

THE CHEMISTRY OF PODOPHYLLOTOXIN

By

Edward H. Price

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of the University of Maryland in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy**

1949

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TABLE OF CONTENTS

	Page
INTRODUCTION.....	1
HISTORICAL.....	2
The 1-Phenyltetralins.....	3
The Lignanes.....	6
Iscolivil.....	8
Isolariciresinol.....	14
Conidendrin.....	20
The Peltatins.....	24
Discoegenol.....	25
Synthetic Work.....	26
Dehydroguaiaretic Acid.....	26
Compound XV.....	26
The Action of Formalin.....	29
An Analog of Picropodophyllin.....	30
DISCUSSION.....	31
EXPERIMENTAL.....	42
Podophylletoxin, XLVII.....	42
Picropodophyllin, XLVIII.....	42
Preparation of the Acetate of Podophylletoxin.....	43
Preparation of the Acetate of Picropodophyllin.....	43
Preparation of alpha-Apopicropodophyllin.....	44
Preparation of the Sodium Salt of Podophyllie Acid.....	45
Acetylation of Sodium Podophyllate.....	47
Attempted Tritylation of Podophylletoxin.....	48
Attempted Esterification of Sodium Podophyllate.....	49

Preparation of the Trihydroxy Compound, L _a	49
Preparation of the Tri-para-nitrobenzoate of L _a	51
Preparation of the Anhydro Compound, LI _a	51
Preparation of the Mono-para-nitrobenzoate of the Anhydro Compound, LI _a	52
Preparation of the Methyl Ether of Anhydro Compound, LI _a	52
Preparation of the Benzoate of LI _a	53
Hydrolysis of the Benzoate of LI _a to LI _a	54
Pyrolysis of the Benzoate of the Compound LI _a	54
Attempts to Prove the Presence of a Secondary Hydroxyl Group in Pieropodophyllin and in the Anhydro Compound, LI _a	54
The Preparation of Trihydroxy Compound, L _b and the Anhydro Compound, LI _b	58
A Sound Preparation of the Trihydroxy Compound, L _b	60
Preparation of the Tri-para-nitrobenzoate of Compound L _b	61
Preparation of the Mono-para-nitrobenzoate of the Anhydro Compound, LI _b	62
Preparation of the Benzoate of Pieropodophyllin, XLVIII.....	62
Preparation of the Benzoate of Podophyllotoxin, XLVII.....	63
Preparation of beta-Apopieropodophyllin, XLIX.....	63
The Preparation of Desoxypieropodophyllin, LII.....	65
Preparation of the Dihydroxy Compound LIII.....	67
Preparation of the Di-para-nitrobenzoate of the Dihydroxy Compound, LIII.....	67
Preparation of the Desoxyanhydro Compound, LIV.....	68
Attempts to Dehydrogenate the Compounds, LI _a to LIV.....	69
Ultra-Violet Absorption Spectra.....	70
OBSERVATIONS AND CONCLUSIONS.....	93
BIBLIOGRAPHY.....	102

INTRODUCTION

The drug podophyllin, formerly used as a cathartic, is of interest in dermatology⁵⁷. It has been demonstrated that application of podophyllin to normal skin causes severe disturbances in the cell pattern leading to disintegration of the chromatin mass, and the discovery has been made that podophyllotoxin, the most active constituent of the podophyllin resin, has activity against cancerous tissue. Further study of this substance is indicated.

This research was started with the purpose of determining, if possible, the portion of the podophyllotoxin molecule which was responsible for the activity of the drug. It was also of interest to lower the toxicity of the molecule thereby rendering it more useful.

Podophyllotoxin has been transformed into certain closely related compounds several of which have been submitted for biological testing through the National Chemical and Biological Coordination Center. The results of the biological tests have not yet been made available, but it is understood from private communications that at least one of the compounds has interesting properties and is worthy of further investigation.

HISTORICAL

It has been established with a reasonable degree of certainty that pedophyllotoxin has the structure given by XLVII, although synthesis of the compound has not been accomplished. A thorough review of the proof of structure for pedophyllotoxin has been made recently by Sterling⁶⁸ and is therefore omitted here. A review of the literature has revealed that a number of compounds are known which have a 1-phenyltetralin structure, but are not closely related to pedophyllotoxin. These compounds are listed in the following Table I together with a brief notation of their manner of synthesis as obtained from Chemical Abstracts.

TABLE I

1-Phenyltetralins

Compound	Synthesis	Bibliographical Reference
1-phenyl-3,4-dihydronaphthalene	The reaction of phenylmagnesium bromide with tetralone	1
1-(2-biphenyl)-3,4-dihydro-naphthalene	The reaction of the lithium alkyl of 2-iodobiphenyl with tetralone	2
1-(2-biphenyl)-4-methyl (and 3,4-dimethyl)-3,4-dihydro-naphthalene	The reaction of the lithium alkyl of 2-iodobiphenyl with the corresponding tetralone	3
1,4-diphenyl-1-hydroxy-naphthalenone -2	The reaction of phenylmagnesium bromide with 2-methoxy-1,4-antraquinone	4
1,4-diphenyl-1,2,3,4-tetrahydro-1-naphthoic acid	The reaction of furoic acid with benzene in the presence of aluminum chloride	5
1,4-dihydroxy-1,2-diphenyl-3-methyl-1,2-dihydronaphthalene	The reaction of phenylmagnesium bromide with 2-methyl-1,4-naphthoquinone	6
1,2-diphenyl-4-oxo-1,2,3,4-tetrahydro-1-naphthol	The reaction of phenylmagnesium bromide with 1,4-naphthoquinone	7
1-phenyl-4-oxo-1,2,3,4-tetrahydro-2-naphthaleneacetic acid	The action of hydrofluoric acid on beta-diphenylmethylglutaric acid	8
6-hydroxy-7-methyl-1,1,4,4-tetra-phenyl-1,2,3,4-tetrahydro-naphthalene	The dialkylation of ortho-cresol by 1,1,4,4-tetra-phenyl-1,4-butanediol in the presence of aluminum chloride	9
1,4-dihydro-1,4-diphenyl-1,4-naphthalenediol	The reaction of phenylmagnesium bromide with 1,4 naphthoquinone	10,21

TABLE I (Continued)

1-Phenyltetralins

Compound	Synthesis	Bibliographical Reference
1,1-diphenyl-2,3-dimethyl-4-keto-1,4-dihydronaphthalene	The dehydration of 1,4-diphenyl-1,4-dihydro-2,3-dimethyl-1,4-naphthalenediol	11
1,3-(1,2)-diphenyl-1,2,3,4-tetrahydro-1-naphthol	The reaction of phenylmagnesium bromide with 3-(2-) phenyltetralone	12
1,2,3,4-tetrahydro-4-phenyl-1,2-naphthalenedicarboxylic acid	The condensation of 1,1-diphenylethylene with maleic anhydride followed by treatment of the product with hydrobromic acid in acetic acid	13
1,2,3-triphenyl-1,4-dihydronaphthalene	The action of lithium metal with toluene followed by a sodium and alcohol reduction of the product	14
4-para-anisyl-1,2-dihydronaphthalene	The reaction of para-anisylmagnesium bromide with tetralone	15
1-phenyl-1,2,3,4-tetrahydro-4-keto-2-naphthoic acid	The reaction of acetyl chloride with 2-methyl-2,3-diphenylsuccinic acid in the presence of aluminum chloride	16
1-phenyl-1,2,3,4-tetrahydro-2-naphthoic acid	The action of sulfuric acid on alpha-benzoyloxy-gamma-phenylbutyric acid	17
1,3-diphenyl-2-keto-1,2-dihydronaphthalene	The condensation of dibenzyl ketone with salicylaldehyde	18
4-tolyltetralone	The action of aluminum chloride on gamma-tolylbutyryl chloride	19

TABLE I (Continued)

2-Phenyltetralins

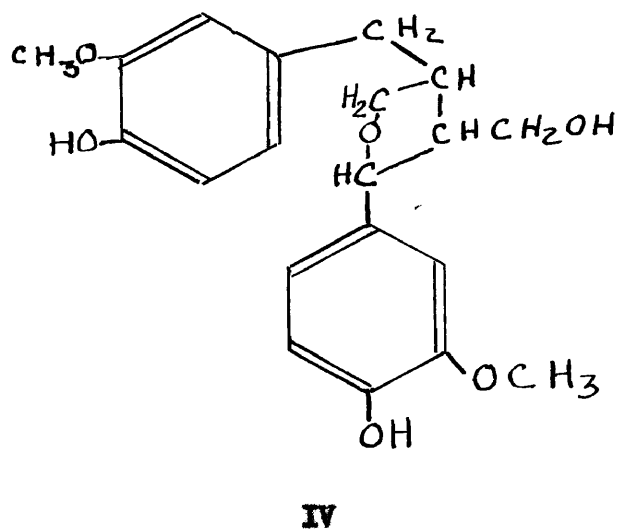
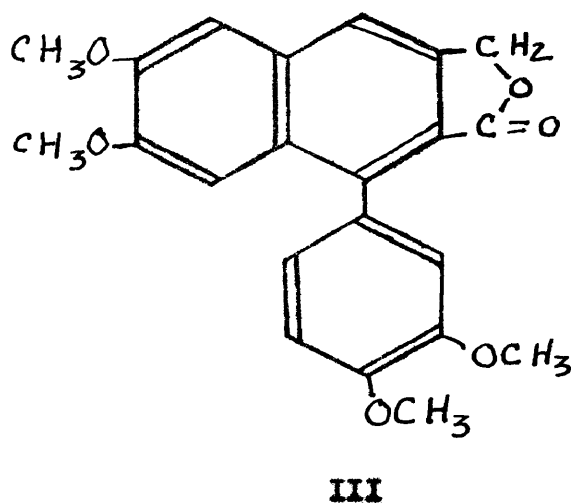
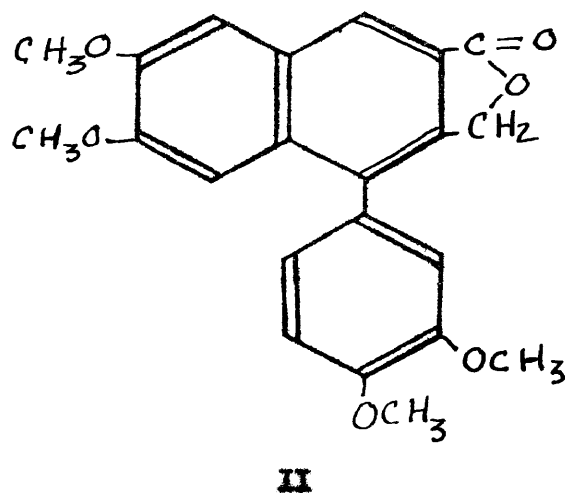
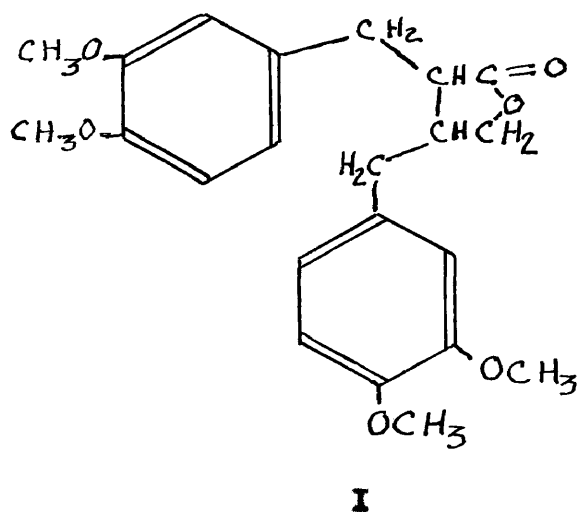
Compound	Synthesis	Bibliographical Reference
1,2,3,4-tetraphenyl-1,4-dihydro-1,4-naphthalenediol	The reaction of phenylmagnesium bromide with 2,3-dichloro-1,4-naphthoquinone	20
1,2-dihydro-2,3-dimethyl-1,2-diphenyl-1,4-naphthalenediol	The reaction of phenylmagnesium bromide with 2,3-dimethyl-1,4-naphthoquinone	22
1,2,3-triphenyl-1,2-dihydro-1,2-naphthalenedicarboxylic anhydride	One of a series of compounds formed by the reaction of sodium with tolane	23
1-(3,4,6-trimethoxyphenyl)-1,2,3,4-tetrahydro-3-hydroxy-methyl-2-naphthoic acid lactone	Obtained by the extraction of the seeds of <u>Marandia oulfera</u> , <u>Linn.</u>	24
1-para-tolyl-7-methyl-3,4-dihydronaphthalene	The reaction of para-tolylmagnesium iodide with 7-methyl-3,4-dihydro-1-(2)-naphthalenone	69
1,2,2,3-tetraphenyl-1,4-dihydroxy-1,2-dihydronaphthalene	The reaction of phenylmagnesium bromide with 2,3-diphenyl-1,4-naphthoquinone	70
1,2,3,4-tetraphenyl-1,4-dihydroxy-1,4-dihydronaphthalene	The reaction of phenyllithium with 2,3-diphenyl-1,4-naphthoquinone	70
2,2,3,4-tetraphenyl-1-keto-1,2-dihydronaphthalene	The dehydration of 1,2,3,4-tetraphenyl-1,4-dihydroxy-1,4-dihydronaphthalene	70
1,2,2,3,4-pentaphenyl-1-hydroxy-1,2-dihydronaphthalene	The reaction of phenylmagnesium bromide with 2,2,3,4-tetraphenyl-1-keto-1,2-dihydronaphthalene	70

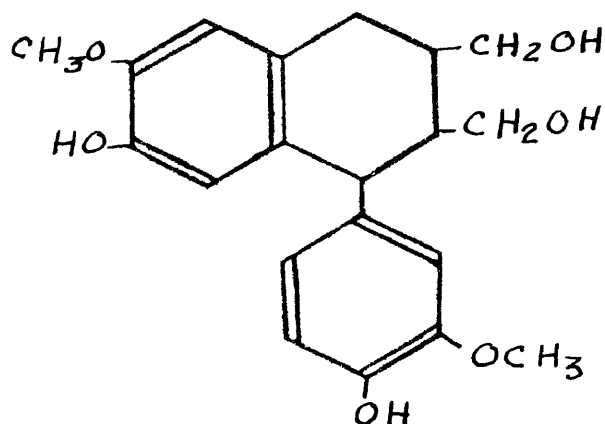
The Lignanes

The substituted 1-phenyltetralin structure is found in podophyllo-toxin²⁵, conidendrin or "sulfite liquor lactone", iscolivil, and isolari-ciresinol. Haworth²⁶ has proposed the name lignan to cover this class of naturally occurring compounds embracing the 1-phenyltetralin structure and also substituted cyclobutane or butyrolactone structures analogous to the tetralins. The lignans occur principally in the heartwood of coniferae and in rhizomes and seeds of many plants. In general, the simple 1-phenyltetralin ring structure is never found as such, for the aromatic rings are substituted by one or more methoxyl or hydroxyl groups or combinations thereof. The substituents on the saturated portion of the tetralin nucleus vary widely and include methyl, hydroxymethyl, carboxyl, and hydroxyl groups in various combinations.

The elucidation of the structures of these compounds has been a particularly difficult problem, inasmuch as all the compounds possess three or more centers of optical activity. As a result, their synthesis has not been possible, and in most cases not attempted; but rather the original material has been degraded by oxidative and dehydrogenation procedures to optically inactive materials which were more easily identified. Compounds of the 1-phenyltetralin type may be oxidized by potassium permanganate to yield substituted ortho-benzoylbenzoic acids, and by alkaline bromine solution to a benzoylbenzoic acid and also a dibasic acid which retains the 1-phenyltetralin structure. It is of interest here to note two very misleading reactions which complicated early work in the lignans. One is exemplified by the conversion of 1-matairesinol dimethyl ether I²⁷ to a mixture of II and III²⁸ by the action of lead tetraacetate. The other is ring closure of certain butanes to the 1-phenyltetralin structure under

the influence of methyl alcoholic hydrogen chloride, an example of which is the conversion of lariciresinol IV²⁹ to isolariciresinol V. The latter transformation was not recognized by Bamberger³⁵ during his early work on lariciresinol, and as a result many of his derivatives of lariciresinol were actually derivatives of isolariciresinol²⁹.





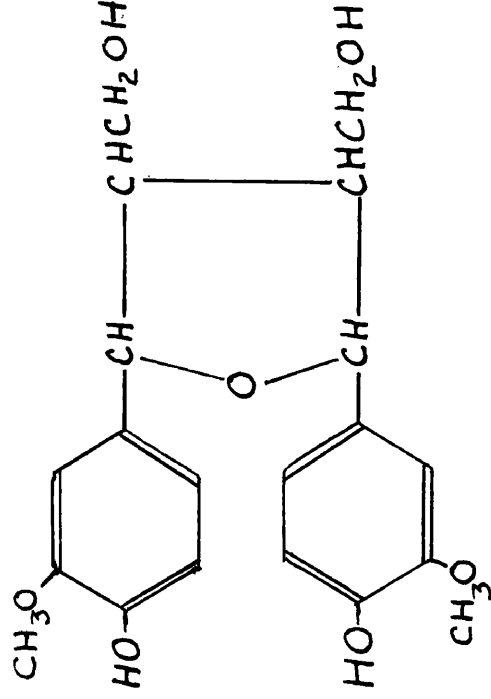
V.*

Iscolivil: Iscolivil has only recently been found to occur in nature when it was isolated by solvent extraction from Olea Cunninghamii³⁰. Until this time it was a synthetic product obtained from olivil, a substance first isolated from the resin of the olive tree by Koerner, et. al.³¹ Koerner established that olivil contained two phenolic hydroxyl groups, two methoxyl groups, and that it could be brominated and nitrated. He determined the molecular formula to be $C_{20}H_{24}O_7$ ³² and recognized that acidic reagents caused the conversion of olivil into a new isomeric substance which he called iscolivil.

Later Vansetti carried on the research which led to the establishment of the structure of iscolivil at the conclusion of which he published an extensive review of his work³³. Oxidation of olivil dimethyl ether in

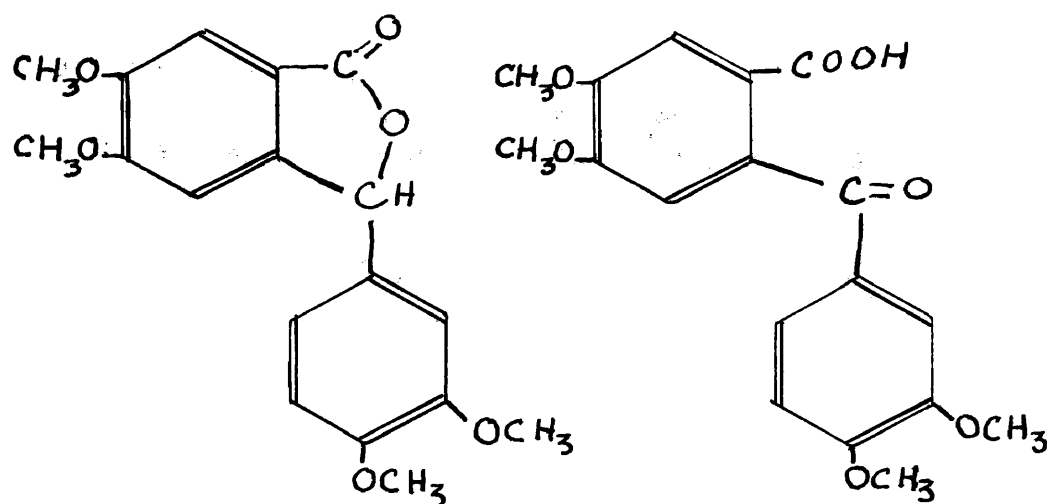
* In writing hydroaromatic compounds the hydrogen atoms have been omitted for the sake of convenience. In all cases the double bonds of the aromatic rings are shown.

Vanzetti's hands yielded oxalic acid and veratric acid, hence he concluded that there were two 1-hydroxy-2-methoxyphenyl groups joined by a chain of six carbon atoms present in olivil. Subsequent studies revealed two alcoholic hydroxyl groups and one etheral oxygen in the saturated carbon chain. Since only one alkylation product was obtained, Vanzetti concluded the molecule was symmetrical, and based on its possible formation by the condensation of two molecules of couiferyl alcohol, he proposed VI as the formula for olivil.



VI

Olivil is converted by acidic reagents into isoolivil, a substance of the same molecular formula, but widely different properties. The dimethyl ether of isoolivil is oxidized by alkaline permanganate solution to give two products, which were identified as the lactone of 3,3',4,4'-tetramethoxy-6-carboxybiphenyl VII and *ortho*-veratrylveratric acid VIII. Structures for compounds VII and VIII were rigorously established by (1)



VII

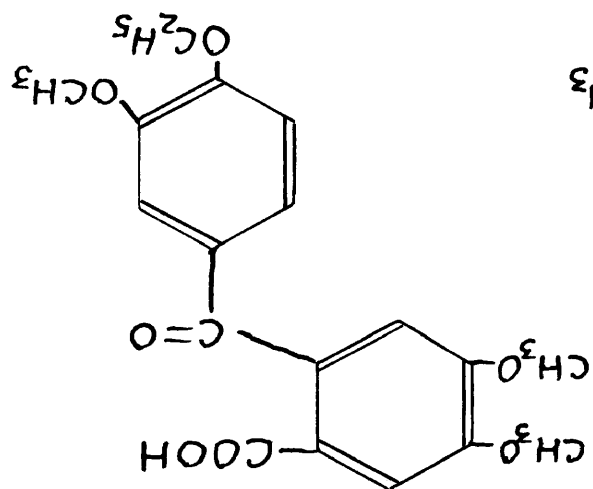
VIII

their interconversion, (2) the decarboxylation of the free acid to diveratryl ketone, (3) the synthesis of the diveratryl ketone, (4) the conversion of VIII to 2,3,6,7-tetramethoxyanthraquinone, which was also synthesized, and (5) analyses and neutral equivalents.

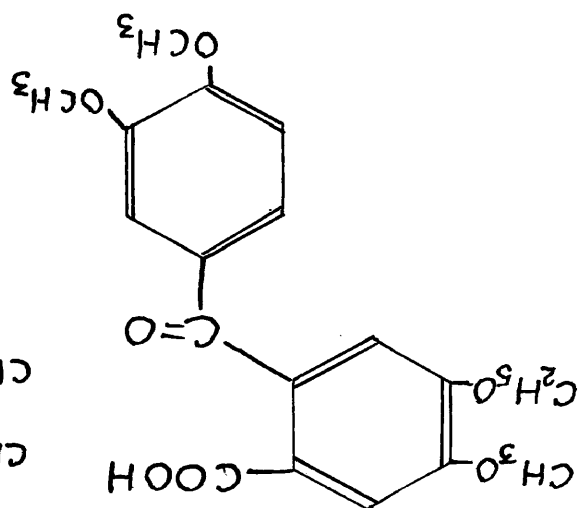
In order to determine the position of the free phenolic groups in iscolivil, Vanzetti and coworkers prepared, diethyl iscolivil, methyl ethyl iscolivil by first ethylating and then methylating iscolivil, and ethyl methyl iscolivil, the ethyl ether from monomethyl iscolivil, Diethyl iscolivil was oxidized to IX and X corresponding to VII and VIII above. These compounds were identified in the same manner as their previous analogs.

Similarly, ethyl methyl iscolivil yielded XI, and methyl ethyl iscolivil yielded XII. From the oxidation products it was concluded that the first phenolic hydroxyl to etherify was the one on the 1-phenyl ring and not on the tetralin nucleus. On this basis, and considering its

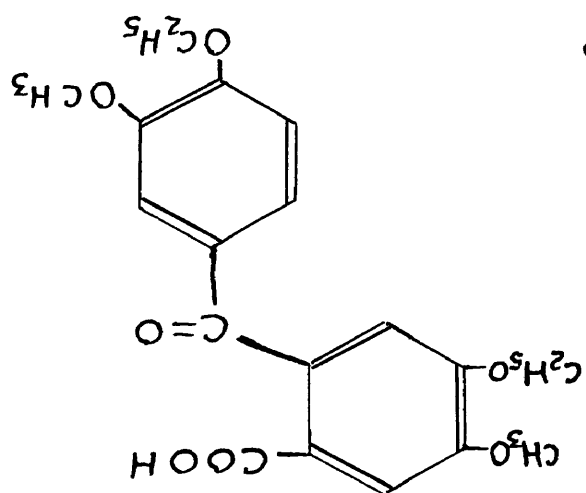
XIX



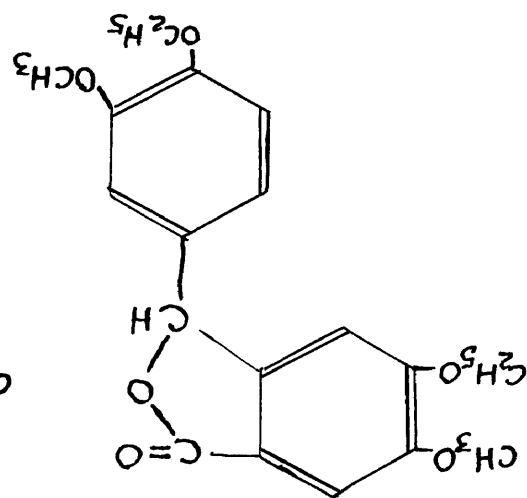
XX



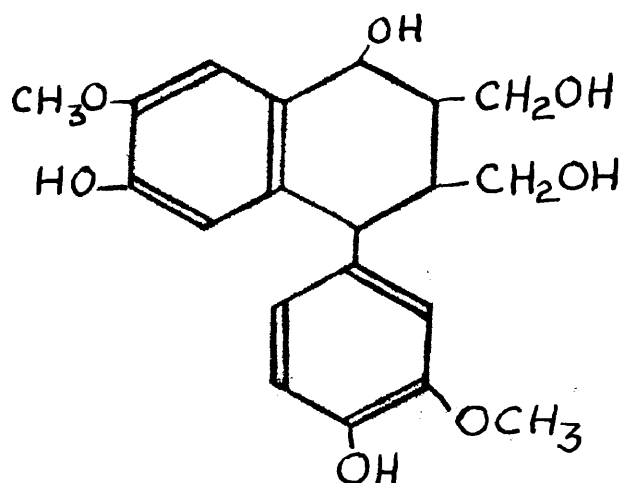
XI



XII



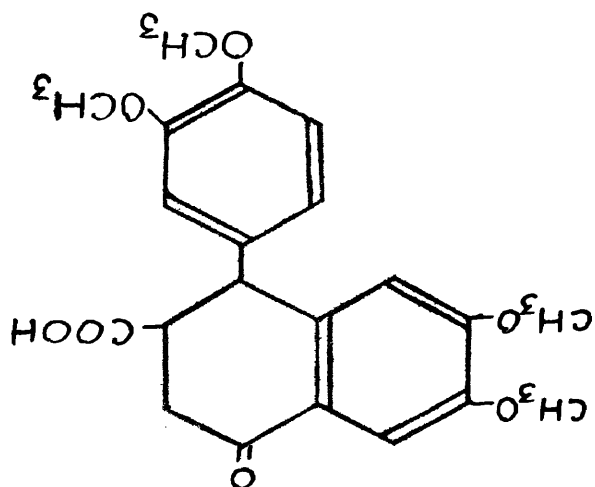
formation from olivil, Vanzetti proposed the formula of iscolivil to be XIII.



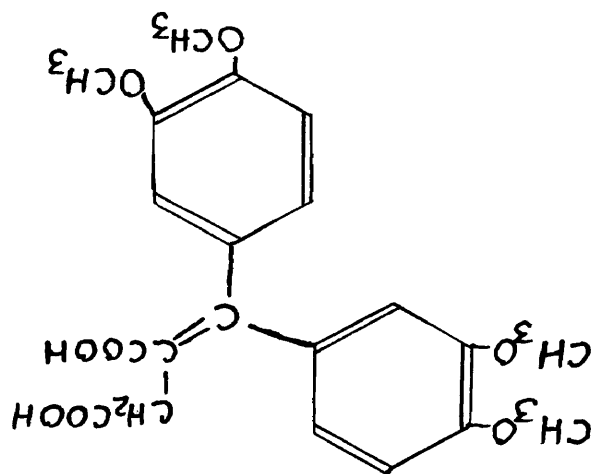
XIII

During the oxidation of iscolivil dimethyl ether there is also formed a small amount of a dibasic acid, which is still optically active, as is its potassium salt, although the latter loses optical activity on standing in solution. For this dibasic acid Vanzetti proposed the structure, XIV, on the basis of the molecular weight determination, methoxyl determination and analyses. Vanzetti further demonstrated that the dibasic acid XIV could be degraded by oxidation to VII and VIII. Structure XIII for iscolivil was confirmed by P. Dreyfuss³⁴ who isolated compound XV after oxidation of iscolivil by chromic acid. XV was shown by direct comparison to be identical with the synthetic lactone of Haworth and Sheldrick²⁸. The synthesis of the lactone XV was best accomplished by the condensation of diveratryl ketone with ethyl succinate to give compound XVI, which on reduction, conversion to the anhydride, and cyclization by aluminum chloride

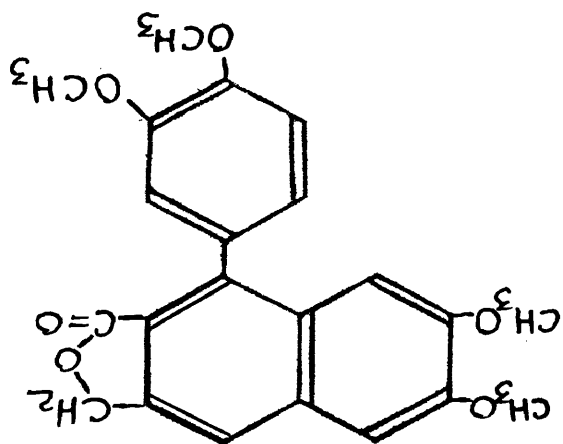
IIIX



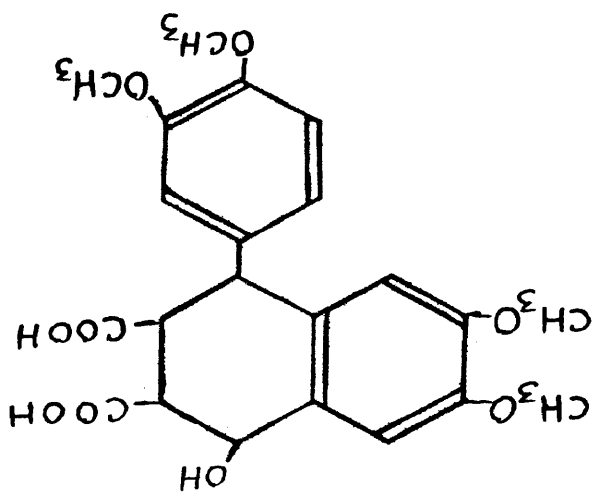
IAX



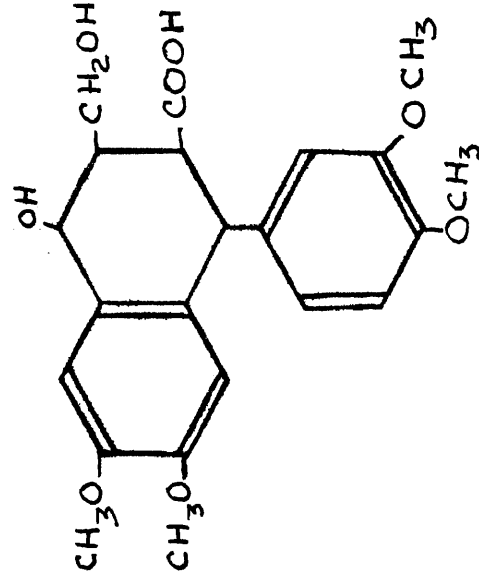
IX



AIX

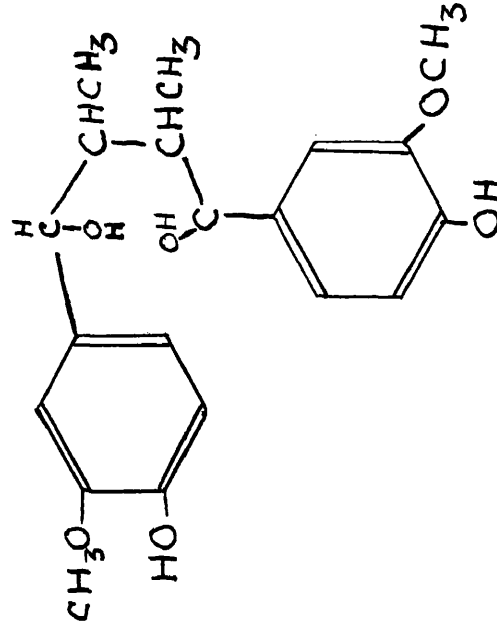


crude product therefrom was dehydrogenated by lead tetraacetate to yield XV.
 compound XVIII. Heating of XVIII brought about lactonization, and the
 by sodium, and the product therefrom reduced by sodium amalgam to give
 yielded XVII. This acid was then esterified, condensed with ethyl formate



XVIII

Isolariciresinol: Early workers, of whom Bamburger was the most prominent, isolated lariciresinol by solvent extraction from the European larch³⁵ and suggested the presence of two methoxyl groups, two phenolic hydroxyl groups, and two alcoholic hydroxyl groups in the molecule, with a molecular formula of $C_{19}H_{22}O_6$. Subsequently Meyer and Jacobsen³⁶ suggested formula XIX as the correct one for lariciresinol without giving any experimental evidence.



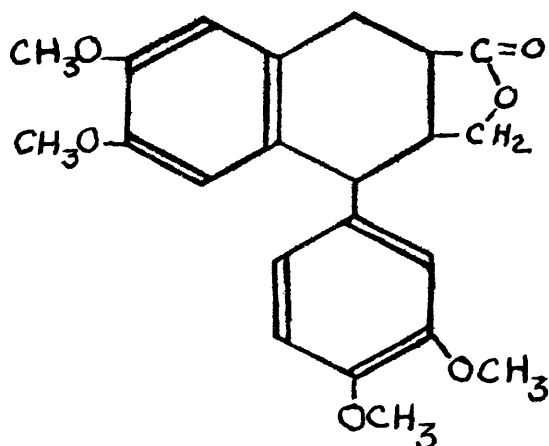
XIX

In 1937 Haworth²⁹ reinvestigated the chemistry of lacticresinol and isolaricresinol, and stated that much of Bambergers early work was invalidated because he failed to recognize that acidic reagents converted lacticresinol, IV, into isolaricresinol, V. As a result, Haworth claims for example that Bambergers acetate of lacticresinol made with acetyl chloride is really the tetraacetate of isolaricresinol, and that on alkaline hydrolysis it yields isolaricresinol. Therefore at the risk of slight discrepancies this review will be confined to the work of Haworth and his collaborators.

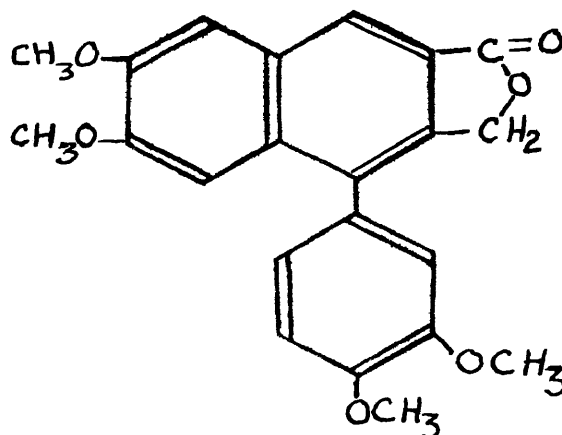
Haworth and Kelly by alkaline treatment of alcoholic extracts of the larch obtained lacticresinol as the potassium salt and then as the free substance. Careful analyses indicated $C_{20}H_{24}O_6$ as the molecular formula, and the presence of two methoxyl groups was demonstrated. Zerewittenoff determinations showed the presence of three hydroxyl groups, of which two were proven to be phenolic. The diethers of lacticresinol are saturated, stable to alkali, give no reaction with ketone reagents and Grignard reagents. Hence the sixth oxygen atom must be etheral and not of the ethylene oxide type.

It was then that the transformation of lacticresinol into isolaricresinol was recognized, and simultaneously it was found that the sixth oxygen atom which was formerly etheral was present as an alcoholic hydroxyl in isolaricresinol. This isomerization was immediately compared to the then known transformation of olivil to isoolivil. The cyclization to a tetralin derivative was confirmed by oxidation studies, wherein it was shown that lacticresinol diethyl ether yielded only 3-methoxy-4-ethoxybenzoic acid on permanganate oxidation, whereas the dimethyl and diethyl ethers of isolaricresinol yielded respectively the substituted benzoylbenzoic acids identical with those already discussed, cf. formulas VIII and X.

The 1-phenyltetralin structure was further established by the oxidation of isolariciresinol dimethyl ether by sodium hypobromite to 1-condendrin dimethyl ether XI, whose proof of structure is yet to be considered. Dehydrogenation of that product by lead tetraacetate was accomplished to yield a naphthalene derivative XXI which had already been synthesized^{28,37}.

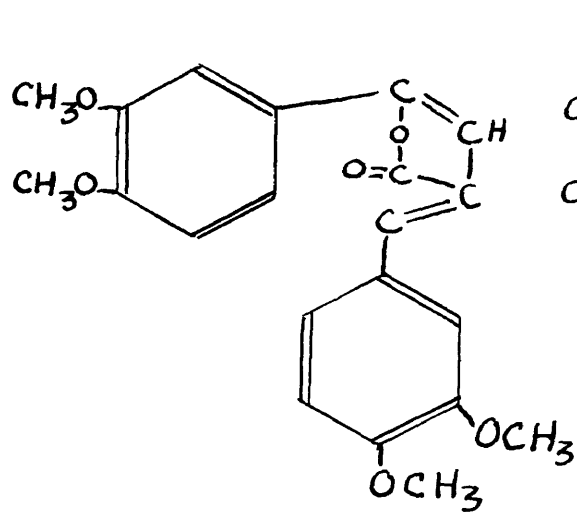


XI

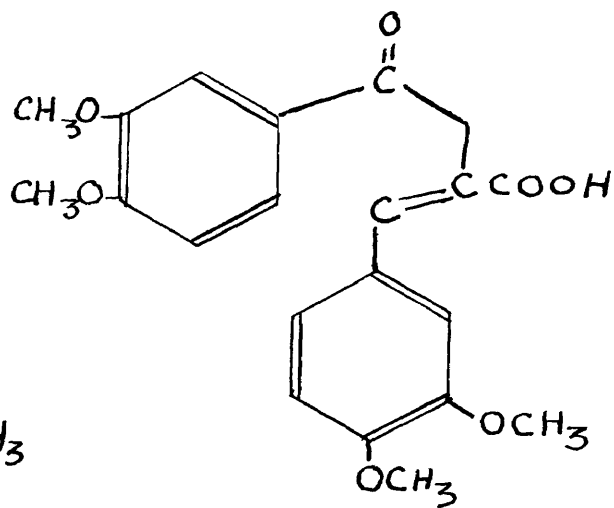


XXI

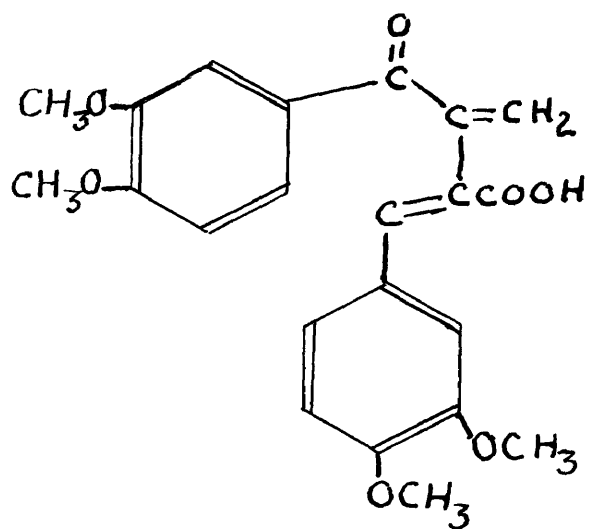
Compound, XXI, was synthesized by the condensation of sodium beta-3,4-dimethoxybenzoylpropionate with veratraldehyde in the presence of acetic anhydride to yield XXII, which on hydrolysis gave XXIII. Compound XXIII on addition of cold alkaline formaldehyde gave XXIV, which in the presence of cold hydrochloric acid in glacial acetic acid underwent cyclization, dehydration, and the addition of hydrogen chloride to the methylene group to give XXV. Alkaline hydrolysis of the latter followed by lactonization gave the desired compound XXI.



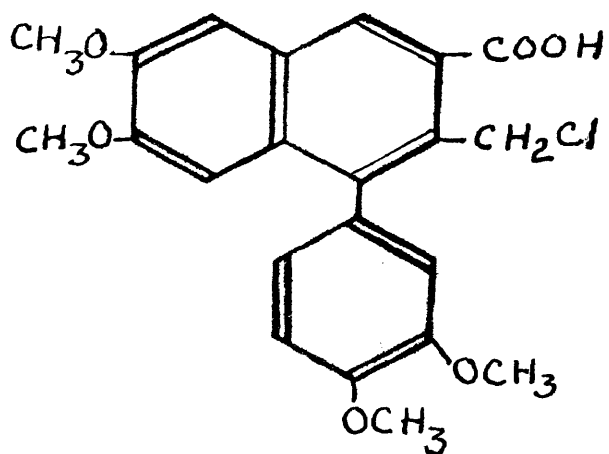
XXII



XXIII



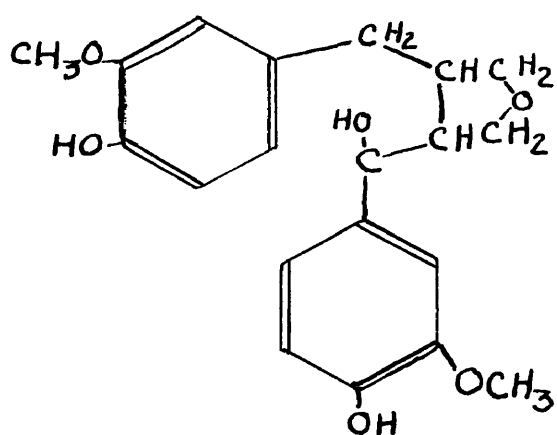
XXIV



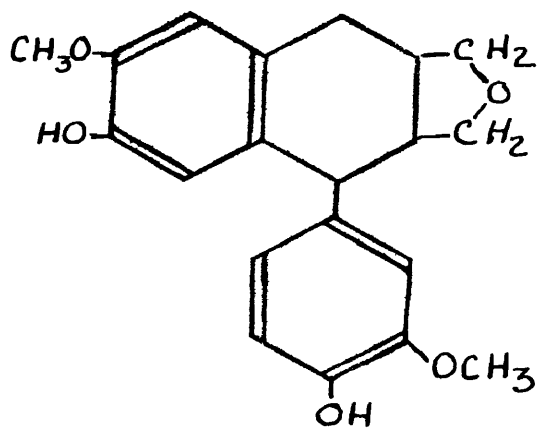
XXV

On this basis, it was proposed that lariciresinol and isolariciresinol are as given in formulas IV and V respectively. Another possibility for lariciresinol is compound XXVI, but this was ruled out, since it was possible to prepare the trityl ether of lariciresinol dimethyl ether, thus indicating the presence of a primary hydroxyl group³⁸. It was also possible to prepare from lariciresinol or isolariciresinol an anhydro derivative XXVII by the action of potassium bisulfate at 180°. This compound is very stable to acids, alkalis, acetylating agents and dehydrogenation by palladium black. Since lariciresinol is transformed into isolariciresinol and not into its anhydro derivative, then formula XXVI is improbable, since it was shown that the anhydro isolariciresinol is not hydrated under the conditions required for the conversion of lariciresinol into isolariciresinol.

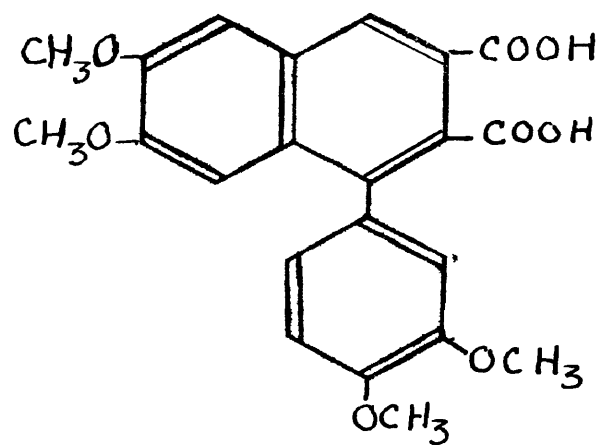
The structure of XXVII was established by dehydrogenation of its dimethyl ether with lead tetraacetate to the corresponding dehydroanhydro-isolariciresinol dimethyl ether, which was synthesized^{28,39}. The oxidation by alkaline bromine solution of either XV or XXI yields XXVIII which on reduction by sodium amalgam, followed by esterification and a Bouveault-Blanc reduction yields XXIX, which is a racemic form of isolariciresinol. Compound XXIX on treatment with potassium bisulfate and subsequent dehydrogenation by lead tetraacetate yields the dehydro derivative of the dimethyl ether of XXVII, identical with that from isolariciresinol. This evidence further established the structure of isolariciresinol.



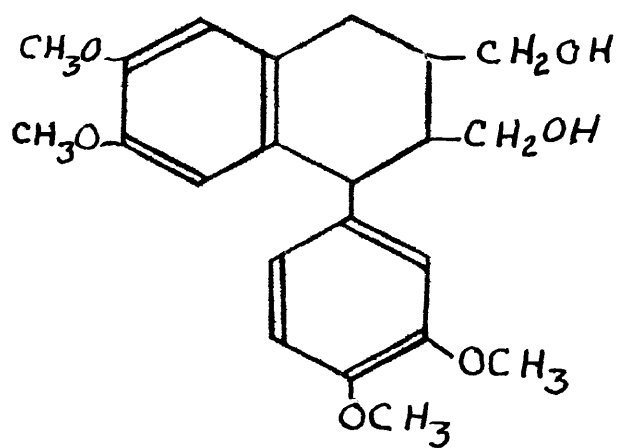
XXVI



XXVII

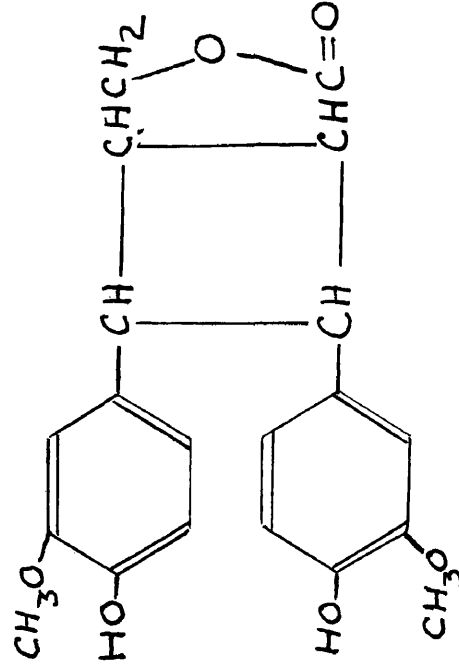


XXVIII



XXIX

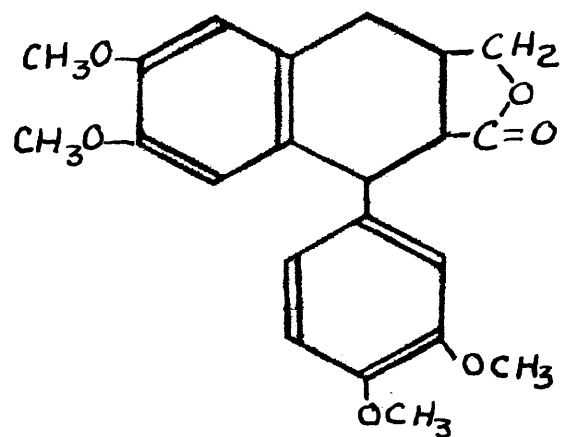
Conidendrin (sulfite liquor lactone or "taugaresinol"); Conidendrin, although reported by early workers, was first investigated by Holmberg⁴⁰ who isolated it from the waste sulfite liquor of paper industries. By extraction with alcohol, ether, or benzene a tarry extract was obtained which, when it was taken up in alcohol, yielded crystalline conidendrin in very small yield. Holmberg determined from the neutral equivalent and elementary analyses that this compound had the molecular formula of $C_{20}H_{20}O_6$, and he further established that it contained two phenolic hydroxyl groups, two methoxyl groups, a lactone group, and was saturated to bromine. Using these facts, he postulated its formation by the condensation of two molecules of coniferyl alcohol, and thus arrived at formula XXX for conidendrin. This formula permitted the observed optical activity of the lactone and its free acid. A further interesting observation was that when his original substance, which had a specific rotation of -195° , was treated with an excess of sodium ethoxide then acidified and recrystallized, and isomer was obtained which had a specific rotation of $+29^\circ$.



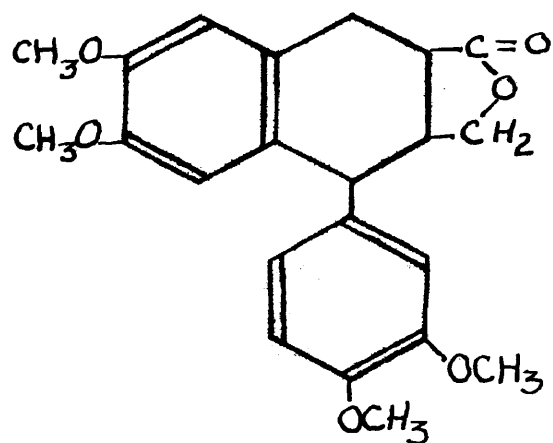
Immediately following this work, Holmberg and Sjöberg⁴¹ prepared the dimethyl ether of conidendrin, and showed that when its free acid was prepared, it did not regenerate pure conidendrin on lactonizing. Further, the isomerization of conidendrin methyl ether to its isomer was carried out as before, and the two corresponding free acids were prepared. None of the substances were enantiomers, but rather diastereomers, so that the presence of at least two asymmetric centers was clearly demonstrated.

By oxidation of the dimethyl ether of conidendrin with alkaline bromine solution, Holmberg obtained oxalic acid, one dibasic acid, another acid and a neutral material. It was then that Erdtman⁴² on oxidation of the same compound by alkaline permanganate obtained a neutral material which he couldn't identify and an acid $C_{18}H_{18}O_7$. The same acid was also obtained by oxidation with alkaline bromine solution by Holmberg's procedure along with the dibasic acid $C_{22}H_{24}O_8$. The acid $C_{18}H_{18}O_7$ gave trinitro veratrol when treated with fuming nitric acid, and 2,3,6,7-tetramethoxyanthraquinone was obtained when the substance was heated with sulfuric acid. Therefore, Erdtman reasoned correctly, the acid must be 6-veratrolyveratric acid, which had been reported several times in the work already cited. Erdtman recognized that the 6-veratrolyveratric acid fixed sixteen of the twenty carbon atoms in the original lactone, and reasoned that since the lactone and the anhydride of the dibasic acid formed so easily, the ring must be five membered. He thereby arrived at the two possibilities for conidendrin dimethyl ether, XXXI or XX, and concluded that the dibasic acid obtained by oxidation must be XXXII.

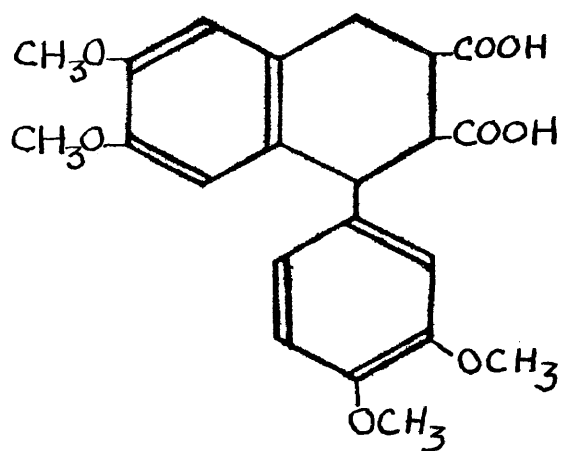
The next step was the dehydrogenation of the dibasic acid obtained from conidendrin. Erdtman was successful only with lead tetracetate, and he thereby obtained the acid XXVIII, already synthesized by Haworth²⁸, and



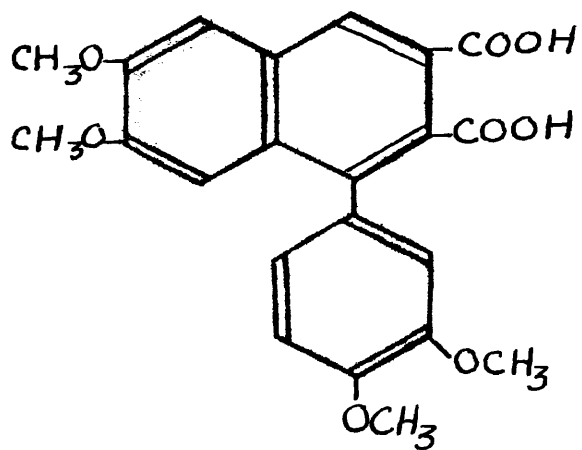
XXXI



XX

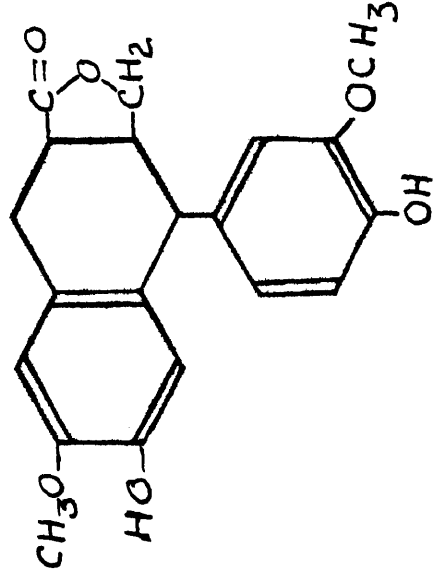


XXXII



XXVIII

later synthesized by the direct dimerization of 3,4-dimethoxyphenylpropionic acid⁴³. By a direct comparison of his product and the synthetic substance, they were proven identical. Erdtman subsequently dehydrogenated conidendrin dimethyl ether to a naphthalene derivative, which procedure Haworth and Sheldrick²⁸ repeated and then oxidized the dehydrogenated compound to XXVIII above. It was then shown by Haworth that the dehydrogenated derivative of conidendrin dimethyl ether was different from XV, and hence must be XXI. Therefore conidendrin dimethyl ether is definitely XX. The positions of the phenolic hydroxyl groups in conidendrin were proven by the oxidation of the diethyl derivative by alkaline permanganate to 5-methoxy-4-ethoxy-2-(3'-methoxy-4'-ethoxybenzoyl) benzoic acid, identical with that prepared by Vanzetti in his research on iscolivil³³. Therefore conidendrin itself is XX_a.

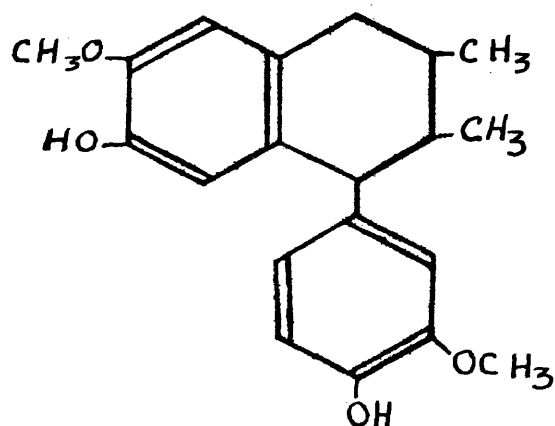
XX_a

Emdte and Schartner⁴⁴ subsequent to Holmberg's work isolated conidendrin directly from spruce shavings by solvent extraction. They repeated some of Erdtman's work and agreed substantially with his early results. They have also confirmed the claim of Kumatsu⁴⁵ that the tsugaresinol is identical with conidendrin. The latter investigators also repeated and confirmed Erdtman's work using tsugaresinol as starting material. The name conidendrin seems to have the widest acceptance, and as such has recently been reported in this country as having been isolated from western hemlock sawdust⁴⁶.

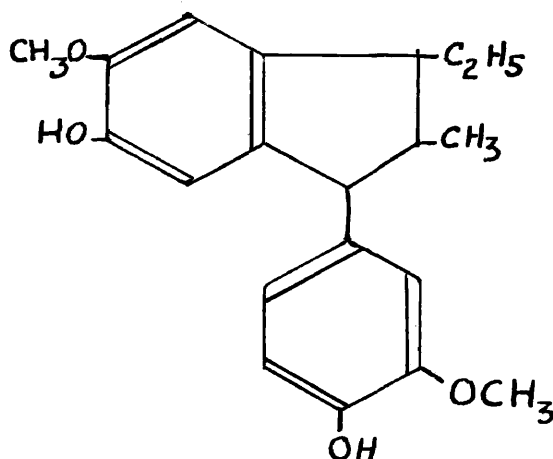
An interesting anomaly regarding the dehydrogenation of conidendrin was reported by Haworth⁴⁷. As already mentioned, conidendrin dimethyl ether can be dehydrogenated to the naphthalene derivative XXI. When this compound is reduced back to the tetralin by sodium and alcohol, one of the four theoretically possible racemates of conidendrin is obtained; and when this racemate is subjected to dehydrogenation again by lead tetraacetate, there is obtained 6,7-dimethoxy-1-veratryl-2-methylnaphthalene, instead of the expected lactone. The structure of the 2-methyl compound has been established by an independent synthesis.

The Peltatins: Two new components of podophyllin resin have been discovered recently. Hartwell^{48,49} has isolated by chromatographic separation of the podophyllin resin two new components, which he calls alpha and beta-peltatin. Alpha-peltatin is found in 9% yield and beta-peltatin in 4% yield. Alpha-peltatin by analysis has the composition, $C_{11}H_{11}O_4$, and contains one methoxyl group per eleven carbon unit. Beta-peltatin by analysis and molecular weight determination has the composition, $C_{22}H_{22}O_8$, and contains three methoxyl groups. Hartwell indicates that these two peltatins are probably isomeric with podophylletoxin, except that alpha-peltatin has one less methoxyl group.

Diisoeugenol: Various investigators have postulated the formation of the lignanes by the condensation of two molecules of eugenyl alcohol. Therefore, artificial dimerization of isoeugenol by methyl alcoholic hydrogen chloride to yield diisoeugenol is of considerable interest. Numerous investigators have worked on this problem, and over a period of years the structure became a choice of XXXIII⁵⁰ or XXXIV⁵¹.

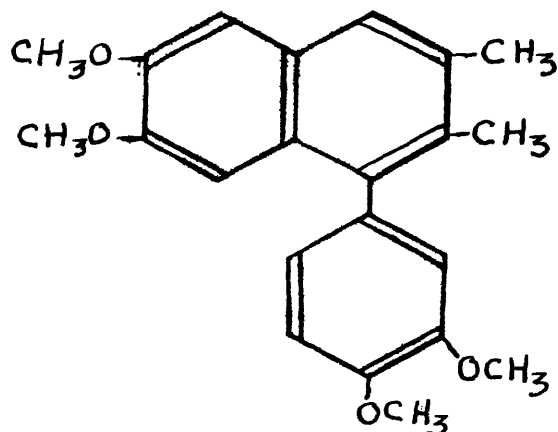


XXXIII



XXXIV

The main argument in favor of XXXIII was that on dehydrogenation with selenium at 280° for twenty-four hours the dimethyl ether of dehydro diisoeugenol yielded the dimethyl ether of dehydroguaiaretic acid, XXXIV, whose chemistry is yet to be considered. It was noted, however, that from diisoeugenol itself, Oliverie⁵⁰ could not isolate any products of dehydrogenation. Mueller⁵⁴ seems to have finally concluded the subject with his synthesis⁵¹ of a compound identical to the diisoeugenol dimethyl ether, and the product has the formula XXXIV.

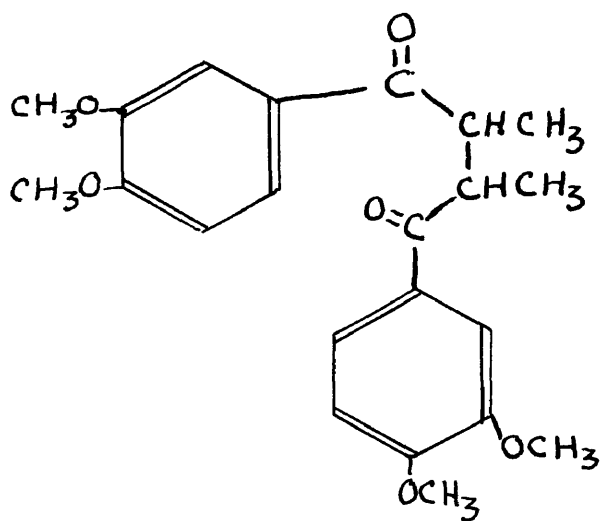


XXXV

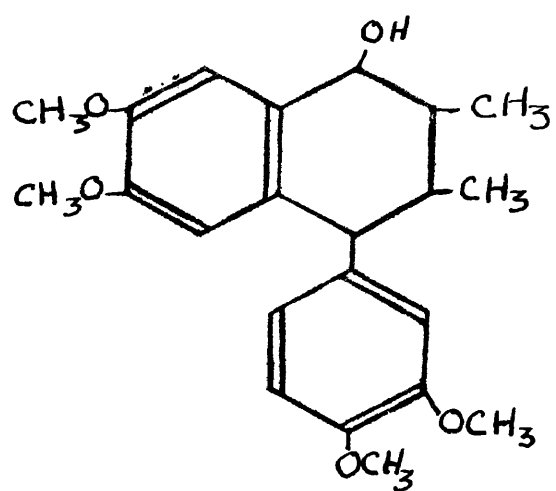
Synthetic Work: Related to the foregoing work are the syntheses of certain key compounds and intermediates necessary to establish structures. The most interesting and pertinent of these are presented here.

Dehydroguaiaretic acid dimethyl ether, XXXV, is important inasmuch as both iscolivil and iselariciresinol will give 8% yields of this substance when heated for twenty-four hours with selenium at 260°. A good synthesis of this compound was achieved⁵² by starting with 3,4-dimethoxy-alpha-bromopropiophenone; treatment of the phenone with copper in xylene gave XXXVI, and the latter was reduced readily to the diol with sodium and alcohol. The diol under the influence of methyl alcoholic hydrogen chloride cyclized to XXXVII, which on treatment with selenium gave XXXV.

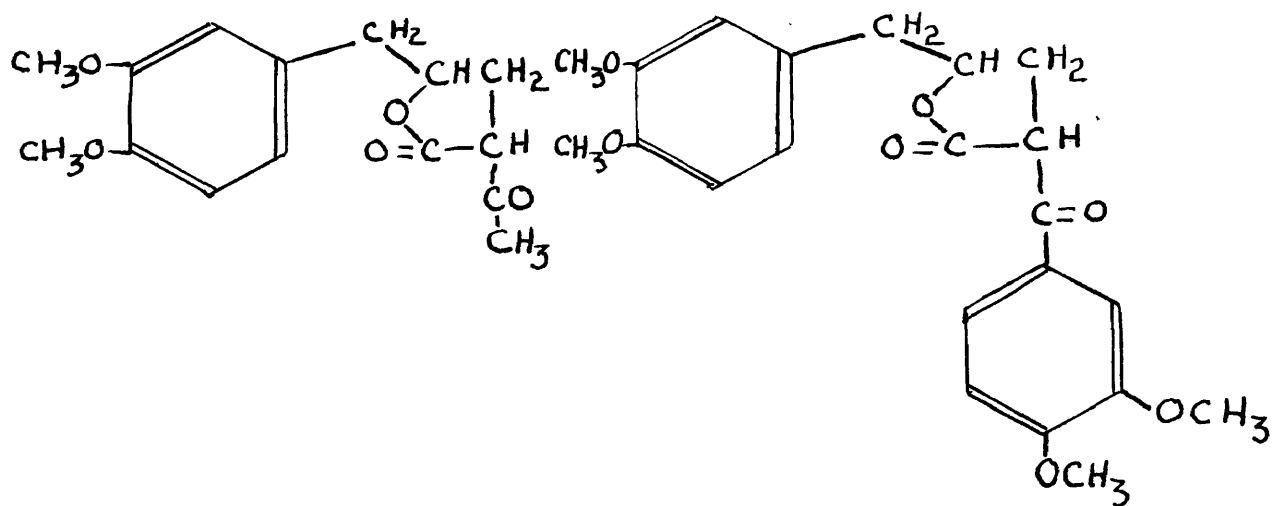
Compound XV: A second synthesis which Haworth considers to be of wide application⁵³ leads to compound XV. Methyl eugenoloxide was condensed with acetoacetic ester to yield XXXVIII, whose sodic derivative, when treated with veratroyl chloride followed by cold alkaline hydrolysis, yielded XXXIX.



XXXVI



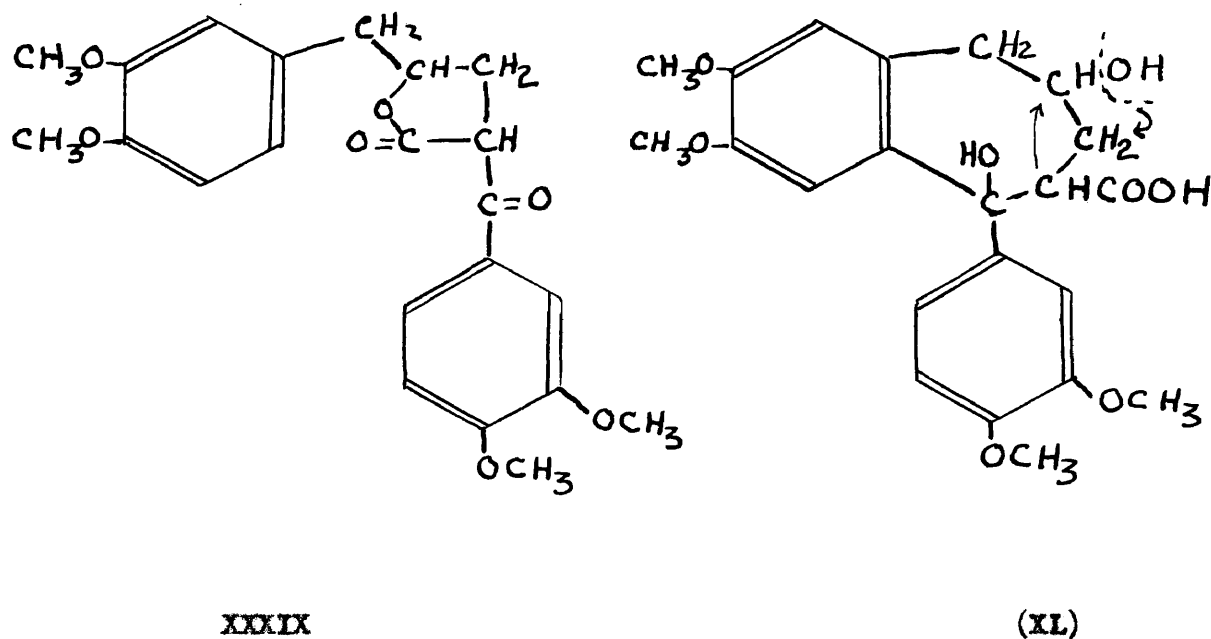
XXXVII

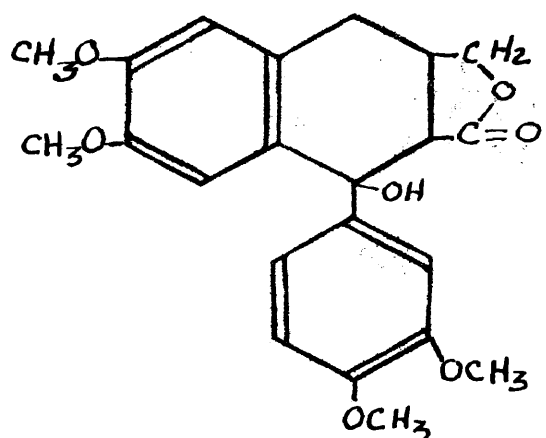


XXXVIII

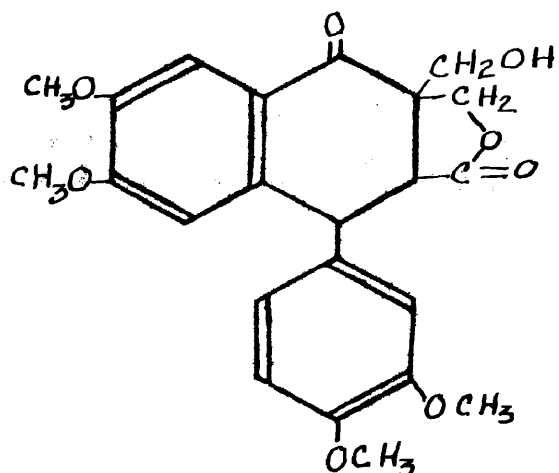
XXXIX

This latter compound on treatment with methyl alcoholic hydrogen chloride underwent a rearrangement to give XL, which was dehydrated and dehydrogenated to XV. The nature of the conversion of compound XXXIX to XL is not too readily understandable, but the fact that XL is dehydrated and dehydrogenated to XV seems to establish the structure of XL, since XV was synthesized by a different procedure. Haworth⁵³ has proposed a mechanism for the formation of XL to be as follows:



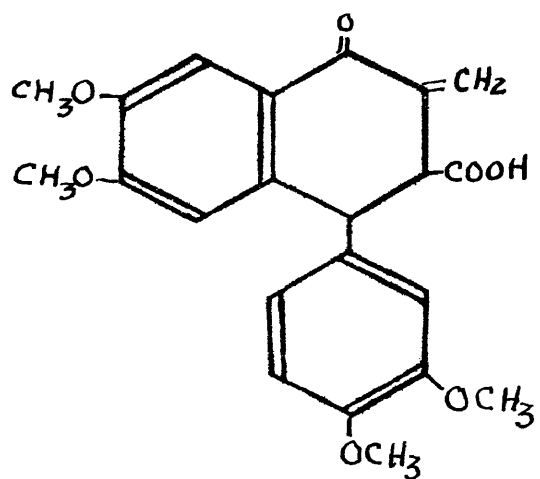


XL

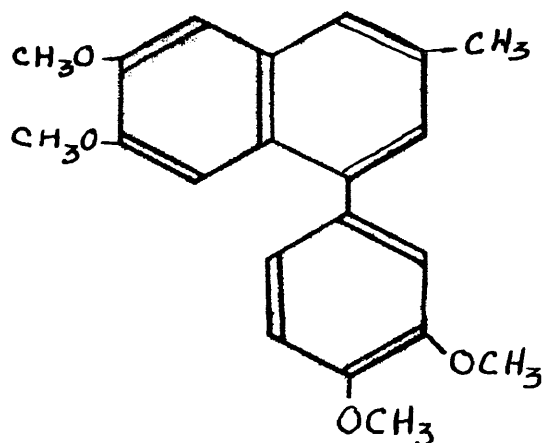


XLI

The Action of Formalin:⁸⁴ Compound XVII, previously prepared, when treated with formalin at room temperature and in the presence of alkali, gave rise to what is probably XLI, and that compound on further treatment with warm alkali, lost formaldehyde to yield XLII. XLI on treatment with zinc and hydrochloric acid gave an oil, which on heating with selenium yielded XLIII.

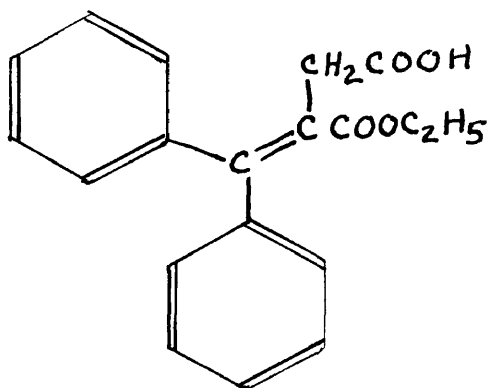


XLII

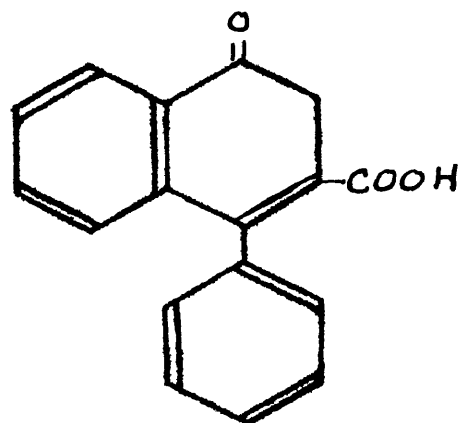


XLIII

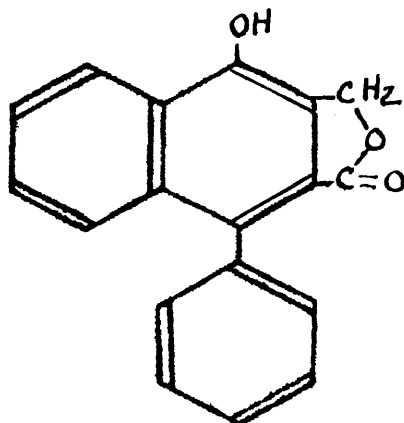
An Analog of Pieropedophyllin:⁵⁵ In an attempt to synthesize a simple pieropedophyllin molecule benzophenone was condensed with succinic acid to give the itaconic acid, which was esterified by ethyl alcohol and then hydrolyzed with one mole of alkali to give XLIV. This compound was cyclized by sodium acetate and acetic anhydride and then hydrolyzed to give XLV, which after reaction with formaldehyde was lactonized to XLVI by concentrated hydrochloric acid in acetic acid. Reduction of this latter compound gave a mixture of isomers from which no compound could be isolated.



XLIV



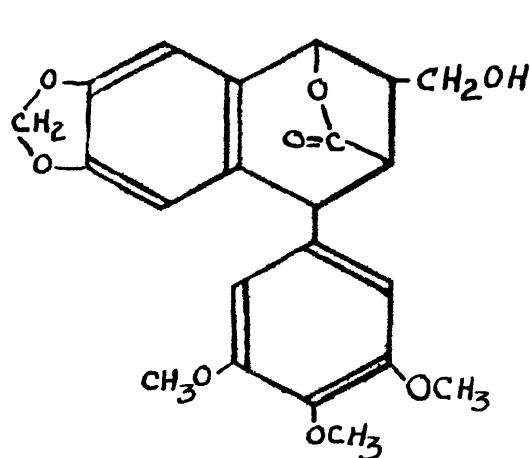
XLV



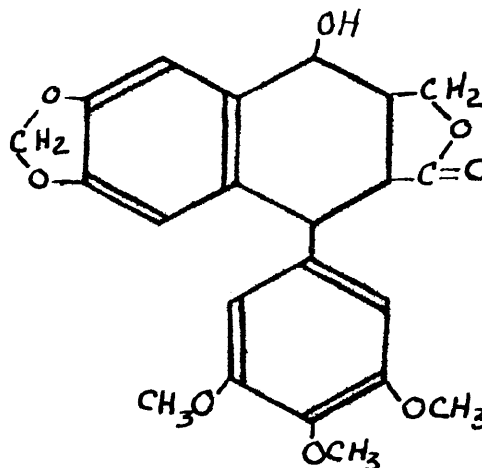
XLVI

DISCUSSION

Podophyllotoxin and its isomer picropodophyllin²⁵ were the compounds subjected to investigation. Podophyllotoxin, XLVII, the much more soluble isomer, has been reported to have various melting points and to crystallize with a wide combination of solvents of crystallization, whereas its isomer, picropodophyllin, XLVIII, is obtained free of solvent of crystallization and has a much more definite and constant melting point.



XLVII



XLVIII

Attempts to recrystallize podophyllotoxin by recommended procedures were unsuccessful in that they did not yield a product which on prolonged drying would give a correct analysis. By sublimation and distillation in high vacuum an anhydrous material was obtained, which was satisfactory for analysis and optical activity determination. It is noteworthy that the specific rotations for both the crude material and the purified podophyllotoxin were somewhat higher than reported in the literature; the reported values are -101° and -108.5° , whereas the value obtained here was -126° .

The instability of the podophyllotoxin lactone posed a problem, since a basic solution would convert the material into its less soluble isomer, pieropodophyllin, which then might undergo a different reaction. A strongly acidic solution was equally detrimental, since it would cause demethoxylation to take place, presumably attacking the labile 4'-methoxyl group. In every case where a reaction failed to proceed with podophyllotoxin, the isolable material therefrom proved to be pieropodophyllin. A further complication lay in the fact that the starting materials and products of most of the reactions were usually so similar in their appearance and solubility characteristics, that unless the reaction proceeded to a good yield, it was often impossible to separate the mixture. The inability to crystallize several of the reaction products contributed to a somewhat low yield of pure material in the preparation of several derivatives. This was particularly true in the case of several para-nitrobenzoyl esters prepared for characterization purposes; it was possible to effect a manner of recrystallization and obtain a solid material which gave a satisfactory analysis, but examination of the solid particles under the microscope failed to reveal any evidence of birefringency.

The acetate of podophyllotoxin was prepared following the procedure of Spath²⁵ both by acetic anhydride and by acetic anhydride in pyridine. Identical products were obtained, and the melting points confirmed those of Spath and Robertson⁵⁶ and were in disagreement with that reported first by Borsche and Nieman²⁵. However, the specific rotation (-143°) was in closest agreement with that of Borsche who reported -135° , whereas Robertson reported -174° .

The acetate of pieropodophyllin was obtained by two methods, one of which was directly from pieropodophyllin by the action of acetic anhydride

and sodium acetate, and the other by the action of acetic anhydride on a suspension of the sodium salt of podophyllic acid in pyridine. The acetate after recrystallization from ethyl acetate gave a melting point different from that reported by previous workers and was the same as that of the acetate of podophylletoxin. However, mixed melting point determination and specific rotations established that these two acetates were different substances. The specific rotation was in agreement with that previously reported.

Pieropodophyllin itself was prepared many times by the procedure of Borsche, namely, by refluxing podophylletoxin with sodium acetate in ethyl alcohol. The product gave the same melting point as that previously recorded, but it had a specific rotation of 0° in contrast to reported values of $+5.3^\circ$ and $+9.3^\circ$. This work, although already reported, was necessarily repeated so that this laboratory would have a measure of the properties necessary to characterize future products.

It is interesting to note that the sodium salt of podophyllic acid, made by opening the lactone ring with alkali, could be approached from either podophylletoxin or pieropodophyllin, and further that its specific rotation is between that of podophylletoxin and pieropodophyllin. A sample of the sodium salt prepared from podophylletoxin and recrystallized had a specific rotation of -78° in water, while that from pieropodophyllin by the same procedure had a value of -90.5° . However, when the podophylletoxin was dissolved in an alcohol, alkali, water mixture and the change in specific rotation observed over a period of time, it was noted that when first read, four minutes after mixing, the specific rotation was -74° ; over a period of ten minutes the specific rotation dropped to a constancy at -89.9° . The hydrolysis of the lactone was therefore exceedingly fast.

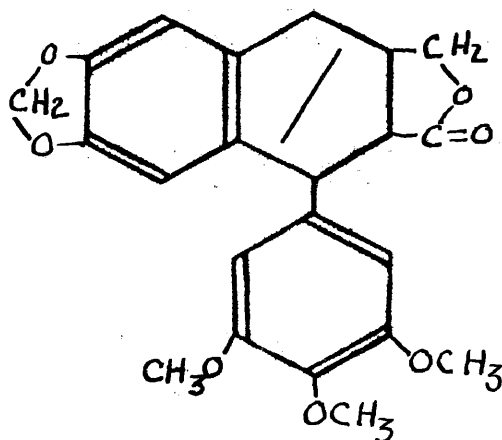
Observations of the specific rotation at periods longer than twenty minutes after mixing were not possible, since the sodium salt precipitated out and made setting the polarimeter impossible. It is regarded as certain that there is only one form of the sodium salt of podophyllinic acid as previously reported by Borsche, whose experimental data, however, were too inadequate to support his statement. The sodium salt was very difficult to burn for analysis; a mixture of the salt and vanadium pentoxide had to be made in order to obtain a satisfactory carbon analysis. As a further proof of the constitution of the salt, its neutral equivalent was determined by titration of the salt in glacial acetic acid with perchloric acid. Former investigators had reported only a sodium analysis.

Early in this research an attempt was made to block the primary alcohol group of podophyllotoxin by the preparation of a trityl ether. This attempt failed, the only products definitely identified were picropodophyllin, triphenyl carbinol, and a compound which might on the basis of its analysis and melting point be triphenylmethyl methyl ether. Subsequently an attempt was made to esterify the sodium salt of podophyllinic acid with phenacyl bromide; this attempt failed, and yielded picropodophyllin. It is highly probable that even if an ester were made, picropodophyllin would result from it in solution by a process of intramolecular transesterification. The methyl ester of this acid had already been prepared by the action of diazomethane on the free acid²⁵, but when an attempt was made to distill the substance, it decomposed to yield picropodophyllin.

Since the acetate of both podophyllotoxin and picropodophyllin are known, it was thought likely that a diacetate might be prepared by the acetylation of the sodium salt of podophyllinic acid suspended in pyridine.

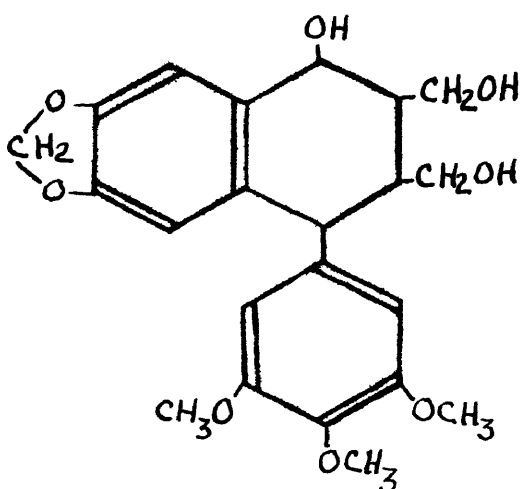
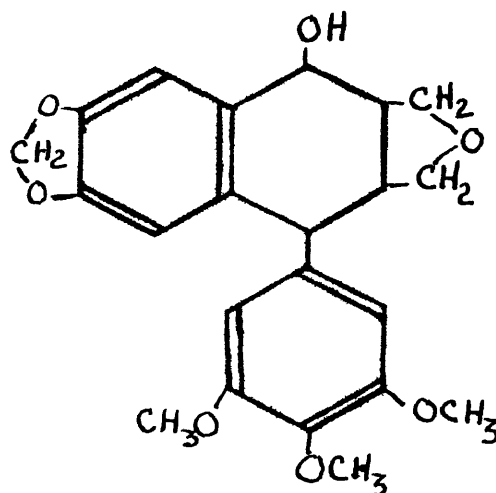
The result of this trial was the formation of the acetate of pieropodophyllin with no evidence of the formation of a diacetate.

On two occasions, by recrystallization of the acetate of pieropodophyllin from acetic anhydride, there was formed alpha-apopieropodophyllin XLIX. This compound itself is of little importance in this research except for its ultra-violet absorption spectrum. The melting point and specific rotation were in excellent agreement with that previously reported^{25,56}. Subsequent attempts to repeat the preparation of alpha-apopieropodophyllin by the above procedure yielded its isomer, beta-apopieropodophyllin, XLIX, which has also been reported previously. The yields on this preparation were poor, and when it became necessary to prepare quantities of the material, a much more satisfactory route was developed by proceeding to the benzoate of pieropodophyllin with subsequent pyrolysis of that compound.



XLIX, alpha, beta
(The diagonal indicates the presence of an unlocated double bond.)

The next step to try to determine the seat of biological activity was to remove the lactone ring, since it was not possible to stabilize the open acid. This was accomplished by reduction of pedophyllo toxin in ether solution by lithium aluminum hydride to form the trihydroxy compound, L_a . Due to the presence of an active hydroxyl hydrogen, the solubility of the starting material in the presence of the reducing reagent was greatly reduced, and therefore the suspension had to be stirred very vigorously to attain a satisfactory reaction. This compound yielded a tri-para-nitrobenzoate by the action of para-nitrobenzoyl chloride in pyridine. The trihydroxy compound was not accomplished until it was recognized that the molecule, when in solution, lost the elements of water to yield the anhydro compound, LI_a , which was actually isolated in a pure state before the trihydroxy compound. Indeed, recrystallization of L_a has never been accomplished, and it was only by decomposing the reduction mixture by base instead of acid and by the careful concentration of the ether solution, that L_a was obtained directly in an analytically pure state. The trihydroxy compound, L_a , is dehydrated to form LI_a by treatment with acidic reagents in alcohol or benzene.

 $L_{a,b}$  $LI_{a,b}$

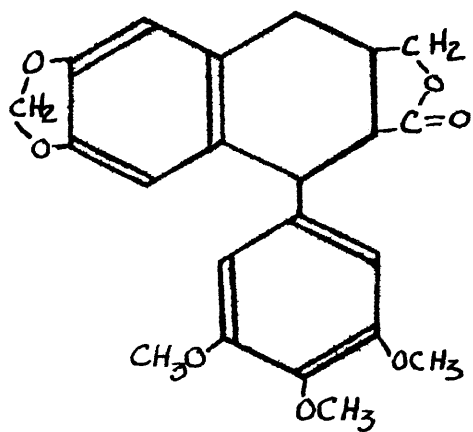
The structure of LI_a was established with a reasonable degree of certainty only after a series of related reactions, but it was early demonstrated by the preparation of its methyl ether, benzoate, para-nitrobenzoate, and the determination of its molecular weight by the Rast method that the compound contained only one free hydroxyl. It was further shown that the compound could not be reduced by palladium at room temperature, nor by Raney nickel at 80° . Direct chemical proof of the existence of the secondary alcohol is lacking, because it was not possible to oxidize LI_a by a theoretical amount of chromic anhydride or potassium permanganate without causing extensive degradation and recovery of large amounts of starting material. Surprisingly, the Oppenauer oxidation failed even using cyclohexanone as the hydrogen acceptor, but this reaction also fails with pieropodophyllin. It was not possible to prepare a halide of either the anhydro compound or pieropodophyllin. The benzoate of the anhydro compound proved to be very stable to pyrolysis, and dehydrogenation attempts using palladium-charcoal catalyst gave only tars and carbonization. An attempted oxidation by benzoquinone in sunlight⁵⁷ also failed.

The reduction of pieropodophyllin by lithium aluminum hydride to LI_b , an isomer of LI_a , proved to be quite difficult. It was finally necessary to dissolve the pieropodophyllin in dioxane and add that solution to an ether solution of the hydride. Attempts at a reduction using the same procedure as for podophylletoxin were successful only on a very small scale due to the extreme insolubility of pieropodophyllin in ether. In addition, the trihydroxy compound from pieropodophyllin proved to be very difficult to crystallize, and it was only after several months of slow evaporation that seed crystals could be obtained. Once the seed

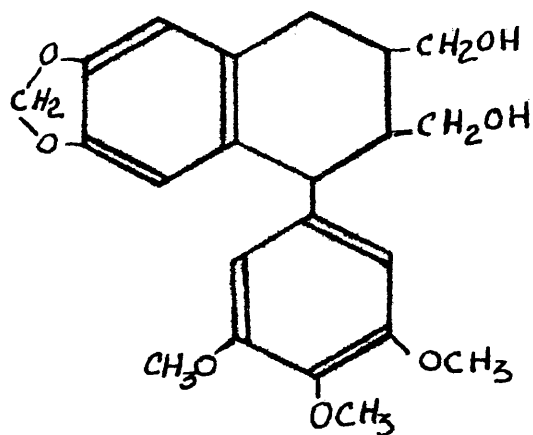
crystals of L_b were available, it was possible to crystallize the compound out of an ether solution, but not if the solution contained an appreciable amount of dioxane. The lower yield of L_b than of L_a is explicable, since it was necessary to remove all dioxane before one could crystallize the compound out of ether, and it was during this removal process that a portion of the compound lost water to form LI_b . The compound LI_b was identified by the same procedure as before. This compound was converted by acidic reagents also to the anhydro form, LI_b , which again was isolated before the pure trihydroxy compound. The anhydro compound from picropodophyllin never has been crystallized, but was identified by the preparation of its para-nitrobenzoate, analysis, and the determination of its molecular weight.

In order to establish the nature of the furan ring present in the two anhydro compounds, the following series of reactions was carried out. Picropodophyllin was benzoylated by benzoyl chloride in pyridine, and the benzoate was converted by pyrolysis to beta-apopicropodophyllin, XLIX. This latter compound was then reduced to desoxypicropodophyllin, LII, which with lithium aluminum hydride gave the desoxydihydroxy compound, LIII, and that in turn by acid treatment was dehydrated to a cyclic ether, desoxyanhydro compound, LIV.

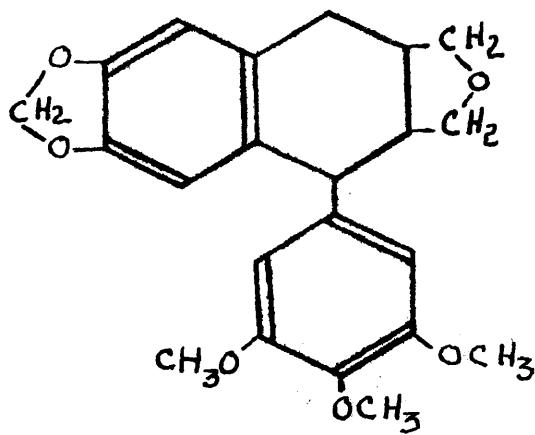
As stated above by vigorous heating of the acetate of picropodophyllin in acetic anhydride one could obtain beta-apopicropodophyllin in consistently poor yields. Robertson had prepared alpha-apopicropodophyllin by the action of sulfuric acid on a solution of picropodophyllin in acetic anhydride, however, on small scale tests this procedure gave rise to much decomposition and coloring and a very impure product. It was then that the benzoate of picropodophyllin was made and converted by pyrolysis to beta-



LII



LIII



LIV

apopicropodophyllin, XLIX, in excellent yields. As a matter of curiosity, the benzoate of podophyllotoxin was made and also converted by pyrolysis to beta-apopicropodophyllin although in somewhat poorer yields. Compound XLIX proved to be surprisingly resistant to reduction; platinum at room temperature failed, and the reduction was finally accomplished with Raney nickel at 60°, but a reaction time of six to seven hours was required. Borsche and Nieman have reported the reduction of apopicropodophyllin by platinum oxide in acetic acid to give a desoxy compound which had a melting point of 169-70°, in contrast to the melting point herein obtained of 199.8-201°. However, Borsche and Nieman did not say which isomer of apopicropodophyllin they started with and did not give their final yield.

The reduction of the desoxy compound, LII, by lithium aluminum hydride proceeded smoothly to give LIII, and although crystalline material could not be obtained, the product was identified by analysis and the preparation and analysis of its di-para-nitrobenzoate. Treatment of the desoxydihydroxy compound, LIII, with acid, under the same conditions as for I_b, gave the desoxyanhydro compound, LIV, which is a crystalline compound.

Attempts were made to dehydrogenate compound LIV in the presence of palladium charcoal catalyst, and it is believed that they were partially successful, although a definite product was not isolated. The resistance of this compound to dehydrogenation is not surprising when it is compared to compound XXVII, which Haworth was not able to dehydrogenate by sublimation from palladium black. At this time the work was concluded, so that other investigations were not made, but indications are that the compound is capable of dehydrogenation, perhaps by the use of lead tetraacetate with which so much success has been had by other workers in the lignan field.

The ultra-violet absorption spectra of the principle compounds developed have been studied with the hope that certain changes in the substituent groups might have produced a change in the spectra. Observations regarding these curves will be discussed later.

Thus far discussion of the nature of the various isomerizations and of the steric arrangement of the groups in pedophylloctoxin has been avoided, since it is believed that this problem can be better discussed after a presentation of the experimental evidence. Following the experimental part, pertinent stereochemical work regarding other compounds of the lignan series will be considered together with information obtained from this research.

EXPERIMENTAL

Podophyllotoxin, XLVII: Podophyllotoxin as obtained from S. B. Penick and Co. was recrystallized from carbon tetrachloride, and also by dissolving the compound in chloroform and adding that solution to a large volume of petroleum ether. Several repetitions of this procedure gave a material of inconstant melting point, and which would not give a satisfactory analysis even after prolonged drying.

A portion of the crude podophyllotoxin was placed in a sublimation apparatus, and it melted and distilled under a pressure of 0.1 to 0.2 microns (McLeod) at a pot temperature of 105-110°. The distillate crystallized on the cold finger and on removal melted at 116.8-119.5°* with sintering at 114.5° to form a thick clear melt. The characteristic fluid meniscus was not obtained.

Anal.** Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.55, 63.55; H, 5.44, 5.44.

(∞) $\frac{25}{D} = -126^\circ$; $C = 0.397$ g./100 ml; chloroform.

All optical rotations herein were measured in a two decimeter, semi-micro, water-jacketed polarimeter tube.

Picropodophyllin, XLVIII: By the procedure of Borsche²⁵, 0.45 g. of podophyllotoxin was dissolved in a mixture of 10 ml. of ethyl alcohol and 5 ml. of 10% sodium acetate solution and heated under reflux for twenty-three hours. At the end of that time the solution was concentrated to

* All melting points recorded herein have been corrected.

** All samples were dried in an Abderhalden apparatus before analysis.

The writer wishes to express his gratitude to Mrs. Mary H. Aldridge and to Mr. Byron Baer for performing the analyses contained herein.

8 to 10 ml, cooled, and filtered to yield 0.36 g. (80% of theoretical) of very fine, white needles of picropodophyllin, which melted at 224.0-225.1° (d). Attempts to obtain a second crop of crystals resulted in only a very gummy precipitate contaminated with a considerable amount of sodium acetate. Several recrystallizations from ethyl alcohol yielded a material which had a melting point of 223.5-224.5° (d).

Anal. Calcd. for $C_{22}H_{22}O_8$: C, 63.75; H, 5.35. Found: C, 63.39, 63.46; H, 5.37, 4.99.

$(\alpha)_D^{25} = 0^\circ$; $c = 0.449$ g./100ml; chloroform.

A second preparation of picropodophyllin by the above procedure also gave a material which had a specific rotation of 0° at $c = 0.694$ g. per 100 ml. chloroform.

Preparation of the Acetate of Podophyllotoxin: Following the procedures of Spath²⁵ the acetate was prepared.

(a) The action of acetic anhydride on podophyllotoxin gave a product which melted initially at 196-198.6°, and after two recrystallizations from methyl alcohol melted at 203.8-204.6° with sintering at 203.1°.

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 63.19, 62.85; H, 5.34, 5.28.

$(\alpha)_D^{25} = -143^\circ$; $c = 0.786$ g./100 ml, chloroform.

(b) By the action of a mixture of acetic anhydride and pyridine on podophyllotoxin, the acetate was obtained in 93% yield; this sample melted without recrystallization at 204.1-205.6° with sintering at 202.6°. A mixed melting point of this product and that of (a) above gave no depression.

Preparation of the Acetate of Picropodophyllin: 0.8 g. (.0019 mole) of picropodophyllin was heated under reflux for two hours with 16 ml. of acetic anhydride and 0.4 g. (0.0048 mole) of sodium acetate, and cooled,

whereupon the solution was added slowly to 60 ml. of cold water. The precipitated acetate was filtered off and after drying weighed 0.61 g., equivalent to 70% of the theoretical yield. The melting point of the crude acetate was 210.6-212.6°. A second crop of amorphous material weighing 0.1 g. was obtained from the mother liquor. The crude acetate was divided into approximately equal portions (a) and (c).

(a) This portion after three recrystallizations from ethyl acetate melted at 203.4-204.6°, and a mixed melting point determination with the analytical sample of the acetate of picropodophyllin, obtained below from the acetylation of the sodium salt of podophyllic acid, showed no depression. A mixed melting point determination with the acetate of podophyllotoxin showed a marked depression to 183-92°.

(∞) $\frac{25}{D} = +19.2^\circ$; $c = 0.600$ g./100 ml., chloroform.

This specific rotation is in good agreement with that of Borsche²⁵ and Robertson⁵⁶.

(b) Preparation of alpha-apopicropodophyllin: Part C, obtained above by action of acetic anhydride and sodium acetate on picropodophyllin, was recrystallized from acetic anhydride after evaporation nearly to dryness (cf. Spath²⁵). The product melted at 233.8-235.2° with sintering at 233°. Two further recrystallizations from alcohol-acetic acid mixture raised the melting point to 235.4-236.3°.

Anal. Calcd. for $C_{22}H_{20}O_7$: C, 66.65; H, 5.08. Found: C, 66.39, 66.61; H, 5.14, 5.09.

On an identical sample (∞) $\frac{25}{D} = -18.1^\circ$; $c = 0.581$ g./100 ml.; chloroform. This rotation is in good agreement with that reported by Robertson, although he reports that a somewhat higher melting point of 244-5° could be obtained by repeated recrystallization of the compound from ethyl acetate.

Robertson had also noted that with cold concentrated sulfuric acid, alpha-apopicropodophyllin gave a brownish red solution which turns purple, whereas the beta-isomer gives a color change of orange to red to purple to violet. This color test has been confirmed on both materials and the above substance must be the alpha-isomer. As stated previously though, attempts to repeat this preparation have invariably led to the beta-isomer, and it is probably due to the presence of small quantities of sodium acetate, which cause the conversion of the alpha form to the beta^{25,56}.

Preparation of the Sodium Salt of Podophyllie Acid; Podophyllotoxin

(0.2g., 0.00048 mole) was dissolved in 3 to 5 ml. of warm alcohol and 0.5 ml. of strong alcoholic sodium hydroxide added. A gel immediately separated which dissolved when the solution was warmed to near the boiling point. After three or four minutes the solution was cooled and a soft white precipitate formed, which with the addition of a little ether and scratching changed to a fine hard crystalline precipitate. The yield was quantitative.

The salt is very soluble in water, its solutions having a pH of approximately 9, and it is stable to a pH as low as 5 without the formation of an immediate precipitate unless the solution is warmed. The compound melts at a very high temperature leaving a hard black residue. An analytical sample was prepared by dissolving the salt in a small amount of ethyl alcohol containing only a few drops of water and then adding small quantities of ether until crystallization started. Analysis of the salt proved to be difficult because it was very resistant to combustion, with the result that the carbon value was very low. A satisfactory analysis was obtained by mixing the sample with vanadium pentoxide and increasing the period of combustion.

Anal. Calcd. for $C_{22}H_{28}O_9Na$: C, 58.14; H, 5.10; Na, 5.06. Found: C, 57.63, 57.83; H, 5.13, 5.20; Na, 5.00, 4.87.

$(\alpha)_D^{25} = -78^\circ$; $d = 1.066$ g./100 ml.; water.

The sodium analysis was made on an identical sample which was subsequently used for the neutral equivalent determination.

The neutral equivalent was determined by the following procedure. A 0.100 N. solution of sodium acetate was prepared by dissolving 1.325 g. of sodium carbonate in 250 ml. of freshly distilled glacial acetic acid; portions of the resulting solution were used to standardize a solution of perchloric acid in acetic acid using bromphenol blue as the indicator. The neutral equivalent of the sodium salt was determined by direct titration of a sample dissolved in glacial acetic acid. (1) 0.3048 g. salt required 8.05 ml. of .0643 N. perchloric acid; N. E. found, 449. (2) 0.3259 g. salt required 8.65 ml. of .0643 N $HClO_4$; N. E. found 447. N. E. calc'd. 454.4.

A sample of the sodium salt of pedophyllic acid was prepared from pieropedophyllin by the same procedure as used for the preparation from pedophyllotoxin. After purification $(\alpha)_D^{25} = -90.5^\circ$; $c = 1.052$ g./100 ml.; water.

An attempt to measure the change in optical activity on opening the lactone of pedophyllotoxin was made. 0.236 g. (.00057 mole) of pedophyllotoxin was dissolved in 20 ml. of 95% alcohol and 1 ml. of 5% sodium hydroxide (0.0013 mole) was added. The volume was quickly adjusted to 25 ml., and the change in optical rotation observed. $c = 0.944$ g./100 ml. Temperature = 25° .

<u>Time (min.)</u>	<u>Obs. rotation</u>	<u>Specific rotation</u>
0	-	
4	-1.39	-74°
6	-1.50	
8	-1.61	
12	-1.70	-89.9°
14	-1.72	
16	-1.70	

The readings were very difficult to make in this procedure and became more so as time progressed due to precipitation of the sodium salt. Several trials to obtain a more suitable solvent mixture were unsuccessful.

Acetylation of Sodium Podophyllate: 0.75 g. (0.00165 mole) of sodium podophyllate were suspended in a mixture of 15 ml. of pyridine and 15 ml. of acetic anhydride and stirred for twenty-four hours by a glass magnetic stirrer. The salt appeared to dissolve readily, and a thin gel which formed could not be dispersed. After twenty-four hours the reaction mixture was poured into a large volume of water, and the precipitate filtered and dried. The yield was 0.72 g. (97% based on the formation of a monoacetate), and the substance melted at 212.6-214.6°.

A portion of this precipitate after three recrystallizations from ethyl acetate melted at 202.6-204.6°. A mixed melting point determination with the acetate of podophyllotoxin showed a marked depression. When this acetate was mixed with the acetate prepared from picropodophyllin, no depression of melting point was observed.

Anal. Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 63.19, 63.01; H, 5.40, 5.40.

Therefore this substance must be the acetate of picropodophyllin.

A second portion of the crude acetate above was recrystallized from acetic anhydride (the solvent used by Borsche and Nieman)²⁵. It was found that the melting point after this treatment remained constant at 212.6-214.2°. This higher-melting material must also be the acetate of picropodophyllin as reported by Borsche and Nieman, Spath, and Robertson, but a completely satisfactory analysis was not obtained on it. A mixed melting point determination with the acetate of podophyllotoxin showed a marked depression, and therefore the substance is probably not the acetate of podophyllotoxin. Hence the result of the acetylation of the sodium salt of podophyllic acid is solely the acetate of picropodophyllin, with no evidence of a diacetate. Analysis of the higher melting material:

Calcd. for $C_{24}H_{24}O_9$: C, 63.15; H, 5.30. Found: C, 62.86, 62.75; H, 5.32, 5.35.

Attempted Tritylation of Podophyllotoxin: (a) 0.186 g. (0.00045 mole) of podophyllotoxin was dissolved in 2.5 ml. of dry pyridine and 0.194 g. (0.00070 mole) of trityl chloride was added. The solution was allowed to stand protected from the atmosphere for seventy-two hours at room temperature, and then diluted with 15 ml. of water. An oil separated which solidified on cooling and was filtered off. This solid could be separated into picropodophyllin and triphenyl carbinol, and another substance which from the results of (b) below is probably trityl methyl ether.

(b) The same procedure was repeated except that the solution was heated under reflux for forty-eight hours. In this case the third substance was identified by analysis and melting point as triphenylmethyl methyl ether. Only a small quantity of product which melted at 82.8-83.2° and gave the following analysis was obtained.

Anal. Calcd. for $C_{20}H_{18}O$: C, 87.57; H, 6.60. Found: C, 87.36, 87.22; H, 6.72, 6.70.

The literature lists a melting point of 82° for trityl methyl ether. It was not possible to obtain or identify any other substance from the reaction.

Attempted Esterification of Sodium Podophyllate: Using the method of Shriner and Fuson⁵⁸, 0.3 g. (0.00066 mole) of the sodium salt of podophyllic acid was dissolved in 0.4 ml. of water and 1 drop of dilute hydrochloric acid was added. Eight ml. of alcohol and 0.3 g. (0.0016 mole) of phenacyl bromide were then added, and the solution was heated under reflux for one and one-half hours. After the mixture had stood overnight, a small amount of precipitate (0.2 g.) had formed. This substance was identified as picropodophyllin, although a slight impurity which could not be removed by crystallization was present. An additional quantity of picropodophyllin was isolated from the filtrate. There was no evidence of any other product.

Preparation of the Trihydroxy Compound, L₂: In the first preparation, 2.0 g. (0.0048 mole) of dry podophyllotoxin was placed in a Soxhlet extractor and extracted into a solution of 0.22 g. (0.0058 mole) of lithium aluminum hydride in 500 ml. of ether in a one-liter flask. The reaction mixture was stirred and protected from the atmosphere by drying tubes. At the end of seven hours all the podophyllotoxin had been extracted into the reaction; however, stirring and refluxing were continued for one hour longer. At the end of that time the excess hydride was decomposed by the cautious addition of water, and when hydrogen was no longer evolved, 100 ml. of 10% sodium hydroxide was added, and the mixture stirred until the ether layer was clear. The layers were separated, and the aqueous layer was placed in a liquid-liquid extractor and the ether in the boiler. Extraction of the aqueous phase was continued for forty hours during which

time two substances separated out of the ether solution. One substance, consisting of very fine feathery needles, was later recognized as picro-podophyllin resulting from unreduced podophyllotoxin. The second substance separated as hard rectangular plates and was the trihydroxy compound. The ether solution was decanted from both these substances and concentrated to 100 ml.; on cooling there separated 0.5 g. (25% of theoretical) of hard rectangular plates of the trihydroxy compound. The compound melted on a hot-stage under the microscope at 198.5-200.5° and remelted at 198-200°.

Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.12; H, 6.25. Found: C, 62.89, 63.20; H, 6.28, 6.28.

(∞) $\frac{25}{D} \pm 0^\circ$; $n_D = 0.512$ g./100 ml.; chloroform.

Subsequent preparations were modified to give much improved yields. 10.0 g. (0.024 mole) of podophyllotoxin were placed in a Soxhlet extractor on a glass wool plug instead of a conventional thimble. Extraction into a solution of 3.0 g. (0.078 mole) of lithium aluminum hydride in 3000 ml. of ether required approximately five hours. Heating and stirring were continued for four hours additional. It is very important in this reaction that stirring be very vigorous. At the end of the reaction time, excess hydride was decomposed cautiously by the dropwise addition of water, and then by the addition of 400 ml. of 10% sodium hydroxide. As soon as the ether layer cleared it was decanted carefully, and the alkaline layer washed by swirling and decantation with three or four successive 400-ml. portions of ether. Rapidity in separating and washing the alkaline layer is important since the trihydroxy compound often crystallizes very quickly. The combined ether solutions, after being concentrated to 700 to 800 ml. and cooled, yielded on filtration and scraping of the sides of all the vessels 7.54 g. (75%) of the trihydroxy compound, L_2 , identical with previous preparations. By washing the flasks with alcohol and combining the

washings with the ether filtrate and concentrating them to 15 ml., a second crop (8%) of slightly impure material of melting point 188-194° could be obtained. The impurity is undoubtedly the anhydro compound, LI_a, formed by dehydration of L_a, for this latter substance is converted very easily to the anhydro compound.

Preparation of the Tri-para-nitrobenzoate of L_a: 0.2 g. (.00048 mole) of the trihydroxy compound dissolved in 5 ml. of dry pyridine was mixed with 0.5 g. (0.0027 mole) of freshly prepared para-nitrobenzoyl chloride. The solution was allowed to stand for three hours at room temperature and was then poured into 20 ml. of water, cooled, and the soft gummy precipitate separated by filtration. Recrystallization of this derivative was very difficult due to the tendency of the ester to separate as an oil, so that a measure of the yield could not be made. Two recrystallizations from an ethyl acetate-alcohol mixture gave a yellow solid which softened and melted at 130-140° and eventually formed a fluid miniscus at 150° (d). A third recrystallization from absolute alcohol reduced the melting range to 125-135° (d), and a fourth recrystallization from a benzene alcohol mixture gave a material of melting point 130.6-134.6° (d) which formed a miniscus at 150° (d). Examination under the microscope failed to show any birefringency.

Anal. Calcd. for C₄₃H₃₅O₁₇N₃: C, 59.65; H, 4.08; N, 4.85. Found: C, 59.95, 60.00; H, 4.47, 4.45; N, 5.08, 4.85.

(α)_D²⁵ = -31°; c = 0.629 g./100 ml.; chloroform.

Preparation of the Anhydro Compound, LI_a: 2.0 g. (0.0048 mole) of trihydroxy compound L_a were digested in alcohol for one and one-half hours with 0.05 g. of para-toluenesulfonic acid, whereupon the solution was concentrated to 50 ml. and cooled. 1.6 g. of crude anhydro compound precipitated out. A second crop which weighed 0.2 g. was collected after

concentrating the mother liquors to 10 ml. and cooling. Recrystallization of the combined first and second crops of crude material from alcohol gave 1.65 g. (83% of the theoretical) of the anhydro compound, LI_a , which melted at 256.3-257.3° with slight sintering at 253°. A second crop of 0.1 g. of impure crystals could be obtained.

Anal. Calcd. for $C_{22}H_{24}O_7$: C, 65.98; H, 6.04. Found: C, 65.78, 65.91; H, 6.25, 6.19.

M. W.: Calc'd., 400.4; Found (Rest), 385, 409

$(\infty) \frac{25}{D} = +13^\circ$; $c = 0.489$ g./100 ml.; chloroform.

Preparation of the Mono-para-nitrobenzoate of the Anhydro Compound, LI_a :

0.3 g. (0.00075 mole) of LI_a was dissolved in 4 ml. of warm dry pyridine, and 0.6 g. (0.0032 mole) of para-nitrobenzoyl chloride was added. The solution was heated under reflux for ten minutes and then poured into 10ml. of water. The oil which separated solidified on cooling to a soft gummy precipitate. The precipitate was recrystallized from alcohol, triturated with sodium bicarbonate solution and recrystallized again from alcohol. The melting point of the yellow solid obtained was 192.5-94.5° (d). A qualitative test for nitrogen was positive. After one more recrystallization the melting point was 193.1-195.0° (d), and at this point there was 0.2 g. of material, so that the initial yield must have been nearly quantitative. One further recrystallization gave pale yellow crystals which melted at 194.7-196.1° (d), with sintering at 193.0°.

Anal. Calcd. for $C_{29}H_{27}O_{10}N$: C, 63.38; H, 4.95, N, 2.55. Found: C, 63.15, 63.46; H, 5.46, 5.14; N, 2.56, 2.55.

$(\infty) \frac{25}{D} = -46^\circ$; $c = 0.474$ g./100 ml.; chloroform.

Preparation of the Methyl Ether of Anhydro Compound, LI_a : 0.55 g. (0.0014

mole) of the anhydro compound was dissolved in 100 ml. of dry refluxing toluene and stirred by a magnetic stirrer in the presence of a small piece

of sodium (approximately 0.3 g., 0.013 mole) for twenty-four hours. A precipitate gradually formed in the flask. After the reflux period, the solution was cooled to approximately 80° and 8 ml. (18.4 g., 0.13 mole) of methyl iodide was added slowly in twenty ml. of dry benzene as a diluent. The solution was then heated under reflux and stirred for three hours more, cooled to 60° and filtered from the precipitated sodium iodide. Concentration of the solution to a volume of 10 ml. produced no crystals even on cooling, therefore, the toluene was steam-distilled off; the yellow solid which remained behind melted at 155-65° and was very soluble in most solvents. Recrystallization of the substance from a large volume of petroleum ether (90-100°) yielded 0.4 g. (72%) of soft yellow crystals of the methyl ether. Its melting point was 155-169.5°. Further recrystallizations did not improve the melting point to any great extent. For analysis, a sample was sublimed under a pressure of 0.1 - 0.2 micron (McLeod) at a pot temperature of 150-155°. The sublimate retained the light yellow color, but the melting point was raised to 167.1-173.6° with sintering at 163°.

Anal. Calcd. for $C_{23}H_{26}O_7$: C, 66.65; H, 6.31. Found: C, 66.90, 66.89; H, 6.38, 6.45.

$(\infty) \frac{25}{D} = + 3^{\circ}$; $n_D = 0.450$ g./100 ml.; chloroform.

Preparation of the Benzoate of Li_2 : 0.3 g. (0.00075 mole) of Li_2 was dissolved in 2 ml. of dry pyridine and 6 drops of benzoyl chloride were added. The solution was heated under gentle reflux for ten minutes, cooled, and 3 ml. of ethyl alcohol added. Addition of this solution to 20 ml. of water caused an oil to separate, and after the aqueous solution was heated to boiling to remove most of the pyridine, the oil solidified. Recrystallization from alcohol gave 0.32 g. (85%) of white crystals which melted at 169.6-171.6°. A further recrystallization gave a product which

showed the same melting point. A mixed melting point determination with starting material showed a depression.

Anal. Calcd. for $C_{29}H_{28}O_8$: C, 69.04; H, 5.60. Found; C, 69.05, 68.85; H, 5.91, 5.87.

$(\infty) \frac{25}{D} = -27^\circ$; $c_D = 0.498$ g./100 ml.; chloroform.

Hydrolysis of the Benzoate of LI_a to LI_b : 0.21 g. (0.00042 mole) of the benzoate of the anhydro compound was dissolved in 5 to 8 ml. of warm ethyl alcohol, and 0.5 ml. of 5% sodium hydroxide (0.00065 mole) was added. The solution was heated under reflux for two hours, then added to 10 ml. of water and cooled. The precipitated white crystals were filtered off, and there was obtained 0.17 g. (theoretical yield) of the compound LI_b . The melting point was $256.8-58.8^\circ$, and a mixed melting point determination with the anhydro compound, LI_a , showed no depression.

Pyrolysis of the Benzoate of the Compound LI_a : The benzoate was completely stable to sublimation in vacuum; therefore, 0.15 g. of the benzoate was heated under nitrogen to $250-260^\circ$ for one hour. The cooled melt was a yellow glass, but it readily crystallized when alcohol was added. After one recrystallization from alcohol, there was obtained 0.11 g. (73%) of starting material of melting point $172.1-173.1^\circ$. There was no indication of extensive dehydration.

Attempts to Prove the Presence of a Secondary Hydroxyl Group in Pieropodophyllin and in the Anhydro Compound, LI_a : (a) 0.3 g. (0.00075 mole) of the anhydro compound, LI_a , was stirred with 0.4 g. (0.0016 mole) of aluminum t-butoxide in 5 ml. of dry cyclohexanone⁶⁰ at 60° . At the end of one and one-half hours a thick paste had formed, so that 5 ml. more of cyclohexanone was added and the reaction continued for one and one-half hours longer. At the end of that time, a small quantity of water was added, and the mixture made acid with sulfuric acid. The cyclohexanone was then

removed by steam distillation, and the light colored residue removed by filtration and dried. By trituration with alcohol, the color was removed, and there was obtained 0.2 g. of slightly impure starting material, which had a melting point of 251-254°. One recrystallization gave pure material which melted at 254.0-56.3° with sintering at 252.3°. A mixed melting point determination with starting material showed no depression.

A repetition of the above reaction at a temperature of 60° for one and one-half hours and then at 100° for two hours produced quantitatively only slightly impure starting material.

(b) 0.6 g. (0.0015 mole) of pteropodophyllin and 0.8 g. (0.0032 mole) of aluminum t-butoxide were heated with stirring in 30 ml. of cyclohexane at 60° for five hours. The reaction mixture was taken up in chloroform, and the inorganic salts were extracted. The organic liquids were removed by steam distillation, and from the residue after one recrystallization from alcohol there was obtained 0.5 g. of pteropodophyllin.

(c) 0.3 g. of anhydro compound, Li_a , was subjected to hydrogenation for four hours at 225° under 5300 p.s.i. pressure over copper chromium oxide catalyst. On working up the reaction, a glass was obtained which could not be caused to crystallize by solvent action or by sublimation. A sample of the sublimate gave the following analysis, which is not in agreement with any reasonable product: C, 69.50%; H, 8.66%.

(d) Attempted dehydration by potassium bisulfate was unsuccessful. After the compound Li_a had been mixed intimately with the bisulfate, an attempt was made to sublime out the organic material under high vacuum.

The mixture became a tar at 250°, and no appreciable sublimation occurred.

(e) 0.5 g. (0.0013 mole) of the anhydro compound, Li_a , was placed in an open beaker together with 1.5 g. (0.014 mole) of benzoquinone in fresh

dioxane. The solution was stirred and irradiated by ultra violet light⁵⁷, whose wavelengths were approximately 2700-3000 Å, for one hour, and then without radiation for one-half hour. This alternation was continued for eight hours; after working up the reaction mixture, there was obtained 0.45 g. of starting material.

(f) Numerous attempts to oxidize the anhydro compound or picropodophyllin to the corresponding tetralone were made by using potassium permanganate of potassium dichromate or chromic anhydride in glacial acetic acid or in acetone. In most cases a theoretical amount of oxidising agent was used; however, one could recover as much as 60% of the starting material after all the oxidising agent was used up. This fact indicated extensive degradation, and that the tetralone first formed (assuming the point of lability was the secondary alcohol) was more easily oxidized than the alcohol itself. In no cases could a single or even reasonably clean mixture of products be obtained from the amorphous residues. Ketone tests were, of course, made, but they were seldom more than slightly positive.

An attempt was also made to study the relative rates of oxidation of podophyllotoxin, picropodophylloctoxin, and the anhydro compound, Lia, at 25°. Equi-molar quantities of each compound were dissolved in acetic acid, and to that solution was added the amount of potassium permanganate theoretically necessary to oxidize the compound to the corresponding aldehyde or ketone. All oxidations took place immediately giving rise to dark solutions, so that the amount of unused permanganate could not be determined by visual observation. However after one and one-half hours, the sample of picropodophyllin was worked up and a quantity of unchanged picropodophyllin in excess of 40% was obtained. In the case of the anhydro compound 80-90% of the starting material was recovered. In the case of

pterodophyllotoxin, the high solubility of the compound precluded any reasonable measure of the amount remaining unchanged, since a small quantity was used at the start.

(G) Attempted preparation of halides:

(1) The anhydro compound, LI_a, was suspended in thionyl chloride in benzene, stirred, and a stream of dry air was drawn thru the solution for one hour to entrain the hydrogen chloride⁵⁹. Then additional thionyl chloride was added and the solution stirred for twenty-four hours. At the end of this time an excess of pyridine was added, and the mixture was heated under reflux for four hours. On working up the product, it was evident that a reaction had taken place, but a homogeneous product could not be obtained. An analysis of one likely fraction did not yield a result reasonable for either a halide or a chlorosulfonic ester.

(2) The anhydro compound, LI_a, was dissolved in liquid hydrobromic acid, and the excess acid allowed to evaporate slowly overnight. A small amount of demethoxylated product was obtained, but a qualitative analysis for halogen was negative.

(3) Pteropedophyllin was dissolved in pyridine and thionyl chloride added. The reaction was allowed to stand for three days at room temperature, was then warmed on a steam bath for two hours, and then allowed to stand for three more days. On working up the reaction mixture, a 70% yield of pure pteropedophyllin was obtained, and the remainder although not obtained pure, was in all probability mostly pteropedophyllin.

(4) 0.3 g. of the anhydro compound, LI_a, was dissolved in thionyl chloride, and the thionyl chloride slowly evaporated. A product (0.32 g.) was obtained which could be recrystallized from petroleum ether (90-100°).

and which gave a positive qualitative analysis for sulfur and halogen. However, the analysis was not reconcilable with that calculated for the chlorosulfinic ester, the alkyl halide, or the sulfite ester.

(5) The action of phosphorous pentachloride in pyridine was also investigated, but the only products obtained were very water soluble and judged to be phosphoric esters. None were obtained sufficiently pure to warrant analysis.

The foregoing experiments serve to further illustrate the extraordinary stability of the alkyl oxygen bond of this supposedly benzyl-type alcohol.

The Preparation of Trihydroxy Compound, L_{h_3} , and the Anhydro Compound, LI_{h_3}

5.0 g. (0.012 mole) of pieropodophyllin were dissolved in 250 ml. of dioxane (the dioxane had been purified and dried for hydrogenations, and was distilled directly from lithium aluminum hydride) and added over the course of forty-five minutes to a vigorously stirred solution of 1.5 g. (0.039 mole) of lithium hydride in two liters of ether boiling under reflux. Refluxing and stirring were continued for several hours, at which time the excess hydride was decomposed by the cautious addition of water. 200 ml. of 10% sodium hydroxide was then added, and stirring continued until the ether layer was clear. The ether was immediately decanted, and the alkaline layer washed by swirling and decantation by two 500-ml. portions of ether. The combined ether solutions, which had a volume of 3,000 ml., were stored under nitrogen for further treatment as described below.

(a) 360 ml. of the above solution were evaporated to a volume of 100 ml. under nitrogen. The specific rotation was determined on this solution to be $(\alpha)_D^{25} = -66^\circ$; $c = 0.537$ g./100 ml. of solution.

(b) A portion of the above solution from (a) was set aside at room

temperature in an Erlenmeyer flask stoppered with a slotted cork so that a slow rate of evaporation was achieved. As the ethereal solution evaporated to near dryness, more ether was added and the evaporation allowed to repeat itself. This procedure was continued for a month, and at the end of that time no crystals had appeared. Therefore, the solution was then allowed to evaporate to dryness, and the residue was allowed to stand for three weeks. At the end of that time the crystals had appeared. The solid was then muddled with ether and removed by filtration to yield fine white needles, which melted with ebullition at 134.4-141.4°.

Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.12; H, 6.25. Found: C, 63.23, 63.14; H, 6.39, 6.34.

(∞) $\frac{25}{D} = -77^\circ$; $c = 0.339$ g./100ml.; chloroform.

(c) 600 ml. of the original solution from the lithium aluminum hydride reduction was concentrated to 40 ml., and then 150 ml. of benzene was added and the mixture distilled fractionally up to the boiling point of benzene. After the addition of 0.05 g. of para-toluenesulfonic acid, the solution was heated under reflux for twenty-eight hours with the returning solvent passing through a tube of Drierite. Concentration of the resulting solution after the period of heating yielded no crystals, therefore, the mixture was evaporated to dryness under vacuum and yielded a slightly colored oil which could not be crystallized from a variety of solvents. The oil was heated under reflux for ten minutes with alcoholic-aqueous sodium hydroxide and evaporated again to dryness. The residue was taken up in ether, filtered from inorganic materials, and many attempts were made to effect crystallization. It was not possible to crystallize this compound; analyses run on the glassy product demonstrated that the compound was the expected anhydro compound, Li_3 . The yield was 0.65 g. (65% over all), and the glassy substance melted to a clear thick melt at

89.0-95.0° with sintering at 86°.

Anal. Calcd. for $C_{22}H_{24}O_7$: C, 65.98; H, 6.04. Found: C, 65.89, 66.17; H, 6.43, 6.25.

$(\alpha)_D^{25} = + 75^\circ$; $c = 0.463$ g./100ml.; chloroform.

Molecular weight determinations by the Rast method gave values of 454 and 459, which though not as close as desirable to the theoretical value of 400.4, clearly demonstrate that the compound is not dimeric. Examination of the compound under the microscope failed to give any indication of birefringency. A repetition of the above preparation of the anhydro compound failed to give a crystalline product, although an analytically pure sample was obtained.

A Second Preparation of the Trihydroxy Compound, L_b : 10.8 g. (0.026 mole) of picropodophyllin was dissolved in 500 ml. of dry purified dioxane and added slowly over the course of forty-five minutes to a vigorously stirred solution of 3.0 g. (0.078 mole) of lithium aluminum hydride in 3500 ml. of ether boiling under reflux. Refluxing and stirring were continued for seven hours. Excess hydride was decomposed as before with 400 ml. of 10% sodium hydroxide; the volume of the combined ether solution and washings totalled 4600 ml. Two-thirds of this solution (equivalent to 7.2 g. of picropodophyllin) was concentrated to a volume of 350 ml., cooled, and seeded with the trihydroxy compound previously obtained. Crystallization did not take place then or on further concentration, presumably due to the presence of the dioxane. Therefore, the solution was evaporated to dryness in vacuo and at a temperature of 50° to yield a slightly colored glassy residue. The residue was taken up in ether, and the resulting solution was concentrated to 250 ml. and seeded with the trihydroxy compound, L_b . Crystallization took place immediately, and after filtration there was obtained 3.8 g. (53%) of white crystals which melted at 160.2-162.2°.

Anal. Calcd. for $C_{22}H_{26}O_8$: C, 63.12; H, 6.25. Found: C, 63.01, 62.84; H, 6.51, 6.44. $(\alpha)_D^{25} = -67^\circ$; $c = 0.347$ g./100 ml.; chloroform

Preparation of the tri-para-Nitrobenzoate of Compound L₃: 1.0 g. (0.0024 mole) of picropodophyllin was dissolved in 100 ml. of dry tetrahydrofuran and added slowly over a period of forty minutes to a well stirred solution of 0.4 g. (0.0104 mole) of lithium aluminum hydride in 250 ml. of tetrahydrofuran boiling under reflux. The reaction was continued for one and one-half hours, whereupon excess hydride was decomposed by 2 ml. of water and the solution was separated from the inorganic precipitate by filtration. When the filtrate was concentrated under vacuum, 3 ml. of an oil remained from which crystals could not be obtained. The oil was dissolved in 20 ml. of pyridine, 2.5 g. (0.013 mole) of para-nitrobenzoyl chloride was added, and the mixture was allowed to stand for one hour. At the end of one hour 5 ml. of ethyl alcohol were added, and the mixture was poured into 50 ml. of water, cooled, and filtered. The soft gummy precipitate was recrystallized by dissolving it in a small quantity of benzene and adding that solution to a large volume of boiling alcohol. Concentration of the alcohol removed most of the benzene, and when cloudiness appeared, the solution was chilled rapidly to produce a soft yellow precipitate of the tri-para-nitrobenzoate which melted at 110-120° to a thick glassy mass. A second recrystallization yielded 0.25 g. of the ester, which melted at 118.5-25.5° to a thick glassy mass. This melting range was constant after two further recrystallizations. Examination of the solid under the microscope gave only slight indications of birefringency.

Anal. Calcd. for $C_{43}H_{35}O_{17}N_3$: C, 59.65; H, 4.08; N, 4.85. Found: C, 59.77, 59.60; H, 4.59, 4.30; N, 5.08, 4.83.

$(\alpha)_D^{25} = -29^\circ$; $c = 0.942$ g./100 ml.; chloroform.

$(\alpha)_D^{25} = -27^\circ$; $c = 0.377$ g./100 ml.; chloroform.

Preparation of the Mono-para-Nitrobenzoate of the Anhydro Compound, LI_b:

0.25 g. (0.00062 mole) of the anhydro compound, LI_b, was dissolved in 5 ml. of dry pyridine, and 0.6 g. (0.0032 mole) of para-nitrobenzoyl chloride was added. The reaction mixture was allowed to stand overnight and was then poured into 20 ml. of water, cooled, and filtered. The precipitate was triturated with sodium bicarbonate solution, filtered off, and dried to give 0.45 g. of the crude para-nitrobenzoate which melted at 80° to a thick melt. Recrystallization was effected from alcohol by cooling a dilute solution of the ester very slowly to room temperature and then chilling. Two such treatments gave a material which melted to a clear thick oil at 90-94.9°. Examination of this compound under the microscope failed to reveal any indication of a crystalline nature. The recrystallization process was such that only a small amount of pure material could be obtained, so that a measure of the yield was not possible.

Anal. Calcd. for C₂₉H₂₇O₁₀N: C, 63.38; H, 4.95; N, 2.55. Found: C, 63.60, 63.39; H, 5.33, 5.15; N, 2.81, 2.80.

(∞)_D²⁵ = +66°; c = 0.197 g./100 ml.; chloroform.

Preparation of the Benzoate of Picropodophyllin, XLVIII: 5.3 g. (0.012 mole) of picropodophyllin was dissolved in 70 ml. of dry pyridine and 4 ml. of benzoyl chloride (0.034 mole) was added. The solution was heated on the steam bath for one hour and then concentrated to 30 to 40 ml. This solution was added to 200 ml. of water, cooled, and filtered. The crude benzoate after drying amounted to 6.5 g. (98% of theoretical) and melted at 185-92°. Two recrystallizations from ethyl acetate raised the melting point to a constant value of 200.1-201.6°.

Anal. Calcd. for C₂₉H₂₆O₉: C, 67.17; H, 5.05. Found: C, 66.99, 66.98; H, 5.32, 5.31.

(∞)_D²⁵ = +18°; c = 0.569 g./100 ml.; chloroform.

Preparations of the Benzoate of Podophyllotoxin, XLVII: 2.0 g. (0.0048 mole of podophyllotoxin was dissolved in 20 ml. of pyridine and 1.6 ml. of benzoyl chloride (0.014 mole) was added. The solution was allowed to stand for fifteen minutes at room temperature and then poured into 80 ml. of water and cooled; the oil which formed was separated. Two recrystallizations from alcohol, in which the ester is quite soluble, gave 1.75 g. (71%) of fine white needles which melted at 90-100°. Two further recrystallizations yielded 1.10 g. of the benzoate which sintered at 109° and melted at 112.6-116.6° with the formation of a miniscus at 145°. Further recrystallization did not raise the melting point.

Anal. Calcd. for $C_{29}H_{26}O_9$: C, 67.17; H, 5.05. Found: C, 67.25, 67.40; H, 5.11, 5.10.

(∞) $\frac{25}{D} = -118^\circ$; $c = 0.486$ g./100 ml.; chloroform.

Since this compound was not of preparative importance, no attempt was made to improve the yield, which probably could be done by allowing a longer reaction period, and perhaps by using a different solvent for recrystallization.

Preparation of beta-Apopieropedophyllin, XLIX: This compound was prepared in three ways, (a) by the decomposition of the acetate of pieropedophyllin, (b) by the pyrolysis of the benzoate of pieropedophyllin, and (c) by the pyrolysis of the benzoate of podophyllotoxin. Method (b) was found to be very convenient and to give consistently good yields, in contrast to (a) and is, therefore, the preferred method of preparation. Method (c) was developed when a comparison of the stability of the benzoates of podophyllotoxin and pieropedophyllin was made.

(a) In a process analogous to that of Spath²⁵, 6.9 g. of pieropedophyllin was dissolved in 50 ml. of acetic hydride together with 3.5 g. of fused sodium acetate, and the mixture was heated under reflux for two hours.

20 ml. of acetic acid was then added, and the mixture was diluted with a large volume of water. The crude acetate was filtered off (87%). Attempts to obtain a second crop of acetate yielded only slight amounts of amorphous impure products.

The crude acetate was recrystallized from acetic anhydride with evaporation of the solution to a viscous oil which was heated cautiously until ready bubbling ceased. The oil was taken up in a small amount of acetic anhydride and crystallized to give crude beta-apopicropodophyllin which sintered at 210° and melted at 211°. Two further recrystallizations from acetic anhydride gave pure beta-apopicropodophyllin which had a constant melting point of 212.6-214.6° with sintering at 212°. The yield was only 3.0 g. (45%); second crops of very impure material totalling 9% could be obtained. This compound dissolved in cold concentrated sulfuric acid with production of a color which ranged from orange to red-brown to violet as described by Robertson.

Anal. Calcd. for $C_{22}H_{20}O_7$: C, 66.65; H, 5.08. Found: C, 66.48, 66.24; H, 5.16, 5.08.

$(\infty) \frac{25}{D} = +103^\circ$; $c = 0.466$ g./100 ml.; chloroform.

Robertson has reported a specific rotation of $+118^\circ$ in chloroform; Spath reported $+75^\circ$ in acetone; and Borsche and Nieman reported a rotation of 0° in chloroform, all at comparable concentrations.

(b) 4.9 g. of the crude benzoate of picropodophyllin were heated under nitrogen. At a pot temperature of 210° the mass melted, and the temperature was then raised to 240° for twenty minutes. During this time a sublimate, subsequently identified as benzoic acid, appeared above the melt. The tube was cooled to room temperature, washed with three 5-ml portions of 5% sodium bicarbonate, then with copious amounts of water, and finally with one small portion of alcohol. By the addition of ethyl

acetate to the cake and scratching, crystallization was induced. Digestion of the product with 250 ml. of ethyl acetate for 3 hours, followed by concentration to 40 ml. and cooling, caused the precipitation of 3.2 g. (85%) of pure beta-apopicropodophyllin. The filtrate was concentrated to 10 ml., and 0.5 g. of additional material obtained. Recrystallization of the second crop material from ethyl acetate yielded 0.4 g. (10.5%) of white fine needles of beta-apopicropodophyllin. The total yield of good material by this procedure was in excess of 95%. A sample of the compound after being sublimed for analysis melted at 214.0-215.4° with sintering at 212.8°.

Anal. Calcd. for $C_{22}H_{20}O_7$: C, 66.65; H, 5.08. Found: C, 66.45, 66.32; H, 5.17, 5.21.

Subsequent preparations on larger amounts of material always gave yields in excess of 80%. The temperature should not exceed 250°, and the period of heating should not be in excess of twenty minutes.

(c) 0.3 g. of the benzoate of podophyllotoxin was heated rapidly under nitrogen up to a temperature of 150°, and then over the course of the next fifteen minutes the temperature was raised to 240°. No appreciable amount of sublimation was noticed below 230°, but as the temperature reached 240° sublimation was quite evident. The temperature was held at the higher level for seven minutes, and then the tube was cooled to room temperature. The sublimate was identified as benzoic acid; following the same procedure as in (b) above the product was worked up to yield 0.15 g. (65%) of beta-apopicropodophyllin which melted at 211.2-214.1° with sintering at 210°.

One recrystallization from ethyl acetate gave pure beta-apopicropodophyllin which with the preparation from (b) above gave no melting point depression.

The Preparation of Dexopicropodophyllin, LII: (a) 3.2 g. of beta-apopicropodophyllin were added to a suspension of 0.2 g. of reduced Adams catalyst in 125 ml. of ethyl acetate, and attached to a quantitative hydrogenator.

Reduction failed to take place even after running eight hours. The reaction mixture was filtered to remove the catalyst, placed in a high pressure bomb with Raney nickel and heated to 60° at 1500 p.s.i. pressure for one and one-half hours. The reaction was stopped, and after removal of the catalyst the filtrate was concentrated to 30 ml. and cooled, whereupon 2.2 g. of white crystalline material crystallized. This substance melted at $173-86^{\circ}$ with sintering at 165° . A second crop of material was lost by accident. Judging from the melting point of $169-70^{\circ}$ reported for desoxypicropodophyllin by Borsche and Nieman, the above material was not fully reduced, therefore, the 2.2 g. of crude substance were reduced further for five hours in 150 ml. of absolute alcohol over Raney nickel at 60° and 1500 p.s.i. The catalyst was filtered off, and the filtrate cooled to obtain a precipitate of 1.4 g. of fine white needles which melted at $198.1-200.0^{\circ}$. A mixed melting point with starting material gave a marked depression. Recrystallization from ethyl alcohol gave 1.2 g. of the desoxypicropodophyllin which melted at $199.6-200.8^{\circ}$. One further recrystallization to obtain an analytical sample raised the melting point to $199.8-201.0^{\circ}$. Attempts to obtain a second crop of material from the mother liquors yielded only a glassy substance.

Anal. Calcd. for $C_{22}H_{22}O_7$: C, 66.30; H, 5.57. Found: C, 66.25, 66.39; H, 5.72, 5.67.

(α) $D_{25}^{25} = -114^{\circ}$; $c = 0.498$ g./100ml.; chloroform.

(b) The yield of desoxypicropodophyllin was considerably improved by the following procedure. 5.6 g. of beta-apopicropodophyllin were reduced in 500 ml. of absolute alcohol at 60° over Raney nickel for eight hours at 1600 p.s.i. The solution and washings were filtered free of Raney nickel and concentrated to 200 ml. and cooled, whereupon fine white needles of desoxypicropodophyllin separated. The yield was 4.3 g. (77%)

of a product which melted at 200.0-201.4° and was identical with the previously prepared sample. The filtrate was concentrated to 30 ml. and cooled to yield a small quantity (.15g.) of impure desoxypicropodophyllin which melted at 182.7-191.5° with sintering at 180°.

Preparation of the Dihydroxy Compound, LIII: 1.0 g. (0.0025 mole) of desoxypicropodophyllin was placed in a Soxhlet extractor and extracted into a well-stirred solution of 0.50 g. (0.013 mole) of lithium aluminum hydride in 500 ml. of ether. After six hours the excess hydride was decomposed first by the dropwise addition of water followed by the addition of 70 ml. of 15% sodium hydroxide. The ether layer was decanted, and the alkaline solution washed by decantation with four 150-ml. portions of ether. The combined ethereal solutions were concentrated to 400 ml., but crystallization could not be induced. Further concentration to 15 ml. did not cause crystallization, so the solution was evaporated to dryness in vacuo to give a white glassy residue which melted at 60-70° to a clear thick oil. The yield was 0.80 g. (79%). Attempts to crystallize this glassy material were unsuccessful.

Anal. Calcd. for $C_{22}H_{26}O_7$: C, 65.65; H, 6.51. Found: C, 65.38, 65.39; H, 6.77, 6.79.

(-c) $\frac{25}{D} = + 120^\circ$; $c = 0.544$ g./100 ml.; chloroform.

Preparation of the Di-para-Nitrobenzoate of the Dihydroxy Compound, LIII: 0.20 g. (0.005 mole) of compound LIII was dissolved in 4 ml. of dry pyridine, and 0.5 g. (0.0027 mole) of para-nitrobenzoyl chloride was added. After standing overnight, the reaction mixture was poured into 20 ml. of cold water, cooled, and filtered. There was obtained a soft bright yellow solid, which after recrystallization from alcohol retained its color and amounted to 0.30 g. (86%). The melting point was 92-103° at which temperature the thick glassy melt was clear, but a miniscus did not appear until

135°. In order to effect recrystallization, it was necessary to dissolve the compound in benzene and add that solution to boiling alcohol. Concentration of that solution removed the benzene, and on the appearance of cloudiness the solution was cooled slowly. One more recrystallization gave the di-ester which melted at 97-107° to form the thick clear melt, which in turn gave a fluid miniscus at 117°.

Anal. Calcd. for $C_{36}H_{32}O_{13}N_2$: C, 61.72; H, 4.60; N, 4.00. Found: C, 61.77, 61.97; H, 4.75, 4.69; N, 4.09, 4.10.

$(\infty) \frac{25}{D} = +59^\circ$; $c = 0.472$ g./100 ml.; chloroform.

Preparation of the Desoxyanhydro Compound, LIV: 0.29 g. (0.00072 mole) of LIII was dissolved in 50 ml. of dry benzene and 0.02 g. (0.00011 mole) of para-toluenesulfonic was added. The solution was heated under reflux for twenty-four hours with the returning solvent passing through a tube of Drierite. After cooling, the solution was washed by three 5-ml portions of 5% sodium hydroxide, and then by water until the washings were neutral. The benzene was dried by Drierite, filtered, and evaporated to near dryness. When almost all of the benzene was removed, crystals appeared and rapidly formed into fine white needles which melted at 162.7-164.1° with sintering at 160°. The yield was 0.21 g. (75%). The desoxyanhydro compound was very soluble in most solvents, but it could be recrystallized from methyl alcohol-water mixtures, or cyclohexane. Recrystallization of the above product from a methyl alcohol-water mixture gave pure desoxyanhydro compound which melted constantly at 162.7-163.7° with sintering at 161.7°.

Anal. Calcd. for $C_{22}H_{24}O_8$: C, 68.73; H, 6.30. Found: C, 69.08, 68.95; H, 6.41, 6.41.

$(\infty) \frac{25}{D} = +64^\circ$; $c = 0.497$ g./100 ml.; chloroform.

Attempts to Dehydrogenate the Compounds LI_a and LIV; (a) 0.2 g. of the desoxyanhydro compound, LIV, was heated with 0.04 g. of 10% palladium-charcoal catalyst in a sublimation apparatus. At a high vacuum the desoxyanhydro compound sublimed unchanged. When the same reaction was run a second time at 30 mm pressure, the material melted and distillation took place. The distillate proved to be unchanged anhydro compound obtained in 75% yield. No product could be isolated from the residue. The same result was obtained on the anhydro compound, LI_a.

(b) A dehydrogenation attempt using 0.2 g. of compound LI_a, or LIV and 0.05 g. of 10% palladium-charcoal catalyst in para-cymene as solvent gave practically a quantitative recovery of starting material after a reflux period of two and one-half to three hours.

(c) By the same procedure as (b) above, using diphenyl ether as a solvent and twice the amount of catalyst, compound LIV gave only tarry or oily residues from which no crystalline product could be obtained.

(d) The anhydro compound, LI_a, was heated with the palladium-charcoal catalyst in an atmosphere of nitrogen to 270-280° for one and one-half hours. A clean crystalline product could not be obtained either by sublimation or crystallization from the highly carbonized amorphous reaction mixture.

(f) In this case a somewhat positive indication was obtained that dehydrogenation did take place and that it might be possible to isolate the naphthalene compound. 0.3 g. of compound LIV was heated to 235-50° with 0.05 g. of 10% palladium-charcoal catalyst for forty-five minutes. When the residual cake was dissolved in alcohol and the solution filtered, a solid crystallized out of solution. The material melted from 100-160°.

Sublimation of this material was attended by a large amount of decomposition of the residue, but a small amount of sublimate was obtained which melted at 160-78° with sintering from 145°. A mixed melting point of this material and starting material gave a depression to 140-50°. Sufficient material for further work was not obtained.

Ultra-Violet Absorption Spectra: The ultra-violet absorption spectra of the principle compounds obtained were determined. The curves were very similar except in the case of alpha-apopicropodophyllin where a marked shift of the maxima toward the visible occurred; there was also present in this curve a much greater degree of fine structure than in any of the other curves. It was necessary to use alcohol as the solvent in order to obtain a sufficient concentration of material, and therefore, the lower limit of observation was 229 millimicrons. The extinction coefficients were calculated and are presented in the following pages of this section. The respective graphs of each of the compounds are also included as a group after the experimental data.

PODOPHYLOTOXIN, XLVII

Cell thickness = 1.000 cm $t = 28^\circ$ $c = 1.01 \times 10^{-4}$ molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
380	0.000×10^4	276	0.299×10^4
360	0.000	274	0.269
350	0.000	272	0.243
340	0.000	270	0.219
330	0.004	268	0.192
320	0.007	266	0.164
315	0.009	265	0.156
310	0.013	264	0.145
305	0.062	263	0.139
304	0.091	262	0.133
302	0.175	261	0.129
301	0.241	260	0.127
300	0.294	259 ^{1/2}	0.126
299	0.339	259	0.127
298	0.366	258	0.133
297	0.384	257	0.145
296	0.405	256	0.166
295	0.422	255	0.196
294	0.441	254	0.234
293 ^{1/2}	0.446	253	0.287
293	0.456	252	0.350
292	0.457	251	0.419
291	0.454	250	0.503
290	0.451	248	0.672
289	0.451	246	0.822
288	0.449	244	0.937
287	0.444	242	1.02
286	0.435	240	1.10
285	0.417	238	1.15
284	0.406	236	1.22
283	0.394	234	1.30
282	0.384	232	1.38
281	0.375	230	1.42
280	0.357	229	1.46
278	0.327		

PICROPODOPHYLLIN, XLVIII

Cell thickness = 1.000 cm.

 $t = 27^{\circ}$ conc. = 1.04×10^{-4} molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000x10 ⁴	278	0.349 x10 ⁴
380	0.000	276	0.315
360	0.000	274	0.283
350	0.000	272	0.255
340	0.000	270	0.224
330	0.000	268	0.190
320	0.000	266	0.167
310	0.006	265	0.151
305	0.041	264	0.145
304	0.060	263	0.156
302	0.125	262	0.123
300	0.210	261	0.121
298	0.293	260	0.118
297	0.352	259	0.114
296	0.359	258	0.118
295	0.385	257	0.127
294	0.404	256	0.137
293	0.421	255	0.160
292	0.440	254	0.193
291	0.445	253	0.239
290	0.449	252	0.291
289	0.452	251	0.362
288	0.452	250	0.444
287	0.448	249	0.533
286	0.443	248	0.610
285	0.437	247	0.697
284	0.432	246	0.765
283	0.416	245	0.827
282	0.408	240	1.06
281	0.399	238	1.20
280	0.381	230	1.33
279	0.370	229	1.39

TRIHYROXY COMPOUND, L₂

Cell thickness = 1.000 cm.

t = 25°

c = .976 x 10⁻⁴ molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000 x10 ⁴	278	0.309 x10 ⁴
380	0.000	276	0.276
360	0.000	274	0.254
340	0.000	272	0.217
330	0.000	270	0.202
320	0.000	268	0.173
310	0.008	266	0.155
305	0.074	265	0.144
303	0.154	264	0.132
302	0.200	263	0.126
301	0.259	262	0.120
300	0.309	261	0.115
299	0.338	260	0.111
298	0.362	259	0.110
297	0.376	258	0.115
296	0.388	257	0.120
295	0.417	256	0.135
294	0.436	255	0.156
293	0.439	254	0.185
292.5	0.439	253	0.227
292	0.439	252	0.284
291	0.438	251	0.355
290	0.437	250	0.438
289	0.434	248	0.621
288	0.429	246	0.794
287	0.420	244	0.917
286	0.407	242	1.00
285	0.391	240	1.11
284	0.383	235	1.21
282	0.361	230	1.39
280	0.337	229	1.43

TRIHYDROXY COMPOUND, I_b

Cell thickness = 1.000 cm

t = 27°

c = 1.04 x 10⁻⁴ molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000x10 ⁴	279	0.322x10 ⁴
380	0.000	278	0.307
360	0.000	276	0.283
340	0.000	274	0.253
330	0.003	272	0.231
320	0.003	270	0.208
310	0.008	268	0.180
305	0.054	266	0.157
302	0.156	265	0.147
300	0.258	264	0.137
299	0.307	263	0.128
298	0.333	262	0.122
297	0.351	261	0.113
296	0.369	260	0.108
295	0.386	259	0.103
294	0.404	258	0.104
293	0.422	257	0.106
292	0.423	256	0.116
291	0.422	255	0.131
290	0.413	254	0.163
289	0.417	253	0.193
288	0.416	252	0.249
287	0.412	250	0.400
286	0.402	248	0.608
285	0.389	246	0.776
284	0.375	244	0.938
283	0.365	242	1.04
282	0.359	240	1.10
281	0.349	236	1.30
280	0.336	230	1.57
		229	1.44

ANHYDRO COMPOUND, Li_2

Cell thickness = 1,000 cm.

 $t = 25^\circ$ $c = 1.19 \times 10^4$ molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000 $\times 10^4$	288	0.462 $\times 10^4$
380	0.000	287	0.442
360	0.000	286	0.427
350	0.000	284	0.399
345	0.002	282	0.363
340	0.002	280	0.333
335	0.003	278	0.304
334	0.003	276	0.269
325	0.003	274	0.241
320	0.003	272	0.220
315	0.006	270	0.196
310	0.026	268	0.177
308	0.057	266	0.158
306	0.117	264	0.149
305	0.161	263	0.146
304	0.214	262	0.149
303	0.277	261	0.154
302	0.328	260	0.170
301	0.370	258	0.222
300	0.394	256	0.314
298	0.457	254	0.427
297	0.463	252	0.543
296	0.467	250	0.656
295	0.496	248	0.773
294	0.501	245	0.924
293	0.501	240	1.09
292	0.499	235	1.22
291	0.496	230	1.33
290	0.487	228	1.39
289	0.476		

ANHYDRO COMPOUND, Li_2

Cell thickness = 1.000 cm.

 $t = 26^\circ$ $c = 1.05 \times 10^{-4}$ molar

Wave Length in millimicrons	Extinction coefficient	Wave Length in millimicrons	Extinction coefficient
400	0.000 $\times 10^4$	392	0.512 $\times 10^4$
380	0.000	290	0.502
370	0.000	288	0.486
360	0.000	286	0.459
350	0.003	284	0.432
340	0.004	282	0.413
345	0.008	280	0.383
330	0.050	278	0.353
325	0.067	276	0.319
320	0.086	274	0.293
318	0.090	272	0.268
316	0.085	270	0.245
314	0.097	268	0.216
312	0.105	266	0.194
310	0.115	264	0.177
308	0.133	262	0.172
306	0.166	260	0.176
304	0.236	258	0.214
302	0.327	256	0.280
300	0.398	254	0.393
298	0.446	252	0.524
296	0.484	250	0.657
294	0.512	245	1.02
293	0.517	240	1.20
		235	1.36
		230	1.60

B-RAPHICROPDOPHYLLIN, XLIX (beta)

Cell thickness = 1.000 cm.

t = 28°

c = 1.05 x 10⁻⁴ molar

Wave length in millimeters	Extinction coefficient	Wave length in millimeters	Extinction coefficient
400	0.000 x 10 ⁴	284	0.428 x 10 ⁴
380	0.000	283	0.417
360	0.000	282	0.407
350	0.002	281	0.395
340	0.002	280	0.384
330	0.003	279	0.369
325	0.003	278	0.357
320	0.007	276	0.341
315	0.011	274	0.321
310	0.035	272	0.306
308	0.070	270	0.294
306	0.127	269	0.289
304	0.216	268	0.283
303	0.266	267	0.287
302	0.312	266	0.289
301	0.345	265	0.290
300	0.372	264	0.297
299	0.391	263	0.305
298	0.410	262	0.313
297	0.430	260	0.332
296	0.447	258	0.356
295	0.456	254	0.434
294	0.462	252	0.492
293	0.464	250	0.569
292	0.466	248	0.659
291	0.466	246	0.757
290	0.466	244	0.868
289	0.462	240	1.09
288	0.456	235	1.34
287	0.447	230	1.65
286	0.441	228	1.84
285	0.434		

α -APOPIROPOPHYLLIN, XLIX (alpha)

Cell thickness = 1.000 cm.

t = 29°

c = 1.14×10^{-4} molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000×10^4	306	0.732×10^4	260	0.391×10^4
380	0.000	305	0.724	258	0.441
370	0.000	304	0.717	256	0.526
360	0.004	303	0.716	254	0.652
355	0.005	302	0.716	253	0.737
350	0.006	301	0.716	252	0.833
345	0.007	300	0.708	251	0.983
340	0.027	299	0.708	250	1.05
337	0.059	298	0.702	245	1.67
335	0.094	297	0.697	240	∞
334	0.122	296	0.693	235	∞
333	0.155	295	0.683		
332	0.194	294	0.670		
331	0.238	293	0.657		
330	0.284	292	0.644		
329	0.338	291	0.636		
328	0.403	290	0.626		
327	0.463	289	0.614		
326	0.518	288	0.605		
325	0.561	287	0.596		
324	0.603	286	0.592		
323	0.629	285	0.591		
322	0.652	284	0.586		
321	0.665	282	0.570		
320	0.676	280	0.547		
319	0.696	278	0.519		
318	0.705	276	0.493		
317	0.717	274	0.464		
316	0.732	272	0.428		
315	0.742	270	0.399		
314	0.754	268	0.373		
313	0.763	267	0.363		
312	0.765	266	0.352		
311	0.767	265	0.349		
310	0.763	264	0.352		
309	0.760	263	0.354		
308	0.749	262	0.364		
307	0.737	261	0.373		

 $C = .93 \times 10^{-5}$

260 0.030×10^5
 258 0.083
 256 0.041
 254 0.051
 252 0.067
 250 0.093
 249 0.100
 248 0.110
 247 0.126
 246 0.135
 245 0.151
 244 0.160
 243 0.180
 242 0.186
 241 0.203
 240 0.215
 239 0.226
 237 0.242
 235 0.265
 233 0.276
 231 0.294
 230 0.300
 229 0.309
 226 0.320

DESOKYPIGROPODOPHYLLIN, LII

Cell thickness = 1.000 cm.

 $t = 26^\circ$ $c = 1.0 \times 10^{-4}$ molar

Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000×10^4	288	0.495×10^4
380	0.000	286	0.469
360	0.000	284	0.445
350	0.000	282	0.413
340	0.000	280	0.376
330	0.000	278	0.337
320	0.000	276	0.299
315	0.002	274	0.266
310	0.018	272	0.237
308	0.046	270	0.204
306	0.089	268	0.177
305	0.126	266	0.152
304	0.167	264	0.133
303	0.221	262	0.119
302	0.275	260	0.112
301	0.327	258	0.122
300	0.366	256	0.154
298	0.426	254	0.221
296	0.481	252	0.328
295	0.495	250	0.455
294	0.510	245	0.765
293	0.517	240	0.980
292	0.519	235	1.09
291	0.520	230	1.25
290	0.517	228	1.35
289	0.507		

DESOXYDIHYDROXY COMPOUND, LIII

Cell thickness = 1.000 cm.

t = 27°

c = 1.15 x 10⁻⁴ molar

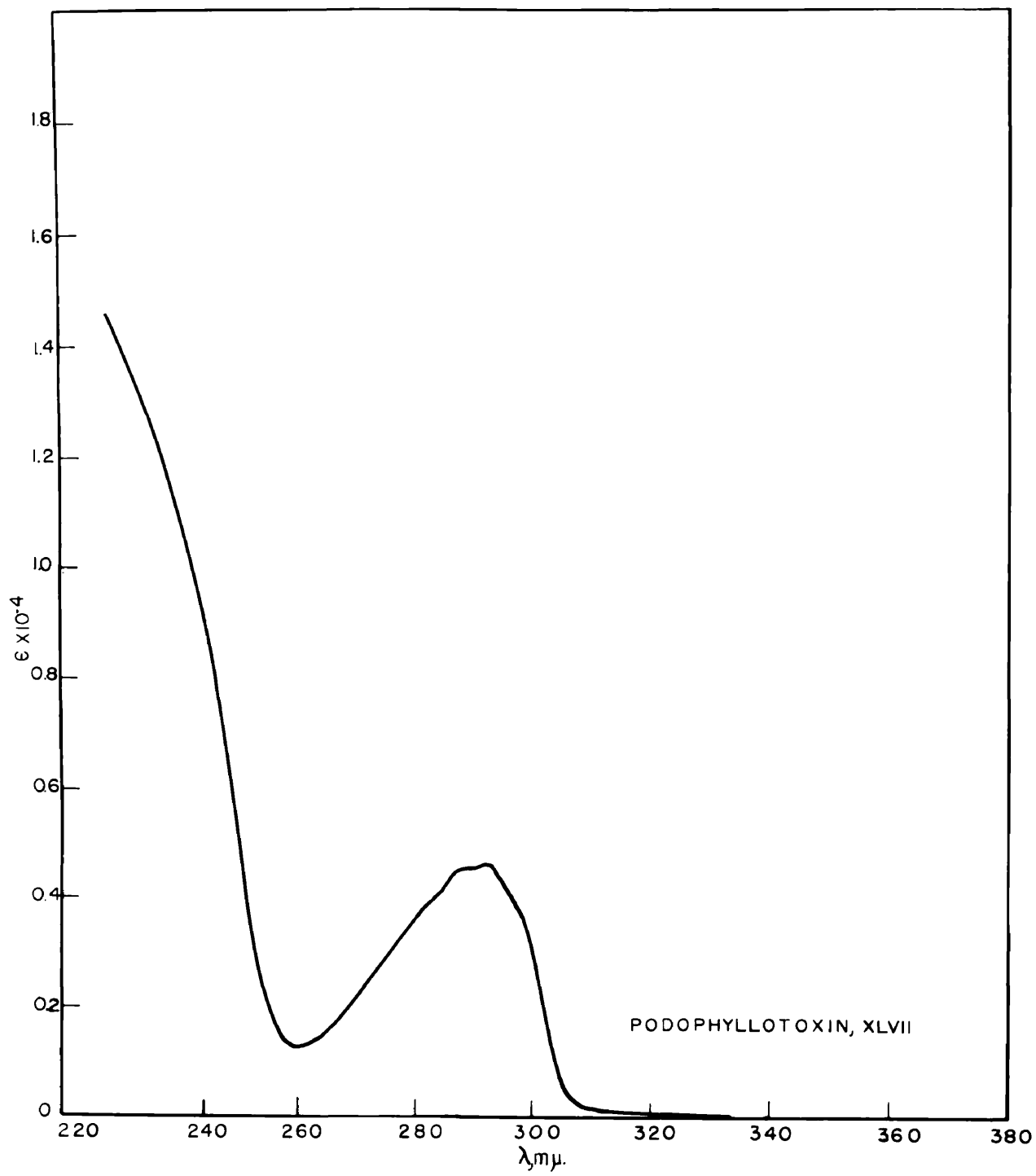
Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000 x10 ⁴	276	0.251 x10 ⁴
380	0.000	274	0.224
360	0.000	272	0.203
340	0.000	270	0.178
320	0.000	268	0.155
317	0.002	267	0.143
315	0.005	266	0.133
310	0.041	265	0.124
308	0.093	264	0.115
306	0.182	263	0.107
305	0.241	262	0.102
304	0.289	261	0.094
303	0.341	260	0.092
302	0.367	259	0.090
301	0.391	258	0.088
300	0.411	257	0.092
299	0.437	256	0.097
298	0.458	255	0.111
297	0.476	254	0.127
296	0.484	253	0.160
295	0.489	252	0.197
294	0.489	251	0.246
293	0.487	250	0.304
292	0.483	249	0.393
291	0.478	248	0.488
290	0.464	247	0.569
289	0.447	246	0.664
288	0.428	245	0.732
287	0.416	244	0.787
286	0.402	242	0.896
285	0.385	240	0.983
284	0.372	238	1.06
283	0.355	236	1.13
282	0.351	234	1.18
281	0.338	232	1.26
280	0.312	230	1.32
278	0.277	229	1.37

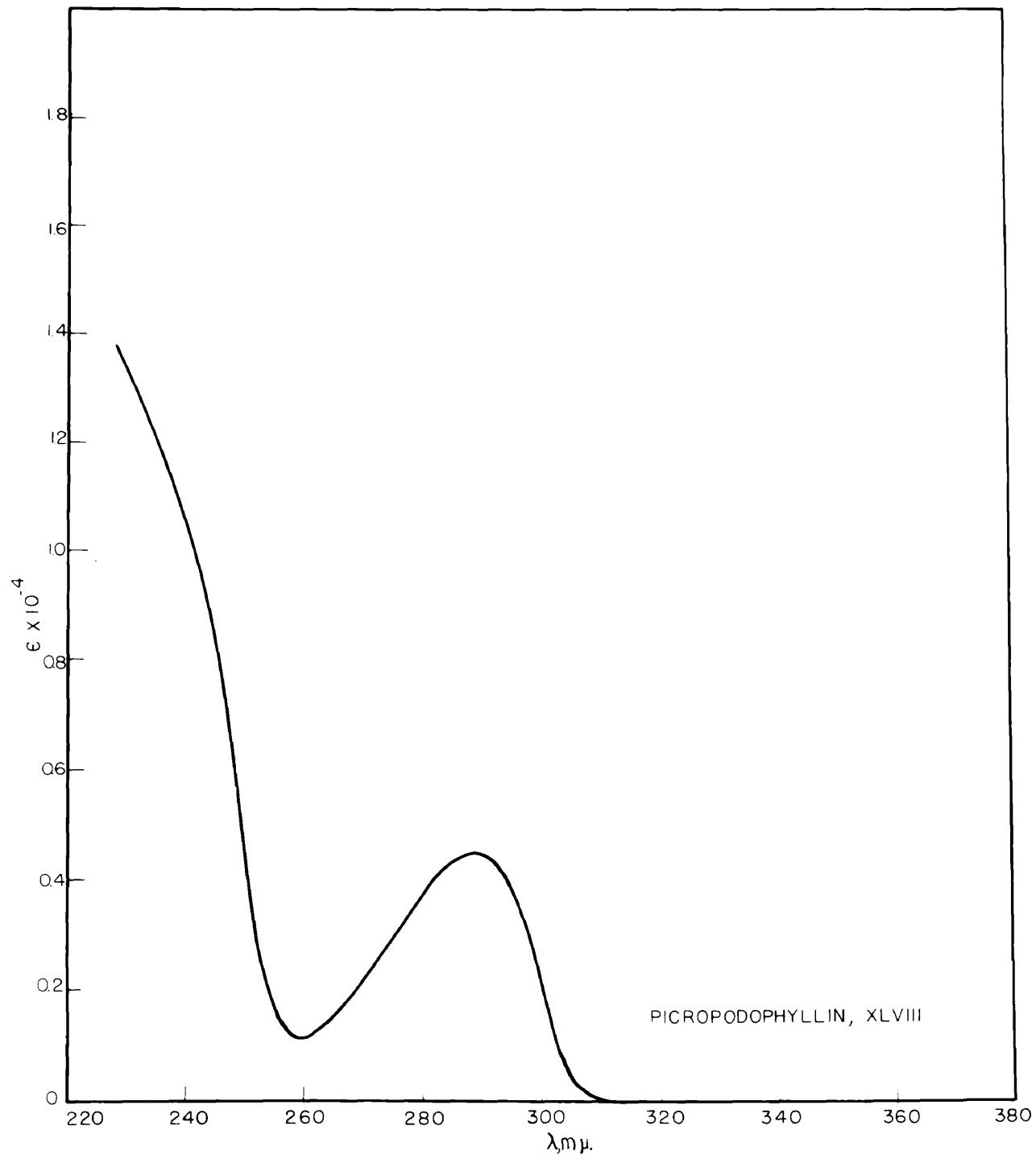
DESORXYANHYDRO COMPOUND, IIV

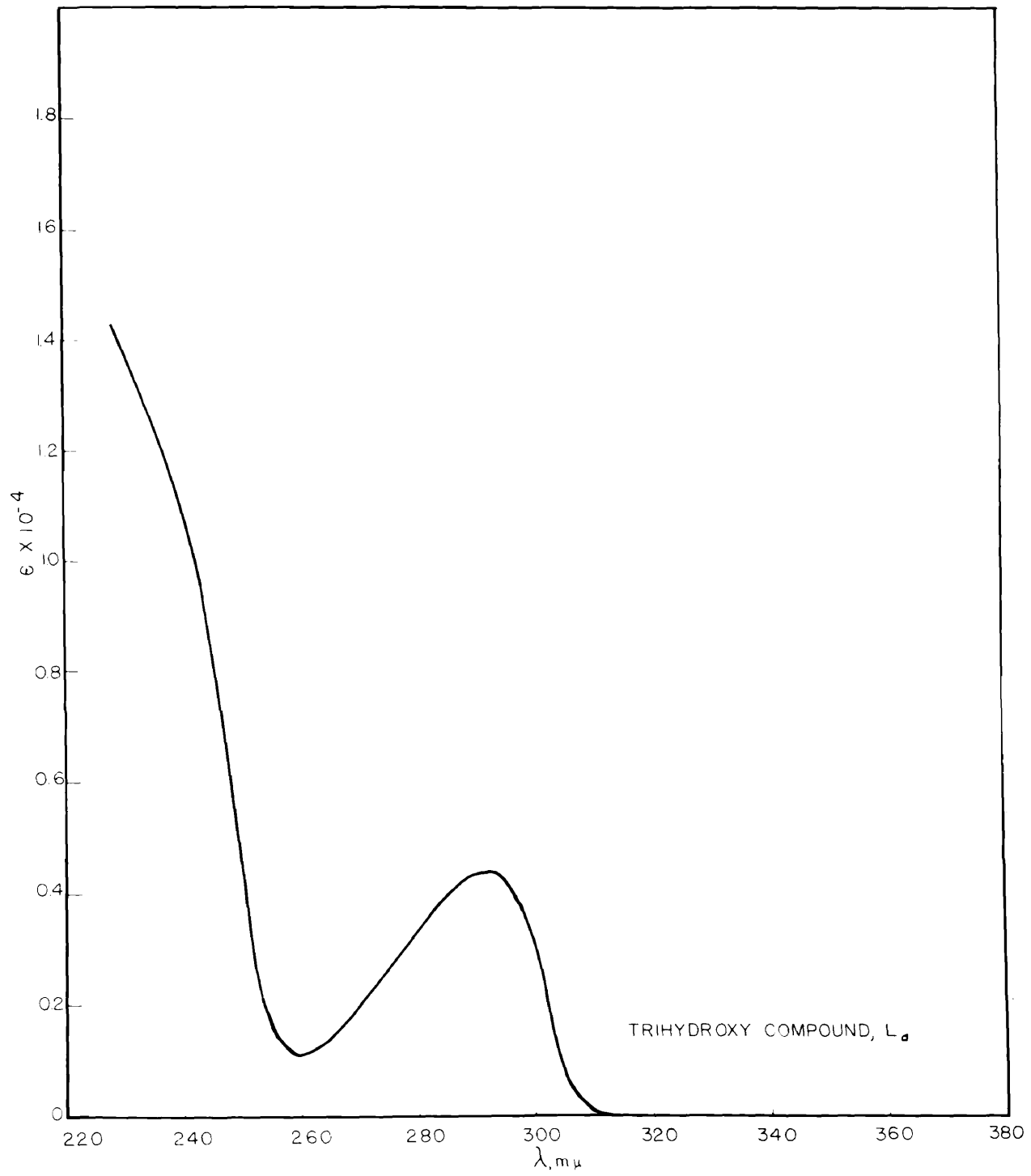
Cell thickness = 1.000 cm.

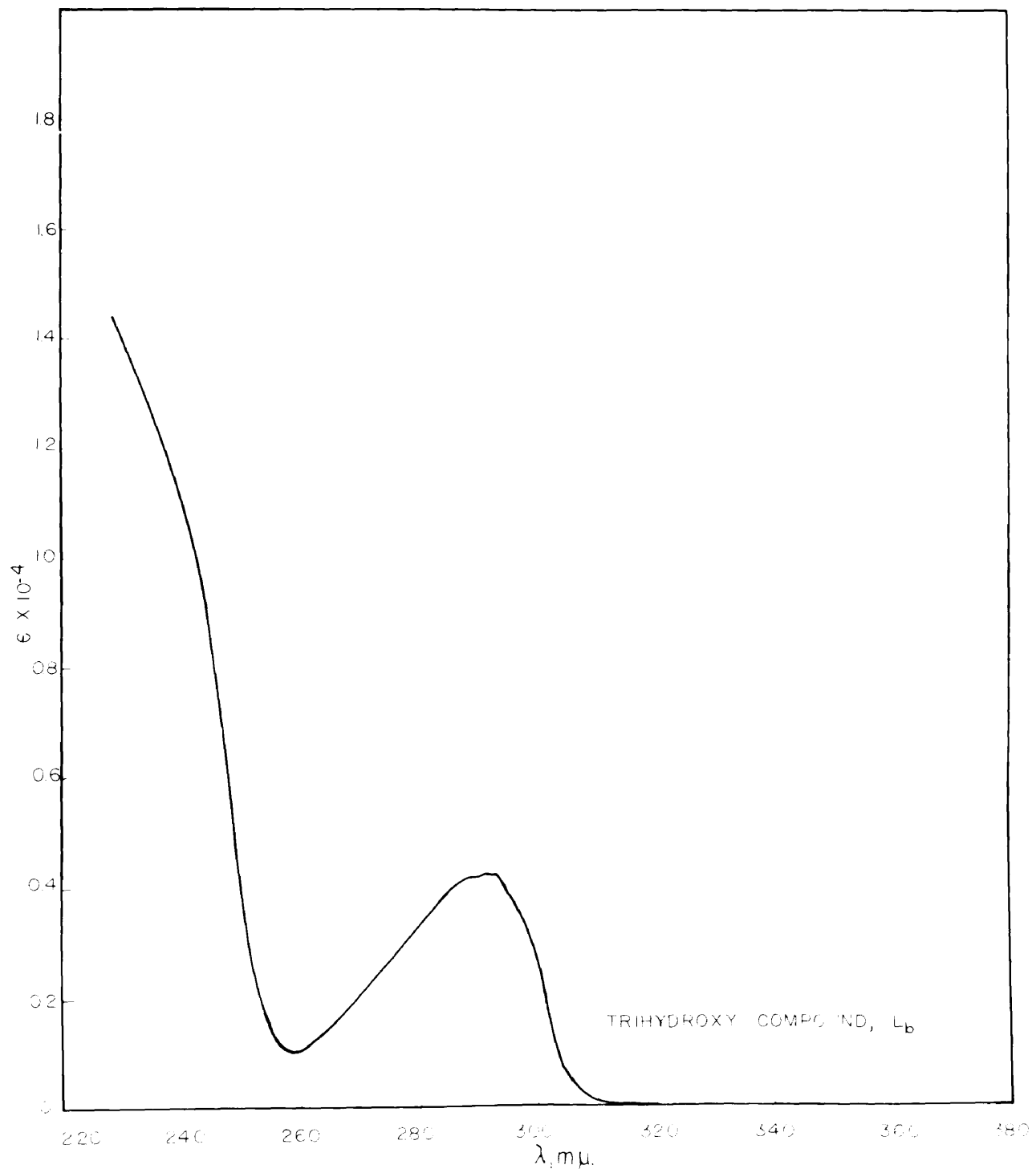
 $t = 27^\circ$ $c = 1.08 \times 10^{-4}$ molar

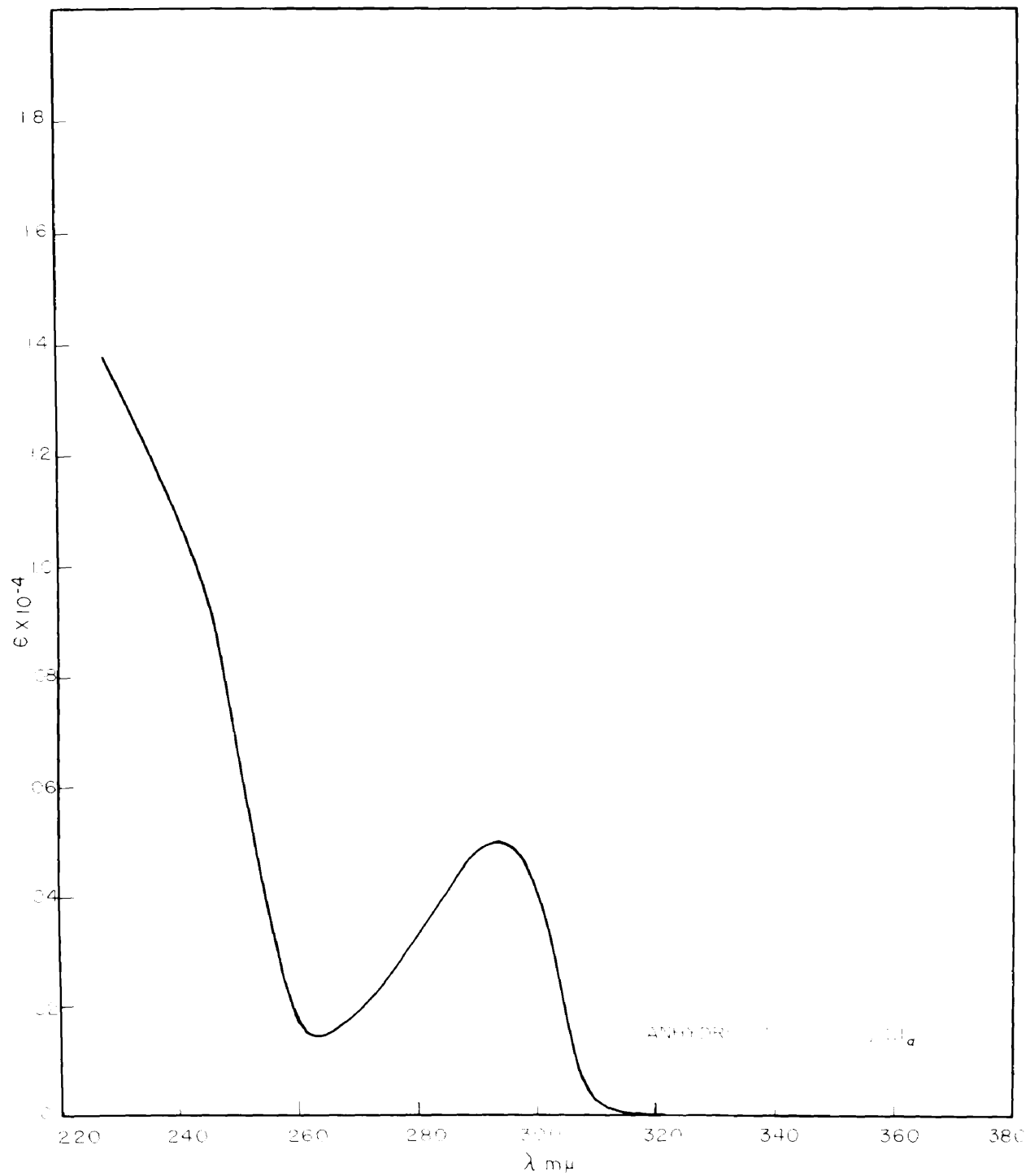
Wave length in millimicrons	Extinction coefficient	Wave length in millimicrons	Extinction coefficient
400	0.000 $\times 10^4$	278	0.293 $\times 10^4$
380	0.000	276	0.257
360	0.000	274	0.228
340	0.000	272	0.204
320	0.000	270	0.180
315	0.002	268	0.159
310	0.027	266	0.137
307	0.078	265	0.128
305	0.153	264	0.118
304	0.203	263	0.108
302	0.299	262	0.100
301	0.337	261	0.096
300	0.362	260	0.093
299	0.395	259	0.088
286	0.419	258	0.087
297	0.448	257	0.090
296	0.461	256	0.094
295	0.474	255	0.105
294	0.479	254	0.131
293	0.481	253	0.160
292	0.479	252	0.200
291	0.476	251	0.262
290	0.471	250	0.323
289	0.462	249	0.393
288	0.448	248	0.465
287	0.431	246	0.600
286	0.413	244	0.731
285	0.396	242	0.838
284	0.381	240	0.935
283	0.366	235	1.06
282	0.355	230	1.21
280	0.321	229	1.26

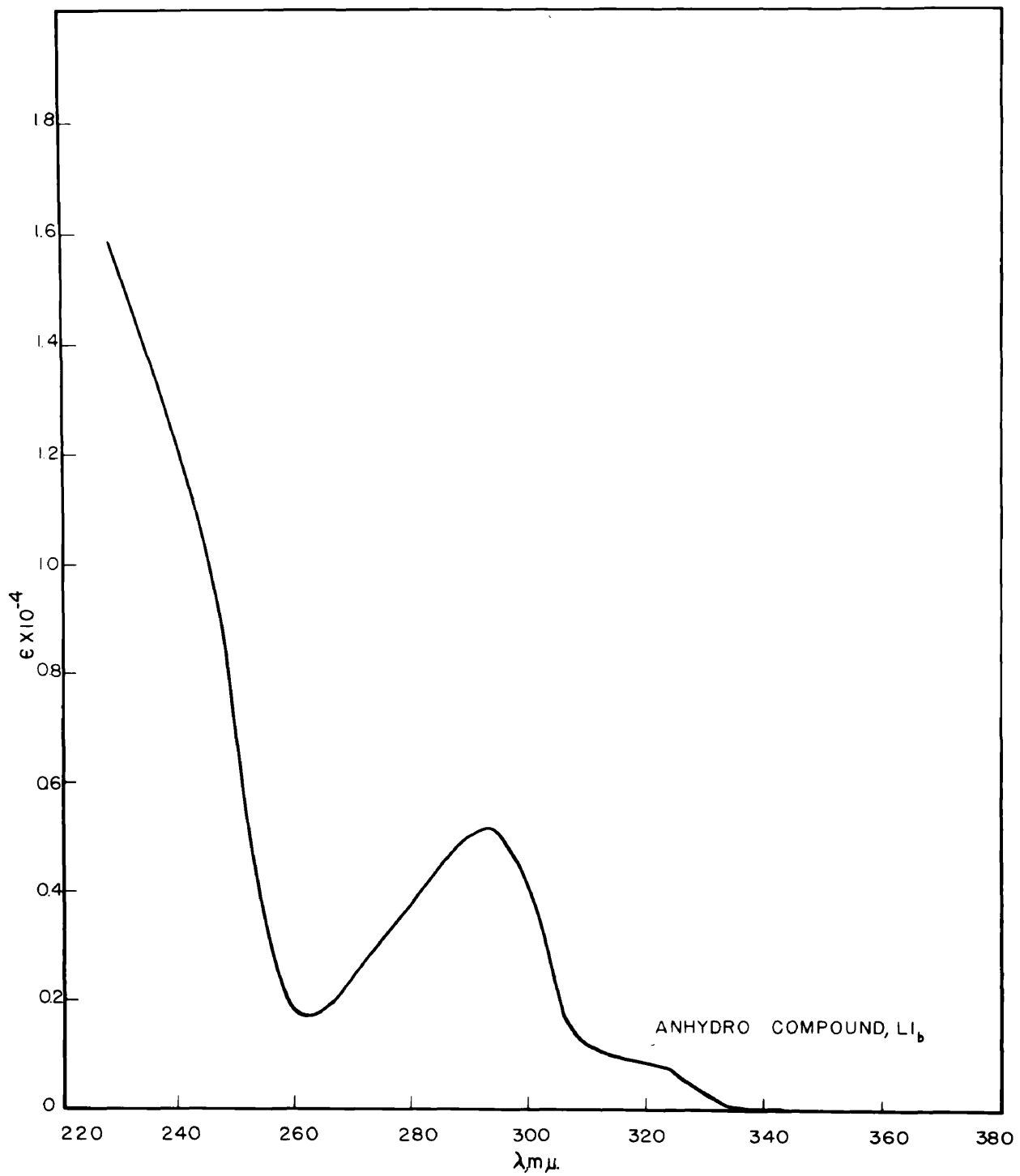


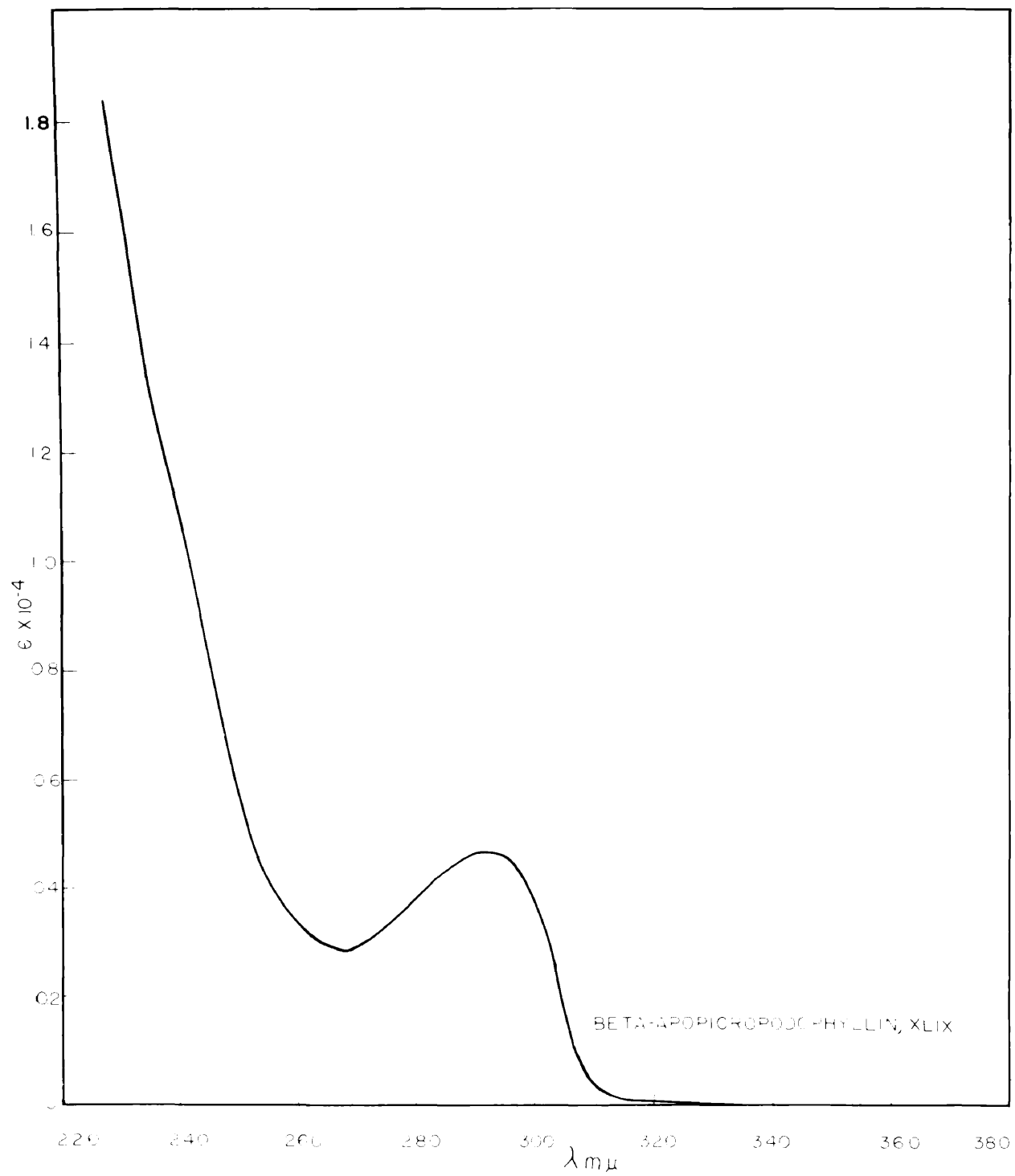


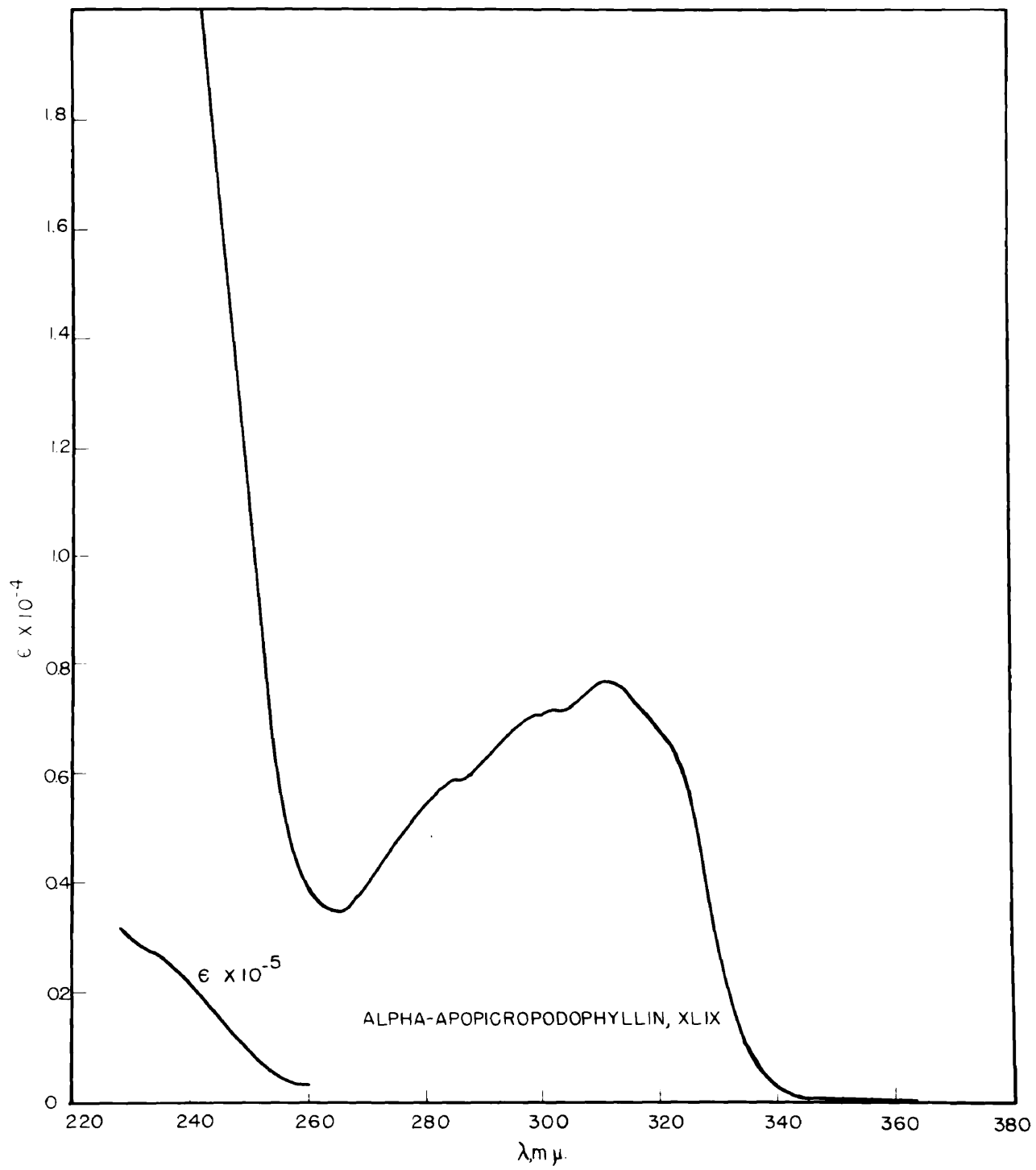


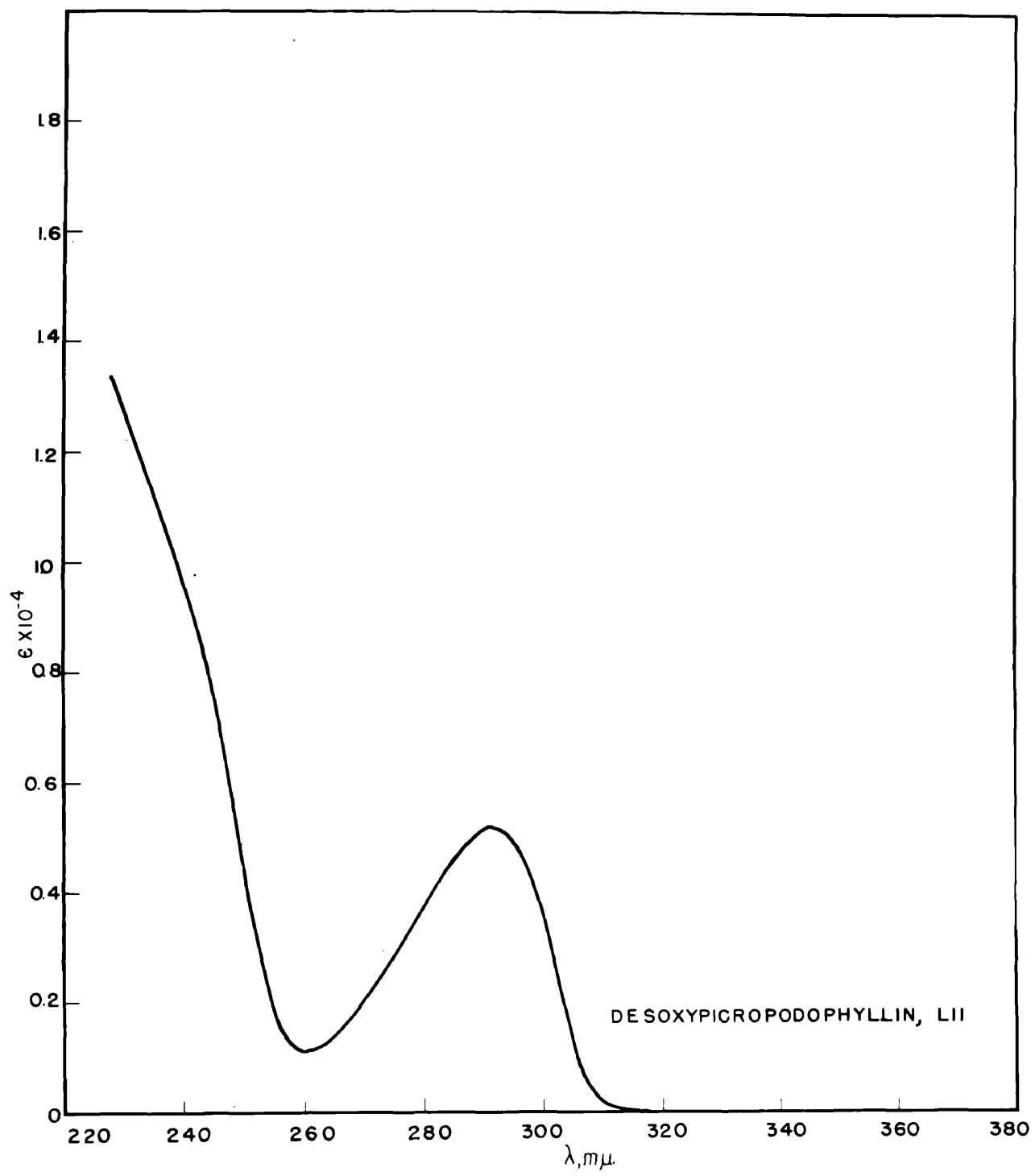


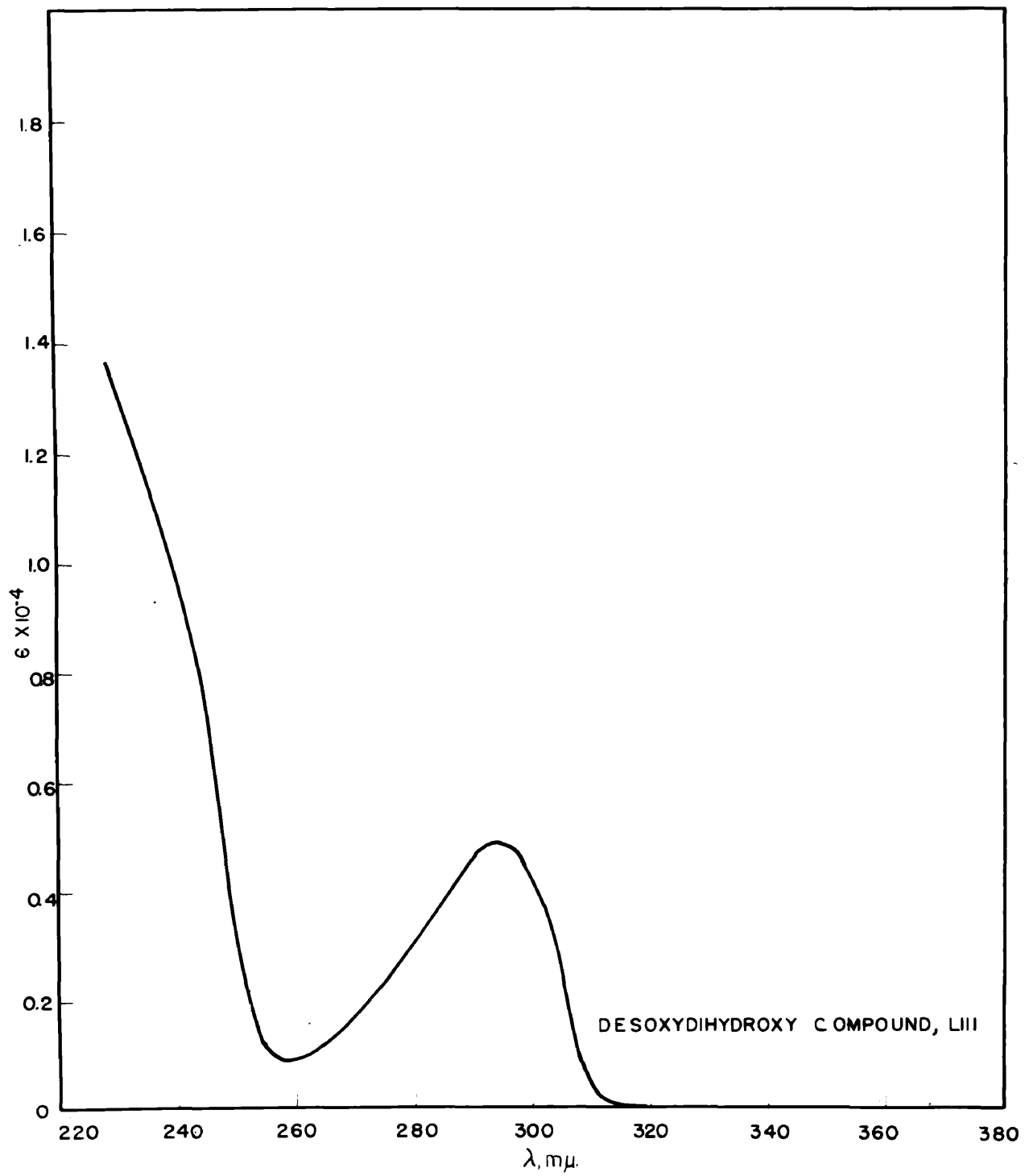


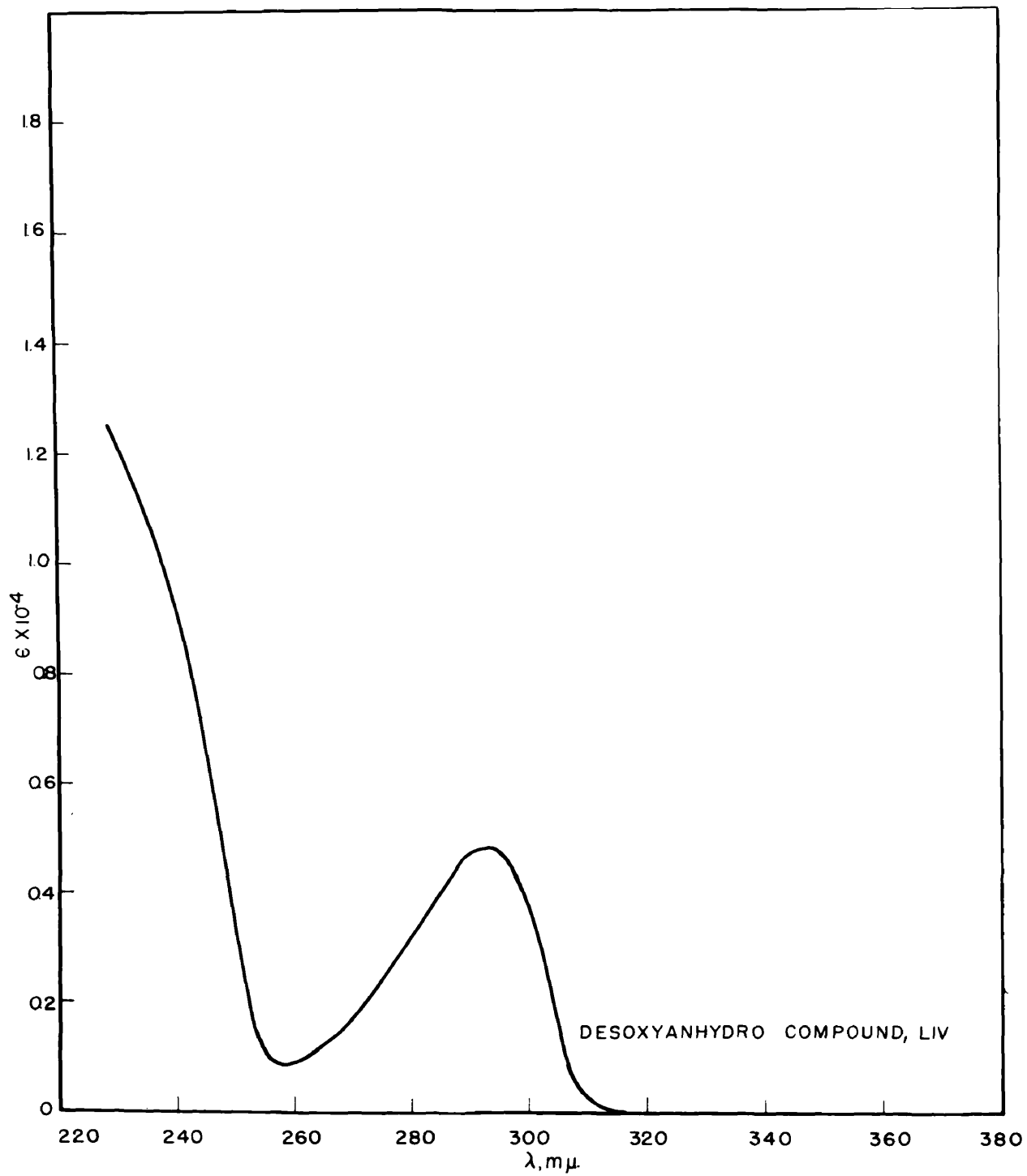






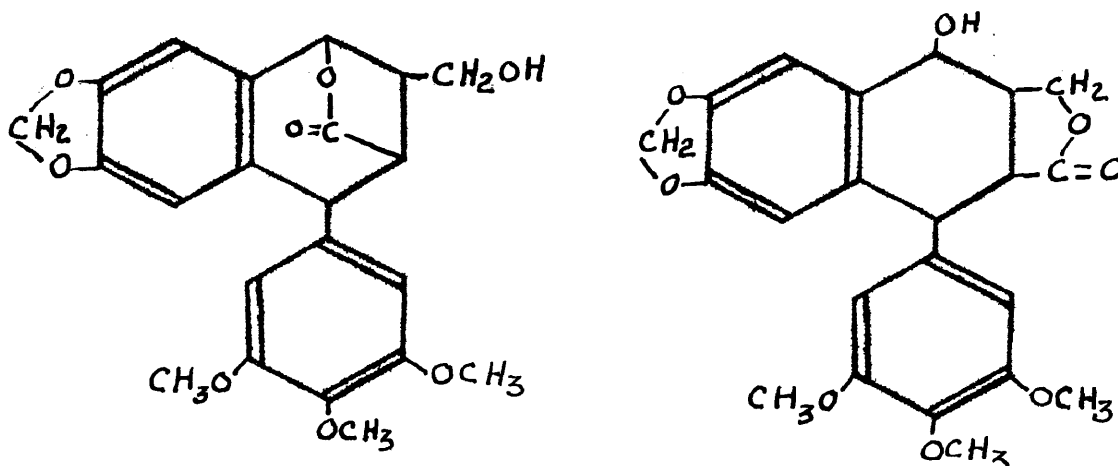






OBSERVATIONS AND CONCLUSIONS

This research has produced the first evidence that there is some change within the molecule of podophyllotoxin, XLVII, when it is transformed into picropodophyllin, XLVIII, other than merely a structural rearrangement of the lactone ring, as has been heretofore assumed.

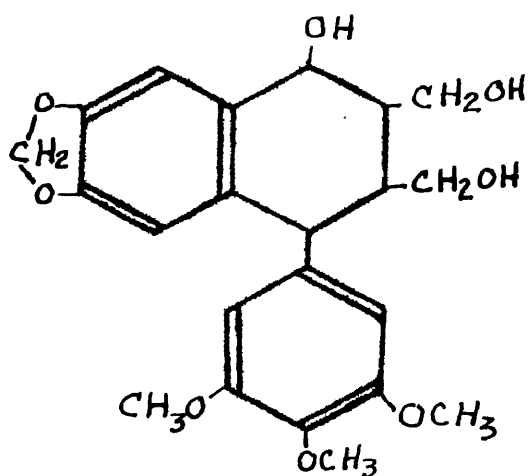
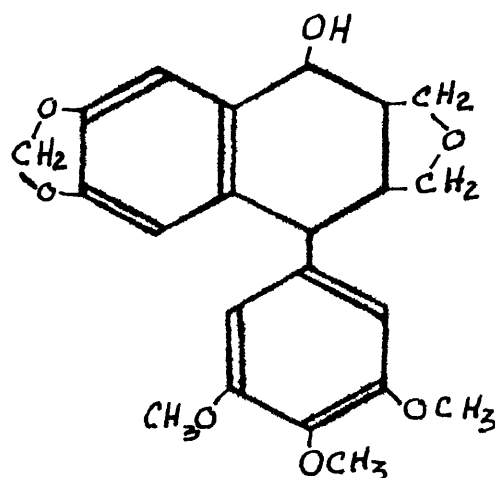


XLVII

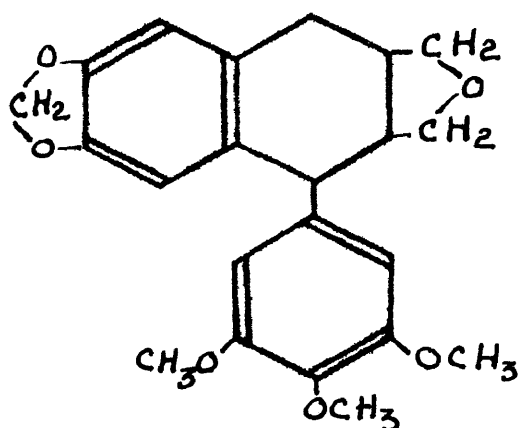
XLVIII

Indeed, in view of the pyrolysis of the benzoate of podophyllotoxin to yield beta-apopicropodophyllin, it might even be postulated that both podophyllotoxin and picropodophyllin have the same lactone arrangement.

Considering the evidence stepwise, there is the indisputable fact that the two trihydroxy compounds, L_a and L_b, derived by the lithium aluminum hydride reduction are different. They can only be different in the configuration of one or more of the carbon atoms in the tetralin ring. For convenience the carbon atoms in the tetralin ring will be referred to as 1,2,3, and 4 proceeding clockwise from top to bottom of the structure XLVII. The stereochemical difference is also born out by the two anhydro compounds, LI_a and LI_b.

I_{a,b}II_{a,b}

The structure presented for the anhydre compounds is substantiated by the following facts. (1) The desoxyanhydro compound, LIV, could be made.



LIV

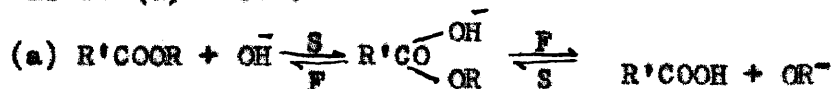
(2) If either C₁ or C₃ epimerized when podophyllotoxin was converted into micropodophyllin, then on subsequent reduction and treatment with acid, the C₁ hydroxyl could not enter into an ether formation with the C₃ hydroxymethyl group. (3) An examination of the molecular model indicated that there would be some greater degree of strain present if the ether

involved the C₁ hydroxyl, than if it were between the two hydroxymethyl groups. (4) As noted in the historical review, it was possible to prepare an anhydro derivative, compound XXVII, of isolariciresinol. It is true that a direct chemical proof of the existence of the C₁ hydroxyl is lacking; however, so is it lacking for pieropedophyllin itself. Furthermore, Vanzetti⁶¹ has stated that he was unable to prove by chemical means the position of the secondary hydroxyl group in isoolivil. He stated that his results along that line although interesting were not decisive due to the sensitivity of the derivatives to acids, which converted them to amorphous substances. By the action of thionyl chloride, Vanzetti was able to obtain a dichloride and two monochloro derivatives of isoolivil. One would assume that the chlorination reaction involves the hydroxymethyl group only.

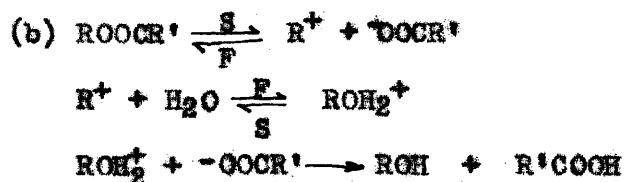
Regarding the conversion of pedophylletoxin to pieropedophyllin, it can be said that either C₁ or C₃ inverts during the conversion, because of the established differences of their respective reduction products. That C₁ or C₃ racemizes during the conversion is extremely unlikely, inasmuch as the yield from the conversion and from the reductions strongly indicate the presence of only one substance, with the possibility of a low percentage of isomers. Hence, the difference in the two trihydroxy compounds lies in either C₁ or C₃.

The conversion of pedophylletoxin to pieropedophyllin by alkali involves first the hydrolysis of an ester; assuming the structure XLVII, there are two possible points of attack for the hydrolysis. Either the alkyl-oxygen bond or the acyl-oxygen bond may be attacked, and there is evidence for the possible occurrence of either type of cleavage in a compound of this type. Normal acyl-oxygen hydrolysis is known to be one

of second order, and all proofs are consistent with a bimolecular mechanism⁶² as in (a) below.



Another possible system is available if the attack is on the alkyl oxygen bond, as is shown in (b). This system is characteristic of a first order reaction, since the first step is rate determining.

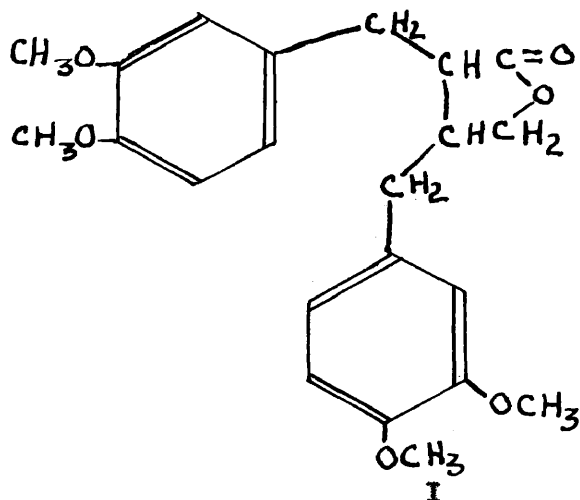


Mechanism (b) is common with esters of an alcohol which has a labile alkyl-oxygen bond. Balfe and his collaborators in a series of articles^{63,64} have investigated this mechanism using esters of optically active allyl alcohols, benzyl alcohols, and benzhydrols. The general rule was that the more activated the alcoholic hydroxyl, the greater the degree of racemization during hydrolysis, and the less concentrated the alkali, the greater the racemization. The degree, of course, varied, but an interesting comparison is afforded by his results with an ester of phenyl methyl carbinol, in which he found that even sodium carbonate gave very slight racemization. However, the optically active sodium salt of the mono-phthalate of 3,4-methylenedioxyphenyl methyl carbinol in a slight excess of sodium hydroxide containing sodium para-toluenesulfinate, yielded 45% of the racemic para-tolyl sulfone of the carbinol. In cases when the hydroxyl group was very active, it was possible to obtain the sulfone by the action of sodium para-toluenesulfinate on the alcohol.

The literature evidence leaves little to choose from in regard to the podophyllotoxin molecule. It is unfortunate that no work was performed using tetralols. However, it is of importance that the alkyl-oxygen split

is attended by racemization of the alcohol. The argument just presented still holds true; it is unlikely that racemization of the C_1 has taken place. In support of the acyl-oxygen split are the following pieces of evidence. (1) The experimental evidence presented in the research regarding the hydrolysis, with a slight excess of alkali in an alcohol-water mixture, of the benzate of the anhydro compound, LI_a , to yield quantitatively the anhydro compound and not an isomer thereof. (2) The failure of the anhydro compound of pieropodophyllin to yield a halide is convincing evidence that this hydroxyl is not like an ordinary benzyl alcohol, and (3) The established arrival at an identical sodium salt of podophyllic acid when approached from either pieropodophyllin or podophylloxin. The conclusion is then that C_1 is not epimerized or racemized, and that C_3 is epimerized when podophylloxin is converted to pieropodophyllin. Then the difference between the two trihydroxy compounds and the two anhydro compounds lies in the configuration around the C_3 atom.

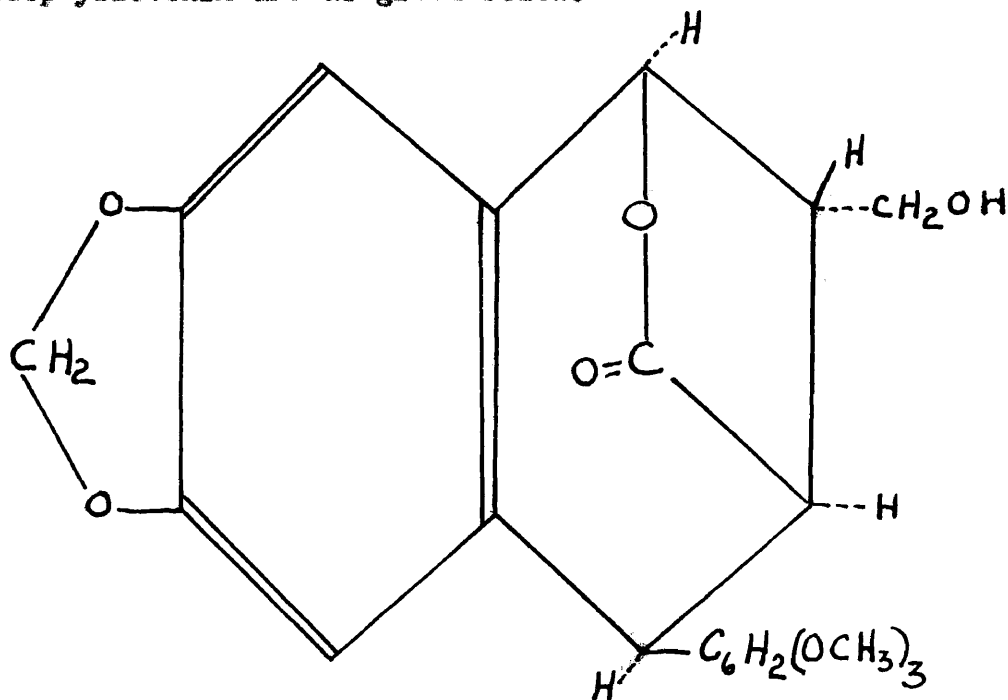
There are several analogies to this isomerization in the compounds related to podophylloxin. Haworth⁵³ has shown that l-matairesinol dimethyl ether, I, by treatment with sodium ethoxide is converted to a



mixture of hydroxy acids, which on being lactonized give rise to two isomeric substances which he called d-iso and l-matairesinol. d-iso-Matairesinol on treatment with sodium hydroxide at 180° will give some of the l-form. His conclusion was that it could only be a case of inversion around the carbon atom alpha to the carbonyl. An even closer analogy is the already cited report of Holmberg that l-conidendrin changes its rotation on treatment with alkali. In this case it was possible to prepare the isomeric free acids.

The cis and trans forms of tetralin-2,3-dicarboxylic acid have been prepared⁶⁵, and it was found that the cis form on treatment of its methyl ester with sodium methoxide gave rise to the trans ester. Surprisingly, when the anhydrides were reduced to the lactones, it was found that the lactone could be opened and closed without causing any isomerization. The investigation was then advanced to the forms possible in the 1-phenyltetralin-2,3-dicarboxylic acids. The four possible racemates were isolated and it was found the the cis (2:3) anhydride was the most stable. All possible forms of the diesters when treated with sodium hydroxide gave rise to a single racemate, which by comparison to the simple tetralin dicarboxylic acids above was judged to be C₂:C₃ trans. It was further concluded that when the esters were subjected to alkaline treatment so that a rearrangement could take place, that the repulsion of similar groups should result in a C₁-C₂ trans, C₂-C₃ trans system. This was supported by the demonstration that one form which was C₂-C₃ trans was very unstable and was probably the C₁-C₂ cis C₂-C₃ trans form. This latter work suffices to prove that the stereochemistry of a 1-phenyltetralin system is much more complicated than the simple tetralin system, and that apparently the phenyl group exerts a strong influence on the arrangement of the most stable form.

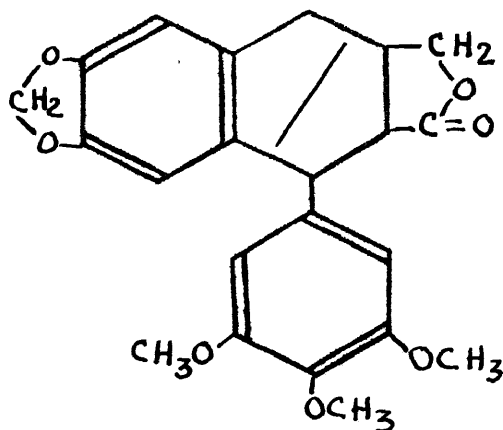
The above observations aid little in definitely establishing the configuration of carbon atoms 1,2,3, and 4 in podophyllotoxin. The starting assumption must be that the C₁ hydroxyl and the C₃ carboxyl groups are cis in podophyllotoxin. This research has shown that the C₃ atom inverts when pieropodophyllin is formed, and therefore in that molecule the C₁ hydroxyl and C₃ carboxyl are trans. The high stability of pieropodophyllin as compared to that of podophyllotoxin, and the fact that it occurs in nature also, suggests that its configuration is predominantly trans in so far as possible. Therefore, in podophyllotoxin the C₁ hydroxyl and C₄ phenyl are probably cis, a fact which would further aid the epimerization of C₃ in the conversion to pieropodophyllin because of the repulsion of the phenyl and carboxyl groups. Nothing can be determined about the configuration around C₂, but based on the repulsion of similar groups, it is likely that the C₁ hydroxyl and the C₂ hydroxymethyl are trans in both podophyllotoxin and pieropodophyllin. It appears most probable then that the arrangement of the groups in podophyllotoxin are as given below.



157887

The determination of the ultra-violet absorption spectra was of little aid in the determination of structure. It was readily apparent from the marked similarity of the curves that the carbonyl group of podophyllotoxin or pieropodophyllin was of little import in the spectra, a fact which is in agreement with a report by Erdtman⁶⁶, who compared the curves of the open chain analogs such as matairesinol, etc., to those of conidendrin and isoolivil. He also found that the open chain compounds gave a maximum at 280 millimicrons and at 230 millimicrons, but when ring closure took place to form the tetralin derivatives, the intensity of absorption increased and the maximum at 230 millimicrons was changed to an inflection point. It was not possible in the present work to detect a maximum at 230 millimicrons, but some indication of an inflection point was found. Of special interest is the similarity of the curves for podophyllotoxin and pieropodophyllin, which indicates a very close relationship in their structures.

The deviation of the curve of alpha-apopieropodophyllin from all the others is noteworthy, but it can not be interpreted beyond saying that there has been a change within the molecule which has increased the lability of the electron system which is responsible for the absorption maximum at 295 millimicrons.



At the same time there has been an appearance of fine structure to a degree which is lacking in all the other curves. An interpretation of this, on the basis that fine structure is indicative of strain within a molecule, would place the double bond of alpha-apopicropodophyllin in the C₂-C₃ position. It has been established⁵⁶ that alkali will cause the isomerization of the alpha-isomer to the beta-isomer, but that both forms yield the same acid which on heating gives only the alpha-form. On that basis it is very unlikely that the double bond of the alpha- or the beta-form is in the C₃-C₄ position, because then it would be conjugated with both the carbonyl and the phenyl ring and should be quite stable. It is probable that the double bond in the beta form is in the C₁-C₂ position, a fact which is supported by the pyrolysis of the benzoate of picropodophyllin to beta-apopicropodophyllin.

There has been presented herein a discussion of the chemistry of podophyllotoxin in an attempt to integrate the results of this research with the facts already known about the compound. In general the results of this research support very well the accepted structure for podophyllotoxin.

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