hydrastic acid easily loses water on heating to give the anhydride.

The studies on the degradation of picropodophyllin concerned an attempt to obtain conclusive evidence regarding the position of the free hydroxyl group in that molecule. A dehydrogenation was attempted using copper chromite as catalyst and ethylene as a hydrogen acceptor. Under these conditions, a secondary alcohol would have led to a ketone. The only identifiable product obtained was dehydroanhydropicropodophyllin although there was inconclusive indications that a ketone had also been formed. The remainder of the work in this section involved the oxidation of picropodophyllin in various solvents with lead tetraacetate. If the hydroxyl group was on carbon 2, formaldehyde would have been liberated. None was detected.

The work on the synthesis of podophyllotoxin was carried out with a view towards proving the structure of podophyllotoxin through synthesis and developing a general method for obtaining similar molecules. The synthesis of podophyllotoxin has not been accomplished although 5,6-methylenedioxy-4-(3,4,5-trimethoxyphenyl)-2-carbomethoxynaphthol-1, an intermediate in the proposed synthesis, was obtained. This was

synthesised by utilising the procedures of Bareckson and Gensler in reaching 4,5-methylenedioxy-2-(3,4,5-trimethoxybenzoyl)benzoic acid. This was methylated with diazomethane followed by a Stobbe condensation with dimethyl succinate. In addition to the naphthol-1, a substituted itaconic acid, a self-condensation product of dimethyl succinate and an unknown substance were also isolated from the Stobbe condensation.