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SOME CONSTITUENTS OF ALYXIA
OLIVAEFORMIS GAUD.

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SOME CONSTITUENTS OF ALYXIA OLIVAEFORMIS

GAUD.

A DISSERTATION SUBMITTED TO THE GRADUATE SCHOOL OF THE
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DOCTOR OF PHILOSOPHY
IN CHEMISTRY
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By

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To my father and mother

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SOME CONSTITUENTS OF ALYXIA OLIVAEFORMIS GAUD.

By Guy Hiram Dority

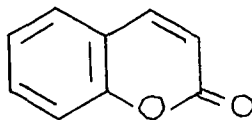
A thesis submitted to the Graduate School of the University
of Hawaii in partial fulfillment of the requirements
for the degree of Doctor of Philosophy.

ABSTRACT

The endemic Hawaiian plant Alyxia olivaeformis Gaud.
(Apocynaceae) had given an apparently satisfactory response to screening
tests for the presence of alkaloids. Since earlier studies of Hawaiian
Apocynaceae had yielded a number of new alkaloids, an exhaustive attempt
was made to isolate alkaloids from A. olivaeformis.

Repeated attempts to obtain alkaloids from this plant,
employing different isolation procedures, were unsuccessful, and it was
concluded that in A. olivaeformis alkaloids are either absent, or
present in only trace quantities. During the course of these investi-
gations, however, two non-nitrogenous compounds were obtained.

One compound was shown to be coumarin (I) by comparison with
an authentic sample.

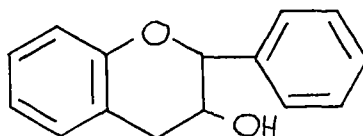


An examination of the volatile extract from fresh A. olivae-formis showed that coumarin is also the major volatile principle of this plant.

The second compound, m. p. 143-145°C., $[\alpha]_D^{27.5} +33.7^\circ$ was soluble in sodium hydroxide and in pyridine. Functional group analysis showed 11.94% methoxyl and 1.06% C-methyl. There was 1.53% active hydrogen. No uptake of hydrogen was observed under conditions which would result in reduction of olefinic centers.

Two empirical formulas were obtained from elemental analyses of the compound: $C_{16}H_{20}O_8$ from samples which were dried under mild conditions, and $C_{16}H_{18}O_7$ from those dried in vacuo at 100°C.

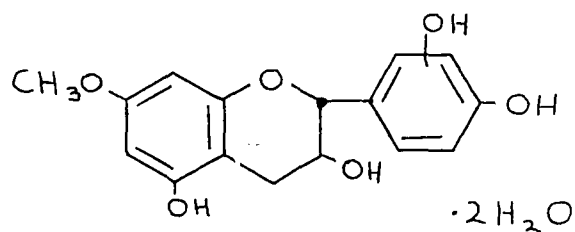
Color tests and spectral data indicated that the compound is a catechin or flavan-3-ol (II).



II

This catechin is a trihydroxymethoxyflavan-3-ol crystallizing with either one or two molecules of water of hydration, depending on the method of drying the solid.

Interpretation of spectral and rotation data and of chemical evidence allows the proposal of a tentative structure III.



III

This structure represents a naturally occurring methoxylated catechin, which is a type of compound not previously reported. The name mailein is proposed for III.

I. INTRODUCTION

A. General Background

The Hawaiian Islands, because of their remoteness from the main continental land areas of the earth, are an area containing an unusually high concentration of endemic plants. Many of these plants are distinct species found nowhere else in the world, and a few, e.g. Isodendrion or Platydesma, are endemic genera.^{1,2}

Such an endemic flora is of interest to the natural products chemist since it may be assumed that some of these plants will contain compounds which plants elsewhere have not elaborated. This was indeed found to be the case. In 1957, Gorman, et al., isolated two new alkaloids, sandwicine and sandwicensine, from the endemic Rauvolfia sandwicensis.³

Extensive investigations into the isolation of alkaloids from Hawaiian plants have been carried out by Scheuer and coworkers. Among the new alkaloids which have been reported are: holeinine,⁴ mauiensine,⁵ platydesmine,⁶ and pilokeanine.⁶ Further work in this general area is in progress.

Progress has also been achieved in the realm of non-alkaloidal constituents of Hawaiian plants. Investigation of Piper methysticum, the 'awa of the ancient Hawaiians, has been carried out by a number of investigators. This research has resulted in the identification of the sedative principle.⁷ In another study, a glycoside was isolated from Morinda citrifolia by Levand in 1962.⁸ This plant, known to the Hawaiians as noni, was used extensively as a medicinal herb and as a

source of dyes in ancient times. The isolation of a new bitter principle from Tacca leontopetaloides was reported in 1963.⁹ This plant, called pia in Hawaiian, was also used medicinally in ancient Hawaii.

From this brief sketch of recent studies of natural products derived from Hawaiian plants it may be seen that the field provides an interesting and fruitful area of research, which only lately has come under detailed investigation.

It may be instructive to consider briefly the criteria which aid in the selection of a particular plant for concentrated study. The first criterion for such a selection is the response of the plant in question to a screening program. Such a program involves the subjection of a number of different plants to a series of qualitative tests, which indicate the presence and relative abundance in the plant of naturally occurring compounds such as alkaloids, steroids, saponins, tannins and the like. Typical screening programs have been carried out by Wall, et al., for steroidal saponins,¹⁰ by Scott, et al., for alkaloids in some Solanaceae,¹¹ by Dominguez, et al., on some Mexican plants,¹² and by Scheuer, et al., on alkaloids of Hawaiian plants.^{13,14,15} The results of the screening programs provide clear evidence that certain plants may contain materials which deserve more intensive investigation.

A second criterion which often aids in the selection of a particular plant as the object of a detailed chemical investigation is the ancient folklore which is associated with the plant in question. Indigenous populations have often developed an extensive native taxonomy of the flora of their territories, together with a pharmacopoeia comprising the uses of these plants in herbal medicines. Remnants of surviving

native lore may often provide clues indicating that a plant merits scientific examination.

When a particular plant gives satisfactory response to the initial screening, the question of availability must be considered. A number of plants endemic to the Hawaiian Islands have become so rare in recent years that they are on the verge of extinction. A notable example is Gardenia remyi. Only a few authentic specimens of this tree are known to exist at this time. Obviously such a plant would be unsuitable for study, since a single collection of a size suitable for chemical work might possibly exterminate the species.

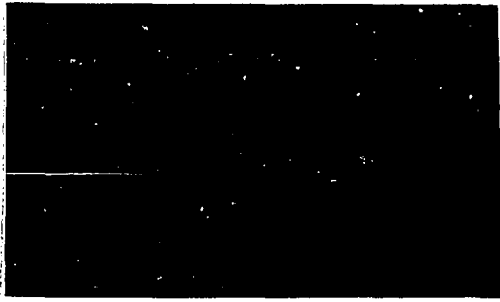
B. Statement of the Problem

The family Apocynaceae is represented in Hawaii by four genera containing endemic species: Rauvolfia, Ochrosia, Pteralyxia, and Alyxia. Investigation of the first two of these has yielded new alkaloids as mentioned in the previous section. Pteralyxia, when subjected to the screening procedure, gave preliminary tests that were not considered to be sufficient evidence that a detailed investigation would be fruitful.¹⁵ The final species, Alyxia olivaeformis Gaud., when subjected to the initial tests gave results which were sufficiently satisfactory to warrant further study.¹⁴ The plant gave positive tests for alkaloids in all parts of the plant which were tested.

The genus Alyxia consists of small trees, shrubs and vines found in tropical regions.¹⁶ Australian species were tested by Webb for alkaloidal components.^{17,18} A number of Malaysian and Indonesian species have been investigated by van Steenis¹⁹ and Bisset.^{20,21}

The species endemic to the Hawaiian Islands, Alyxia olivaeformis, is a long branching vine as may be seen from the photographs in Figure 1. It grows in tangled masses along sheltered areas of the mountain ranges of all the principal islands. It appears to be restricted to damp and cool areas. Collections for this research were made at Kokee on the Island of Kauai, in the Kawaiiki valley on the Island of Oahu, and in both the Panaewa Forest and at Malama-Ki on the Island of Hawaii. It has also been observed by the author at Pauoa Flats on Oahu, and at Kipuka Puaulu, Napau Crater, and in the Kohala mountains on Hawaii. It may be recognized by the form of its growth, its milky sap (a property of all Apocynaceae) and its fruit, which is green to blue-black and olive-shaped, from which fact the species takes its systematic name. The flowers are so small as to be completely unnoticed compared to the vast bulk of the vine forming a single plant (Figure 1).

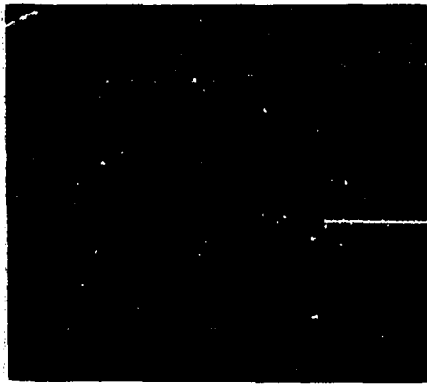
Leaf length appears to divide the species into two types. Alyxia collected on Kauai and Oahu showed an average leaf length in the mature plant of approximately 3-4 cm. A similar leaf length was observed for specimens seen by the author in the Kohala mountains, at Kipuka Puaulu, and at Napau Crater. The same plant collected in the Panaewa and Malama-Ki Forest Reserves on Hawaii, however, has a much longer leaf than have the other specimens. In these locales, the plant possesses mature leaves of 8-12 cm. in length. Alyxia from this area of the Island of Hawaii is commonly known as the fragrant maile which is popular throughout the islands. It is the only type of Alyxia in the Hawaiian Islands which possesses a distinct fragrance. This property of the plant will be discussed separately (see Chapter III-C). The question whether the



Flowers



Entire Vine



Mature Fruit

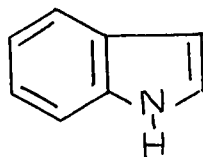


Immature Fruit and Flowers

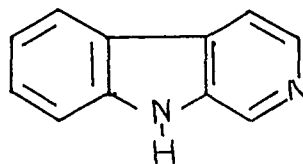
Figure 1. Photographs of Alyxia olivaeformis.

difference in leaf length in Alyxia olivaeformis is due to climatic influence, or whether it represents a variant of the plant itself, was not examined in this study. Further botanical study of this species may be necessary to resolve the problem.

The family Apocynaceae, of which Alyxia is a member genus, is rich in alkaloid-bearing plants.^{22,23} While in some families, such as Rutaceae, the isolated alkaloids belong to several structural types, in the Apocynaceae nearly all alkaloids whose structures have been determined contain an indole nucleus (I).²²



I

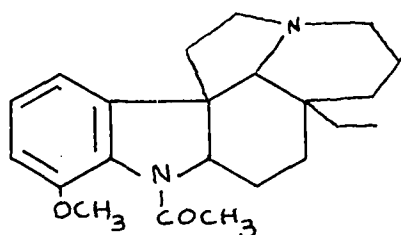


II

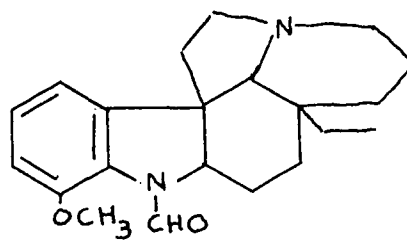
The new alkaloids which have been isolated from other members of Apocynaceae endemic to the Hawaiian Islands have been rather closely related, most of them being derived from beta-carboline (II). This would lead one to a hypothesis that plants which contain these compounds might well be closely related in a chemotaxonomic scheme. Examination of the literature, however, reveals that there is disagreement among authorities in the classification of the Apocynaceae. This is illustrated in Figures 2-4.^{24,25,26}

Alkaloids from Aspidosperma and Vallesia have a different ring structure than those derived from Ochrosia and Rauvolfia, as may be seen by comparing the structures of aspidospermine (III) and vallesine (IV) with ellipticine (V) and reserpine (VI), which have been isolated from member species of these respective genera in the order

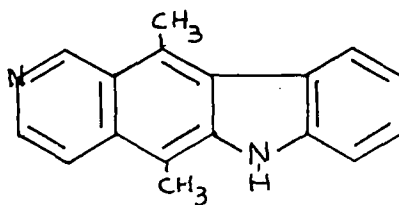
given. Alkaloids isolated from Alyxia, then, by their resemblance to



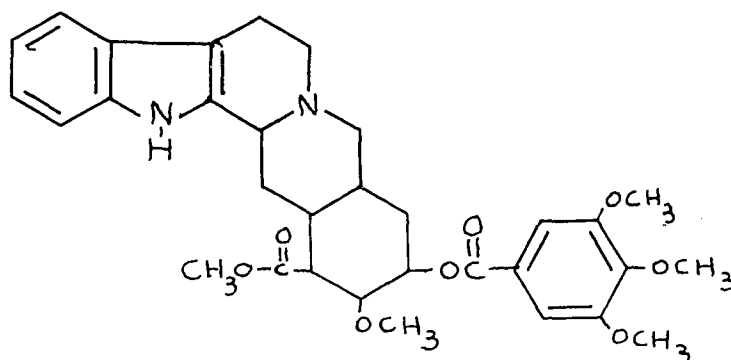
III



IV



V



VI

either group, might furnish valuable chemical evidence in favor of one of the classifications over the others.

A survey of the literature showed that three species of Alyxia, which were native to Australia,^{17,18} and four from Indonesia,^{20,21} were tested for alkaloid content. These species showed weakly positive tests in a screening program. No further attempts at characterization of alkaloidal constituents from Alyxia species seem to have been made.

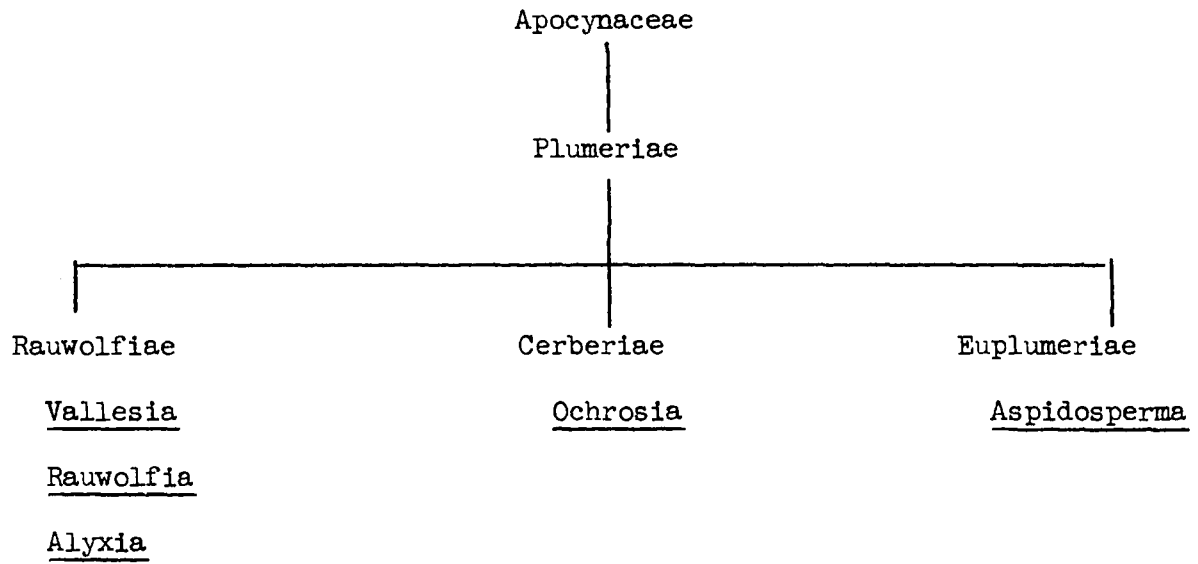


Figure 2. Classification of some Apocynaceae after Bentham and Hooker.²⁴

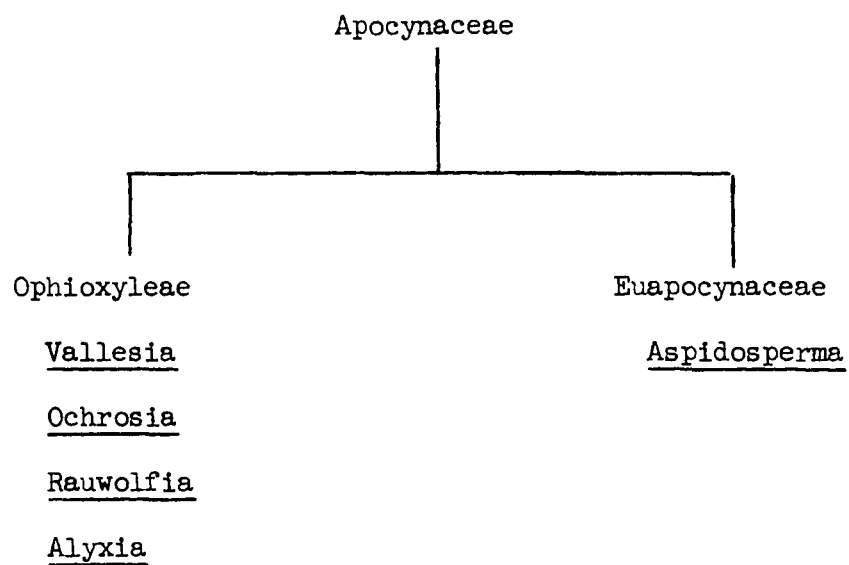


Figure 3. Classification of some Apocynaceae after Endlicher.²⁵

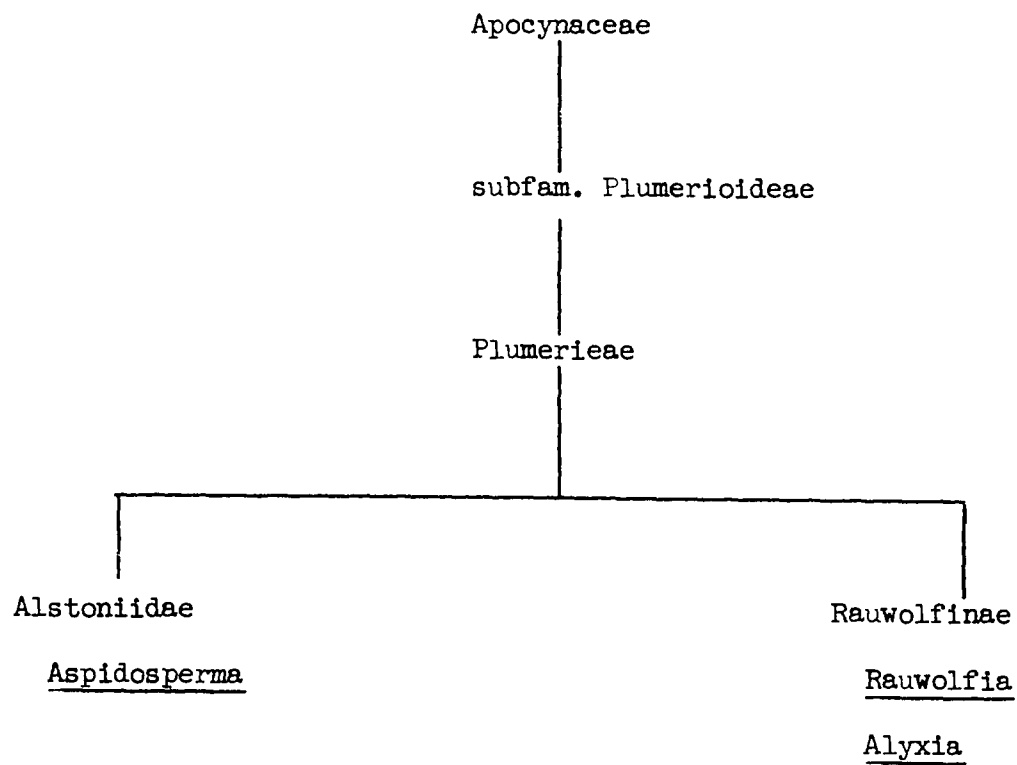


Figure 4. Classification of some Apocynaceae after Engler.²⁶

The original series of screening tests on Alyxia olivaeformis seemed to indicate the presence of alkaloids. Positive tests were reported with all the standard alkaloid reagents.¹⁴ As a result, an exhaustive search for alkaloidal components in Alyxia olivaeformis was undertaken.

II. EXPERIMENTAL

A. General Information

All melting points were determined on a Fisher hot-stage and are uncorrected. Infrared spectra were measured on a Beckman IR-5 Infrared Spectrophotometer as potassium bromide discs containing 2 mg. of the compound in question.

Ultraviolet spectra of D-1 and the catechin samples were determined using a Beckman DB spectrophotometer, equipped with a recorder. Those of D-2 were determined on a Beckman DK-2 Ratio Recording Spectrophotometer.

NMR spectra were obtained using a Varian A-60 Nuclear Magnetic Resonance Spectrometer.

B. Procurement of Plant Material

Collections of Alyxia olivaeformis were made at Kokee on the Island of Kauai, along the Kawaiiki Ditch Trail on the Island of Oahu, and in the Panaewa and Malama-Ki Forest Reserves on the Island of Hawaii. The entire plant was collected in all cases since separate extraction of plant parts is cumbersome when one deals with a vine. The plant material was dried in a forced-draft oven at 60° C. After the material was dried, which was usually accomplished in 48 hours, the entire quantity of plant material from each collection was ground in a Wiley mill to pass a 2 mm. screen. In this manner, a total of 82 kg. of dried maile was collected and processed. This information, together with quantitative data, is summarized in Table I.

For the investigation of the volatile constituents of Alyxia

TABLE I
SUMMARY OF PLANT EXTRACTIONS

Collection	Location	Amounts of Dried <u>Alyxia</u> kg.	Results
1	Kokee, Kauai	3.9	Pilot extraction
2	Kawaiiki, Oahu	10.0	Lost
3	Panaewa, Hawaii	10.6	<u>D-1</u> , 70.6 mg. ($6.83 \times 10^{-4}\%$)
4	Kawaiiki, Oahu	3.0	Pilot extraction
5	Kawaiiki, Oahu	8.1	<u>D-2</u> , 311 mg. ($3.84 \times 10^{-3}\%$)
6	Kawaiiki, Oahu	5.9	Pilot extraction (aqueous)
7	Kawaiiki, Oahu	7.4	Complexes attempted
8	Kawaiiki, Oahu	14.9	<u>D-2</u> , 120 mg. ($8.06 \times 10^{-4}\%$)
9	Malama-Ki, Hawaii	18.4	<u>D-2</u> , 20 mg. ($1.09 \times 10^{-4}\%$)

olivaeformis, the plant material was obtained in the form of 2 kg. of fresh maile leis at the Hilo airport on the Island of Hawaii.

C. Extraction Procedures

A general extraction procedure for the dried plant material is shown in the form of a flow sheet (Figure 5). The material was exhaustively extracted with petroleum ether (boiling range 40-60° C.) in a large Soxhlet extractor in order to remove waxes and other fatty components; this was followed by a second extraction with boiling 95% ethanol.

1. The Petroleum Ether Extract

The petroleum ether extract, which was colored green, was examined for the presence of alkaloids, but gave no reaction with any of the customary alkaloid test reagents (Mayer's reagent, Dragendorff's reagent and silicotungstic acid). Upon concentration of the solvent, a cream-colored waxy solid separated. After 16 g. of this wax was isolated no further investigation was carried out on the petroleum ether fraction. In subsequent extractions this fraction was discarded after recovery of the solvent.

2. The Ethanol Extract

The ethanol extract, when tested for the presence of alkaloids, gave seemingly positive reactions. The solution turned turbid when a drop of test reagent was added to a few drops of the acid soluble portion of the extract.

All batches except number 6 were extracted with ethanol in a similar fashion. The plant material which had been extracted with

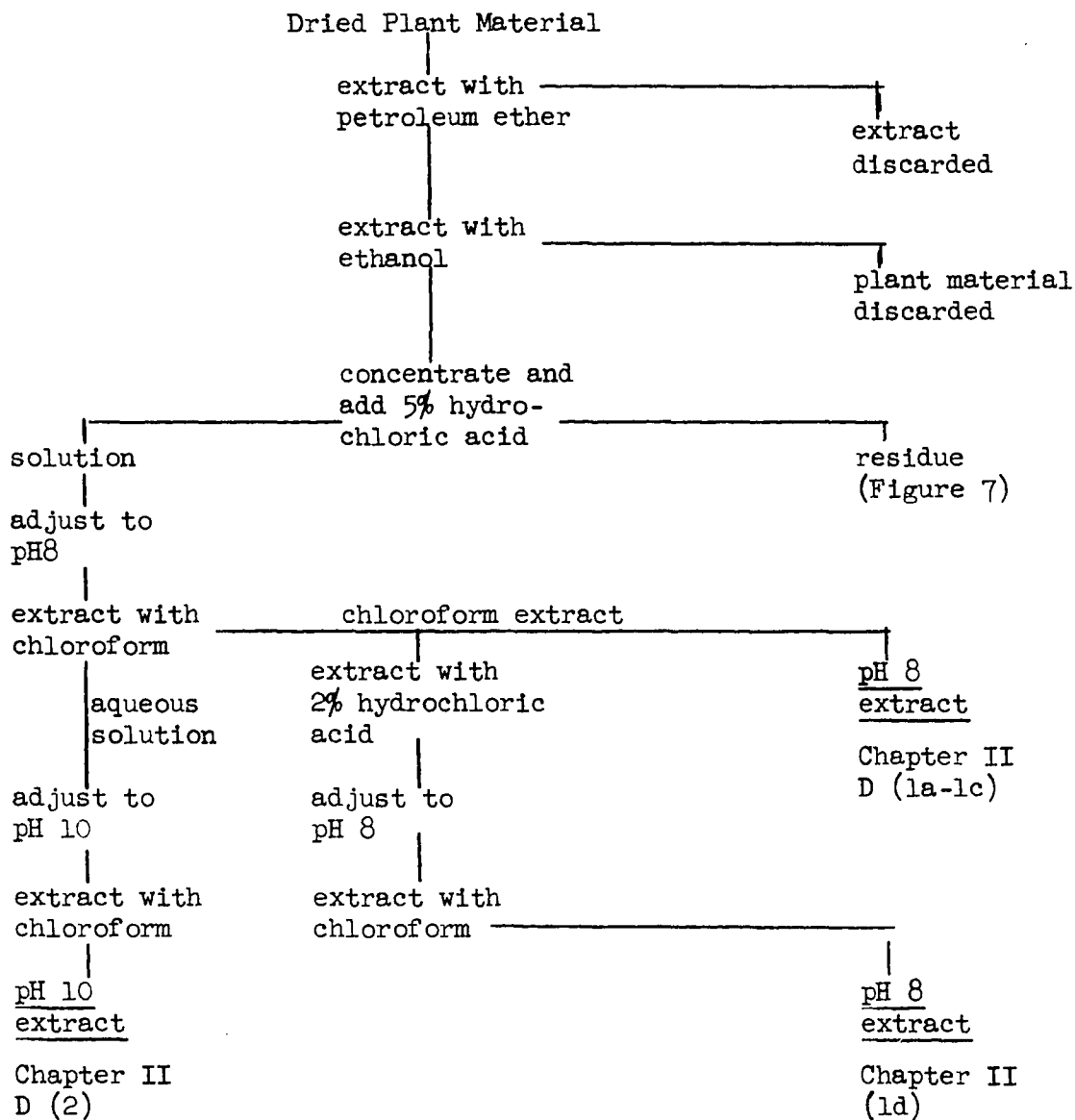


Figure 5. Flowsheet for general extraction procedure.

petroleum ether was re-extracted with ethanol until no evidence of turbidity could be observed upon testing a few drops of the solution in the extraction apparatus with alkaloid test reagents. A time of 36 hours was sufficient for each extraction charge. The ethanol extract was then concentrated and the solvent was recovered. The concentrate, a thick syrup, was then treated with 5% hydrochloric acid solution. The syrup dissolved very slowly in the acid with deposition of a voluminous green solid. This solid could be filtered only with difficulty; upon addition of more acid to the filtrate, further amounts of solid precipitated. This process of filtration and acidification was continued until no further amounts of solid formed, at which point the acid-soluble portion of the ethanol extract was employed in further extraction studies. The green solid was discarded in all cases except batch number 9. A discussion of this particular extract will be furnished in section E of this chapter.

3. Extraction of the Acid-Soluble Portion

In the work-up of batch number 1 the acidic solution obtained as described above was extracted with chloroform at successive pH values of 2, 4, 8, and 10. After each extraction the pH was raised by the addition of aqueous ammonia. Tests with Mayer's reagent of the chloroform solutions indicated that alkaloid-positive material was being extracted at pH values of 8 and 10, with the virtual absence of such material in the extracts from acidic media. The chloroform extracts were dried over anhydrous magnesium sulfate and evaporated in a rotary evaporator. In all cases the residue was a red-brown gum. In all subsequent runs extractions were made only at pH 8 and pH 10. From

batch number 3, 5.7 g. ($5.37 \times 10^{-2}\%$) of residue was recovered at pH 8 and 3.0 g. ($2.83 \times 10^{-2}\%$) at pH 10.

D. Chromatographic Studies

1. The pH 8 Extract

a. Florisil

A portion of the pH 8 extract (2.8 g.) was dissolved in 10 ml. of dry chloroform and placed on a 12 x 1.5 cm. column of Florisil. Using chloroform as the eluant, 10 x 25 ml. fractions were collected. The eluant was then changed to chloroform-methanol (50:50 v/v) and 16 fractions were taken. All fractions were tested with Mayer's reagent. The Mayer-positive fractions 2-5 were combined, dried and evaporated yielding 2.3 g. of a yellow residue. The residue was treated with 5% hydrochloric acid and filtered. The pale yellow filtrate was made basic with sodium carbonate to a pH of 8. This solution was then re-extracted with chloroform. The resulting extract was dried and concentrated, yielding 0.3 g. of a pale yellow oil.

This oil was dissolved in 10 ml. of dry chloroform and passed over a Florisil column as previously described. The initial fraction upon evaporation yielded white crystals which dissolved in 5% hydrochloric acid and gave a turbidity when tested with Mayer's reagent. The second fraction also yielded a trace of crystalline material. The crystals from both fractions had identical melting points of $64-66.5^{\circ}\text{C}.$, and were therefore combined, a total of 70.6 mg. A melting point determination of the mixture showed no depression. This substance was given the code D-1. No other fractions in this separation yielded any material which

TABLE II
CHROMATOGRAPHY OF THE pH 8 EXTRACT ON FLORISIL

<u>Fraction</u>	<u>Eluant</u>	<u>Appearance</u>	<u>Mayer's Test</u>
1	chloroform	Colorless	—
2	"	"	+
3	"	Pale Yellow	+
4	"	" "	+
5	"	" "	+
6	"	Colorless	tr.
7	"	Pale Yellow	tr.
8	"	" "	tr.
9	"	Colorless	tr.
10	chloroform- methanol (50:50 v/v)	Red-brown	tr.
11	"	Lt. red-brown	tr.
12	"	Colorless	—
13	"	"	—
14	"	"	—
15	"	"	—
16	"	"	—
17	"	"	—
18	"	"	—
19	"	"	—
20-26	"	"	—

was either crystalline or responsive to Mayer's reagent.

b. Thin Layer Chromatography

Following the chromatographic separation on Florisil of the first portion of the pH 8 extract, an examination of this extract by thin layer chromatography was carried out. The adsorbents employed were alumina G and silica gel G; chloroform, benzene, and methyl cellosolve were employed as eluants, for a total of six systems. The pH 8 extract was run along with a sample of the crystalline material obtained from the Florisil column, and the plates were developed by spraying them with a solution of modified Dragendorff's reagent.²⁷ The crystalline material appeared on the developed plates as a blue-black spot. Several other components seemed to be present as indicated by other spots. The system which appeared to give the best separation of these spots was that using alumina as the adsorbent and benzene as the eluant. The appearance of this plate is shown on Figure 6.

c. Alumina

As a result of the thin layer chromatography a second chromatographic separation was attempted. In this experiment, benzene was selected as the eluant and basic alumina (Woelm) was the adsorbent. Three gram of the pH 8 extract was treated with 25 ml. of dry benzene. The extract was not completely soluble in benzene, but the resulting yellow solution was placed on a column 1.5 cm. in diameter containing 25 g. of alumina. A total of 57 x 25 ml. fractions were collected as illustrated in Table III.

The first fraction exhibited a brilliant blue fluorescence; it was evaporated to dryness and yielded a pale yellow gum which was

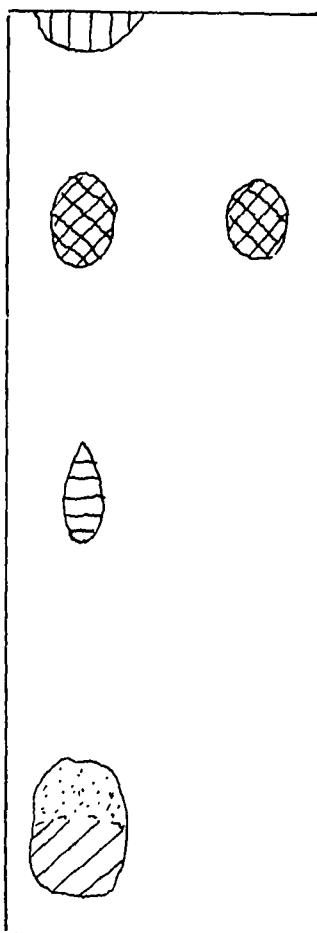


Figure 6. Thin Layer Chromatogram of the pH 8 Extract as Compared with D-1.

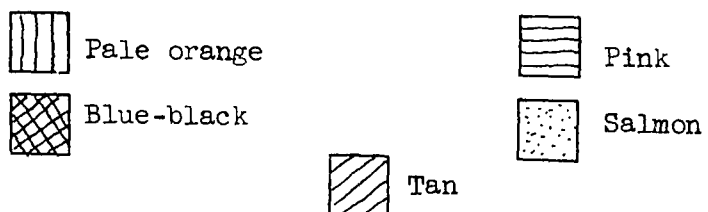


TABLE III
CHROMATOGRAPHY OF THE pH 8 RESIDUE ON ALUMINA
ELUTED WITH BENZENE

<u>Fraction</u>	<u>Appearance</u>	<u>Comments</u>
1	Blue fluorescence	Non-polar gum
2-26	Colorless	No residue
27-29	Colorless	Crystals, m.p. 64-66°C.
30-31	Colorless	No residue
32-48	Pale yellow	Trace of residue, + Mayer's*
49-57	Colorless	No residue
58	Colorless	Trace of residue + Mayer's*

* + Mayer's indicates development of turbidity

insoluble in aqueous systems, but soluble in non-polar solvents. Fractions 27-29 upon evaporation left a crystalline material which was shown by melting point studies to be identical with the crystalline product D-1 obtained from fractions 2-5 of the Florisil separation scheme. A total of 193 mg. of this solid was obtained in this manner. Fractions 32-48 were combined and evaporated, yielding a small amount of yellow oil. Evaporation of the remaining fractions resulted in no significant products, with the exception of Number 58 which yielded a small quantity of oily residue similar in appearance to that found in 32-48. These oils gave turbidity with Mayer's reagent, but were present in quantities too small for further purification.

d. Silica Gel G

(1) Pilot Run

An extraction series, using petroleum ether and ethanol, was performed upon Collection Number 5 in the same manner as the general scheme described above. A single modification, however, was made in the procedure. It had been observed that D-1 was not soluble in 2% hydrochloric acid but was slightly soluble in 5% hydrochloric acid. Accordingly, the ethanolic extract was evaporated, treated with acid, adjusted to pH 8 and extracted with chloroform as in the first procedure. The chloroform extract was then repeatedly shaken with portions of 2% hydrochloric acid until no evidence of yellow color appeared in the acidic solution. This acidic solution was then made basic to pH 8 by the addition of solid sodium carbonate and re-extracted with chloroform. The chloroform extract, upon drying and evaporation, yielded 15.3 g. of a pale yellow solid which gave turbidity when tested with Mayer's reagent.

Thin layer chromatography of this solid using various eluants and adsorbents indicated that the best separation was achieved with a 93:7 mixture of chloroform-methanol as the eluant and silica gel as the adsorbent. A column, 40 x 4 cm., was packed with silica gel G and a solution of 1 g. of the solid dissolved in 5 ml. of the eluant was placed on the top of the column. A total of 132 fractions of 25 ml. each were collected. Each fraction was dried over anhydrous magnesium sulfate and evaporated under vacuum.

Examination of the fractions showed a yellow oil in fractions 10-14, similar in appearance to that obtained in the separation scheme in which alumina was used. No significant residual material was noticed in fractions 15-73. Fractions 73-108 contained a white, glassy solid, which gave turbidity with Mayer's reagent. Combination of fractions 73-108 yielded 0.3 g. of a pale cream-colored solid. No further residual material was obtained in the additional fractions, and chromatography was discontinued after collection of fraction 132.

The solid obtained from fractions 73-108 was recrystallized from water, yielding 14 mg. of white flaky material melting at 143-145° C. Upon careful examination of the flakes, the material was observed to consist of clumps of tiny needles. Turbidity was observed with Mayer's reagent, Dragendorff's reagent, and silicotungstic acid. This solid was coded D-2. When tested by thin layer chromatography, using the same conditions as in the column, D-2 showed an approximate R_f -value of 0.05-0.1. The spot produced by D-2 was a very pale tan color when developed by the Dragendorff's reagent spray, but was a darker tan when the plate was sprayed with a chloroform solution of anhydrous

iron (III) chloride.²⁸

(2) Preparative Separation

A second separation was carried out, using 10 g. of the crude solid material obtained from the modified extraction scheme described in the previous section. Two hundred and forty fractions of 30 ml. each were collected. It was found by thin layer chromatographic studies that fractions 153-240 contained D-2. Accordingly, these fractions were combined and evaporated as before. Recrystallization of the residue yielded 0.2 g. of product, identical with the first sample of D-2.

Thin layer chromatography, using the same adsorbent and eluant as employed in the column, indicated that the other fractions from this separation contained material which should be investigated further. On the evidence of the thin layer chromatograms these fractions were combined as illustrated in Table IV.

The fractions in each grouping possessed a single major component of similar R_f -value on thin layer plates. This was indicated by the presence of a single predominant spot which appeared when the chromatogram was developed with the Dragendorff's reagent spray. The grouped fractions were all yellow-brown syrups, possessing pleasant caramel-vanilla odors. Group 2 appeared to possess the largest amount of material and was therefore selected first for further investigation. It was dissolved in 2 ml. of dry chloroform and run on a 12 x 1.5 cm. column of silica gel G. Fractions of 5 ml. each were collected. A narrow yellow band soon traveled the length of the column and was collected in fractions 9-13, with the largest quantity of residue in fraction 12. The total weight of the residue from these five fractions

TABLE IV
FRACTIONS FROM CHROMATOGRAPHY OF pH 8 EXTRACT ON
SILICA GEL G AND CHLOROFORM/METHANOL (93:7) AS ELUANT

<u>Group</u>	<u>Fractions</u>	<u>R_F (approx.)</u>
1	1 - 29	0.9
2	30 - 37	0.8
3	38 - 49	0.7
4	50 - 57	0.5
5	58 - 63	0.45
6	64 - 83	0.4
7	84 - 102	0.3
8	103 - 125	0.2
9	126 - 152	0.15
10	153 - 240 (<u>D-2</u>)	0.1

was 6.4 mg. Similar separation schemes were carried out for groups 3 and 4 of the original preparative run, with similar results, namely, the separation of several milligrams of yellow syrup as the principal component of each group.

All of these syrups gave turbidity with Mayer's reagent, but all attempts to induce them to crystallize were fruitless. One portion of the syrup from group 2 yielded a very few tiny brown needles on prolonged chilling, but these could not be separated, and no additional material was observed to crystallize in this sample. The use of solvent pairs such as chloroform:hexane mixtures was attempted without success.

Because of the unfavorable responses of groups 2, 3, and 4 of the preparative separation attempt to further purification by secondary chromatographic separation, it was felt that additional operations of this type would be fruitless. Accordingly, no further attempts were made to separate the remaining groups of fractions by chromatography. Subsequent investigations of these groups will be discussed in Part E of this chapter.

2. The pH 10 Extract

The pH 10 extract derived from batch Number 3 was treated in a fashion which was analogous to the work-up of the pH 8 extract of batch Number 3. No crystalline material corresponding to D-1 or D-2 was obtained. No further experiments were performed on this extract, as comparison of the thin layer chromatograms of the two extracts showed them to have qualitatively similar compositions.

E. Attempted Separation of Alkaloidal Components by Complex Formation

1. The pH 8 Extract

An attempt was made to prepare a picrate of the Group 9 fractions listed in Table IV. The material was dissolved in 5 ml. of absolute methanol and 2 ml. of a saturated solution of picric acid in methanol was added. The mixture was concentrated to approximately 4-5 ml. and allowed to cool. No trace of crystalline material was observed in the solution. Further concentration of the solution finally gave yellow crystals together with droplets of tarry material. The crystals, on separation and purification were shown by melting point comparison and infrared spectroscopy to be picric acid.

Following the method of Battersby,²⁹ an attempt was made to prepare a Reinecke complex of Group 8. Again, no crystalline material could be isolated. No further work was done on these fractions.

2. Batch Number 8

This batch was extracted according to the flow sheet shown in Figure 7. After the ethanolic extract of the plant material was treated with 5% hydrochloric acid, a 25 ml. aliquot of the acidic phase was treated with a solution of Reinecke salt following the method of Battersby.²⁹ Turbidity developed immediately, but the precipitate turned out to be a tarry mass which could not be crystallized from the customary acetone-water system used for alkaloid reineckates.³⁰ A second experiment in which the acidic system was saturated with sodium chloride and allowed to stand for two hours before the reagent was added, produced a similar result.

Two other reagents were employed on 25 ml. aliquots of the acidic extract. These were perchloric acid,⁴ and chloroplatinic acid.³¹ In each case a crystalline solid was obtained. The solid resulting from perchloric acid treatment was white, insoluble in water and dilute acids. Its melting point was very high (above 360° C.). Upon examination, the infrared spectrum appeared to be far too simple to be the spectrum of an organic compound. Comparison with known spectra showed the substance to be potassium perchlorate.

Since it is known that both potassium perchlorate and potassium hexachloroplatinate are highly insoluble in water, it was then felt that the solid obtained from the reaction with chloroplatinic acid was in fact potassium hexachloroplatinate. This hypothesis was sustained when a single crystal of the substance, upon ignition in a Bunsen flame, showed a strong potassium flame color and was not observed to char. No further attempts were made to isolate derivatives from Batch Number 8.

F. Ethylene Chloride Extraction

Following the attempts to separate alkaloid complexes from the acidic extract of Batch Number 8, the extract was made basic and extracted with chloroform according to procedures which have been described previously. Upon conclusion of the chloroform extraction the aqueous solution was extracted with ethylene chloride until no more colored material was visible in the solvent.

The ethylene chloride extract was dried and evaporated, yielding 3.75 g. of a white solid, which was contaminated with green coagulations. This solid was examined for the presence of D-2 by thin

layer chromatography, using silica gel G as the adsorbent and a 93:7 mixture of chloroform-methanol as the eluant. The plate, upon development with a spray containing iron (III) chloride in chloroform,²⁸ indicated that D-2 was indeed present in the solid. Accordingly, the solid was run on a silica gel G column in the same manner as described above. Additional D-2 (120 mg.) was obtained in this fashion.

G. The Alkali-Soluble Portion

Figure 7 shows that batch number 8, as had earlier collections, led to acid-insoluble material when the initial ethanolic plant extract was treated with dilute acid. The acid-soluble portion was treated according to the procedures described above and led to the isolation of D-1 and D-2. Attention was now turned to that portion of the ethanolic extract which did not dissolve in dilute acid, and which had been discarded in previous runs.

This material was a greenish-brown amorphous solid, which could be removed from the acidic solution by very slow filtration. The weight of this solid from batch number 8 was not determined as the material was wet. Drying the solid was not attempted as previous observations of similar solids from other collections indicated that drying caused decomposition as evidenced by the appearance of a black coloration. The solid from batch number 8, after washing with water by repeated trituration in a large mortar until the wash liquid was no longer acidic to Hydrion paper, was treated with 6N ammonia solution in which it was almost entirely soluble. A total of 10 l. of dark yellow-brown liquid was obtained. A small quantity of the alkaline solution

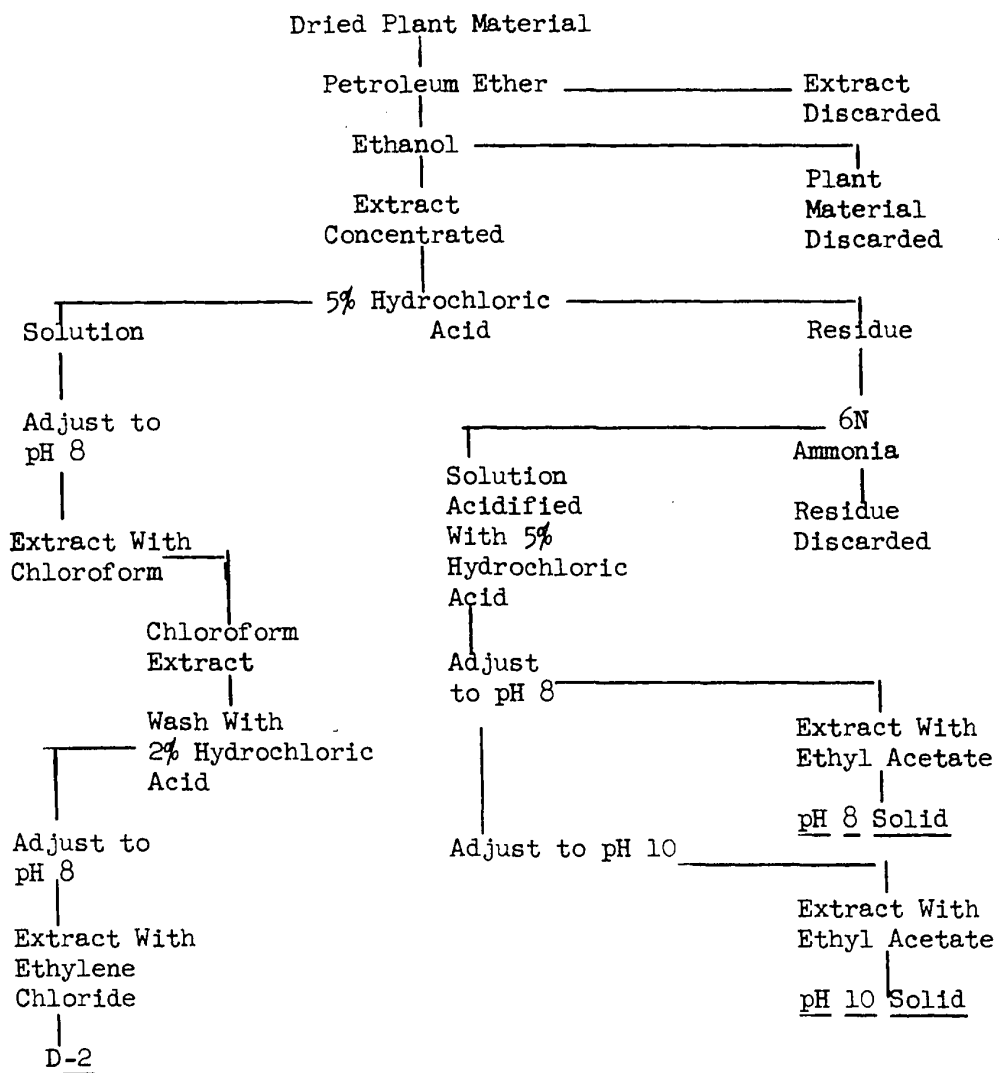


Figure 7. Flowsheet for Extraction of Batch Number 8.

was slowly acidified with dilute hydrochloric acid. A voluminous greenish-brown solid separated. Centrifugation yielded a golden-yellow supernatant liquid which gave a positive test with Mayer's reagent. This test was quite different from the turbidity which was obtained in tests with D-1 and D-2 since it showed definite evidence of coagulation into a solid on standing for five minutes. Previous tests on standing for this period of time gave no solid but rather droplets of an oily scum.

Attempts were made to obtain alkaloid complexes directly from 25 ml. samples of alkaline solution after acidification following the procedures discussed earlier. The complexing reagents employed in this series of experiments were: Reinecke salt, chloroplatinic acid, and a saturated aqueous solution of picric acid. As were the cases earlier, the results were unsatisfactory. Accordingly, extraction procedures were attempted.

H. Extraction of the Alkali-Soluble Portion

Selection of a suitable solvent and pH conditions for extraction of the alkali-soluble portion was carried out in a manner analogous to that employed for the acid-soluble portions. For extraction of 25 ml. samples of the alkaline solution which had been previously acidified and centrifuged, pH values of 2, 6, 8, and 10 were chosen. The solvents chosen for extraction purposes were benzene, chloroform, ethyl acetate, and ethylene chloride. The 16 extracts thus obtained were studied with alkaloid test reagents. Responses of the extracts to these tests showed that optimum results were obtained by the use of ethyl acetate

at a pH of 8. This extract gave a positive test with Mayer's reagent, Sonnenschein's reagent, and silicotungstic acid.

A 2 1. quantity of the original alkaline solution was therefore acidified, filtered, adjusted to pH 8 with sodium carbonate, and continuously extracted with ethyl acetate. After all appearance of color had ceased in the non-aqueous layer of the extraction system (48 hours), the ethyl acetate solution was removed, and the pH of the aqueous solution was raised to 10 by the addition of aqueous ammonia. This material was then re-extracted with ethyl acetate for one day. Upon concentrating and chilling these extracts, a quantity of brown solid was obtained from each. Removal of the solids by centrifugation and concentration of the mother liquors yielded additional quantities of the solids. The final amounts of the solids obtained in this manner were 643 mg. from the pH 8 extraction and 82 mg. from the pH 10 extraction.

I. The pH 8 Solid

1. Chromatography

Thin layer chromatography of both solids was carried out. An illustration of the thin layer chromatogram of the pH 8 solid on an alumina-coated plate, using a 3:1 chloroform-methanol mixture as the eluant, is shown on Figure 8. The plate was developed with modified Dragendorff's spray reagent. The spots indicated by the solid lines are those found in the first portion of pH 8 solid. That given by the broken line indicates the only spot found in later portions of the pH 8 solid. A thin layer chromatogram of the pH 10 solid run under similar

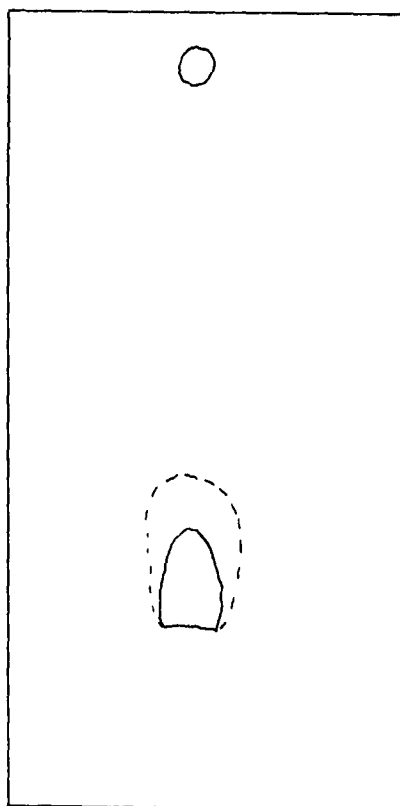


Figure 8. Thin Layer Chromatogram of the pH 8 Solids.

Solid Line----First crop
Broken line---Later crops

conditions showed no spots.

Two hundred and fifty milligram of the pH 8 solid was dissolved in a minimum amount of 3:1 chloroform-methanol mixture. This solution was placed on top of a column 1 cm. in diameter, containing 25 g. neutral alumina (Woelm). Thirty-five fractions of 5 ml. each were collected. A thin yellow band migrated down the column, and was collected in fractions 2, 3, and 4. A darker yellow band, more diffuse than the first, migrated at a much slower rate. This band moved about halfway down the column, at which point it ceased to migrate. No further development of bands was observed in this separation. After fraction number 35 was collected, the column was washed with methanol. A small quantity of yellow solution was obtained.

One drop of fraction 2 when evaporated and dissolved in dilute hydrochloric acid showed an intense blue fluorescence. This acidic solution gave a positive reaction with Mayer's reagent. Fractions 3 and 4 were similar in appearance and test response to number 2, although the reaction was less pronounced. None of the other fractions collected in this separation gave any residue on evaporation. The methanolic wash liquid from the column was tested with Mayer's reagent with negative results.

Fractions 2, 3, and 4 were combined and evaporated, yielding 12 mg. of residue. Thin layer chromatography showed that it contained a single component which was responsive to Dragendorff's spray reagent. This appeared to correspond to the spot on the original plate (Figure 8), having an R_f -value of approximately 0.8-0.9.

Attempts were made to crystallize the material from

fractions 2-4. Ethyl acetate, the parent extraction solvent, gave a brownish powder which was non-crystalline. Benzene, chloroform, hexane, chloroform-hexane, and chloroform-benzene were tried with unsatisfactory results. The material dissolved completely in chloroform and was completely insoluble in the other systems. Addition of hexane or benzene to a chloroform solution of the material produced turbid suspensions which coagulated into oils. During these crystallization attempts the material seemed to decompose, evidenced by a darkening of the substance together with a loss of reactivity towards Mayer's reagent.

A second column, 1 cm. in diameter, was prepared of 50 g. of neutral alumina (Woelm). Five hundred milligram of the pH 8 solid obtained from the alkali-soluble material in the manner described above was placed on this column and eluted in the same manner as in the first separation attempt. Again, only one fraction gave a positive reaction with Mayer's reagent. Thin layer chromatography showed this material to be identical to that from the first separation. As in the previous separation scheme, a second yellow band slowly migrated to a point about 4 cm. from the top of the column. At this point it ceased to advance. Continued elution caused it to become fainter in color until it finally vanished. No trace of material was obtained in any fraction after the first five, which contained the material which was positive to Mayer's reagent and possessed an approximate R_f -value of 0.8-0.9. A total of 250 x 25 ml. fractions were collected in this separation.

2. Attempted Formation of Alkaloid Complexes

a. Picric Acid

The solution of this Dragendorff-positive material was

immediately used in an attempt to obtain a picrate. A procedure similar to that described earlier was followed, but again only picric acid was isolated.

b. Reinecke Salt

A second attempt was made to prepare a Reinecke salt complex of the presumably alkaloidal material in the alkali-soluble extract. Two liter of the original alkaline extract was acidified, filtered, and saturated with sodium chloride. The solution was allowed to stand for two hours after which time it was decanted from the additional precipitate which had formed. This solution was treated with Reinecke salt according to Battersby.²⁹ In this experiment, 1.7 g. of a pink solid was isolated. This material was decomposed according to the method of Asmis, et al.,³² after crystallization of a sample of the solid from acetone/water was attempted without success. The following quantities of reagents were used:

acetone	25.5 ml.
water	8.5 ml.
Ag ₂ SO ₄ sol'n.	78.6 ml. (7.6 g./l.)
BaCl ₂ sol' n.	16.2 ml. (18 g./l.)

The product from this decomposition, amounting to less than 1 mg., proved to be unresponsive to Mayer's reagent. No further attempts were made to isolate alkaloidal components from Alyxia olivaeformis.

J. Volatile Components

1. Isolation

Two kilogram of fresh Alyxia olivaeformis was obtained and

ground with water to a thick slurry in a Waring Blender. The slurry was steam-distilled until 4 l. of condensate was obtained. This condensate was continuously extracted with ether (2 d.).

Upon drying and evaporating this extract a golden-yellow oil was obtained. This oil possessed a powerful aroma, so much so that a tiny droplet on the fingers gave a strong scent of maile. On standing, the oil crystallized into a white solid which could be recrystallized from ether only with difficulty. It was, however, readily sublimable and in this manner 289 mg. of material was obtained. The melting point of the solid was 63.5-64°C. Upon comparing its melting point and infrared spectrum, the solid was found to be identical with the previously obtained compound D-1.

A sample of the crude volatile extract was placed on a Carbowax column at a temperature of 250°C. and a helium flow rate of 0.75 ml./sec., using an Aerograph Model A-90-P gas chromatograph. A single principal peak was obtained, indicating that the only significant volatile component of Alyxia olivaeformis is compound D-1.

2. Analysis of D-1

Calcd. for $C_9H_6O_2$: C, 73.97; H, 4.14.

for $C_9H_8O_2$: C, 72.96; H, 5.44.

for $C_{10}H_{10}O_2$: C, 74.05; H, 6.22.

Found: C, 74.80, 74.21; H, 4.22, 4.45; N, 0.00.³³

Ultraviolet and infrared spectra are reproduced in Figures 9 and 10.

D-1 was only slightly soluble in dilute hydrochloric acid, but was soluble in both concentrate hydrochloric acid and dilute alkali.

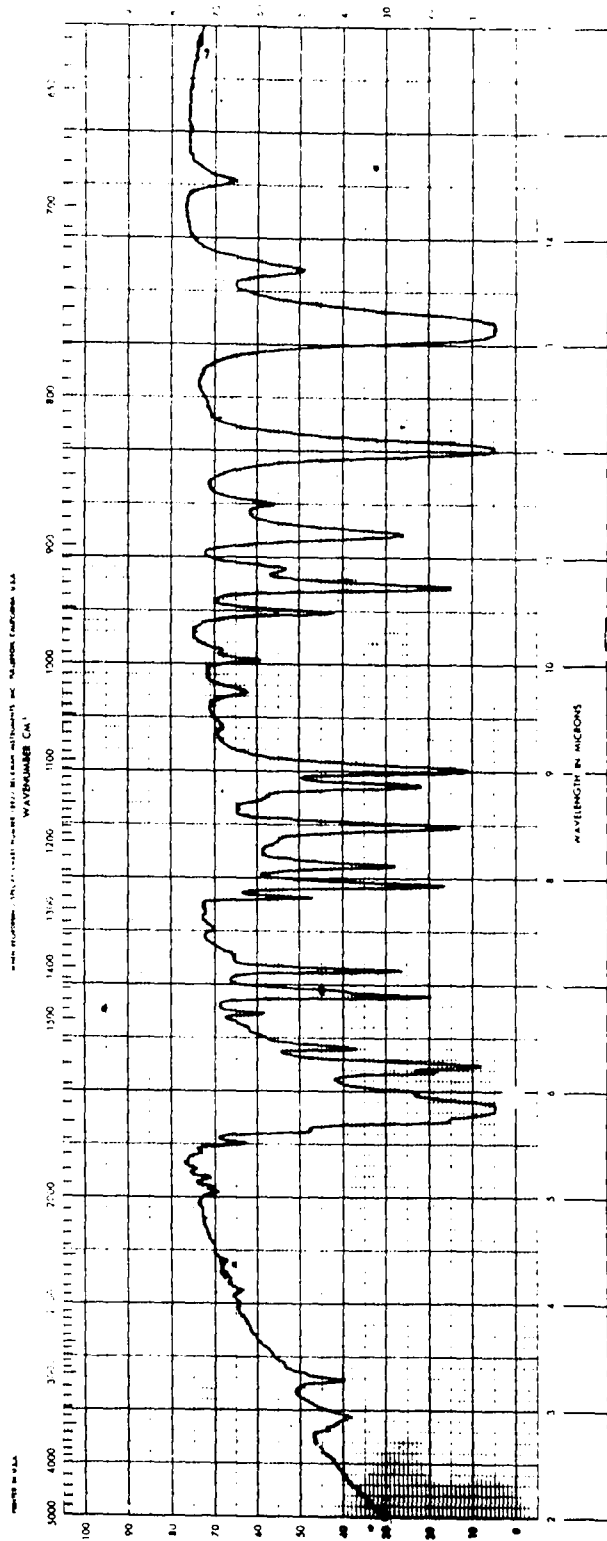


Figure 9. Infrared Spectrum of D-1 in a potassium bromide disc.

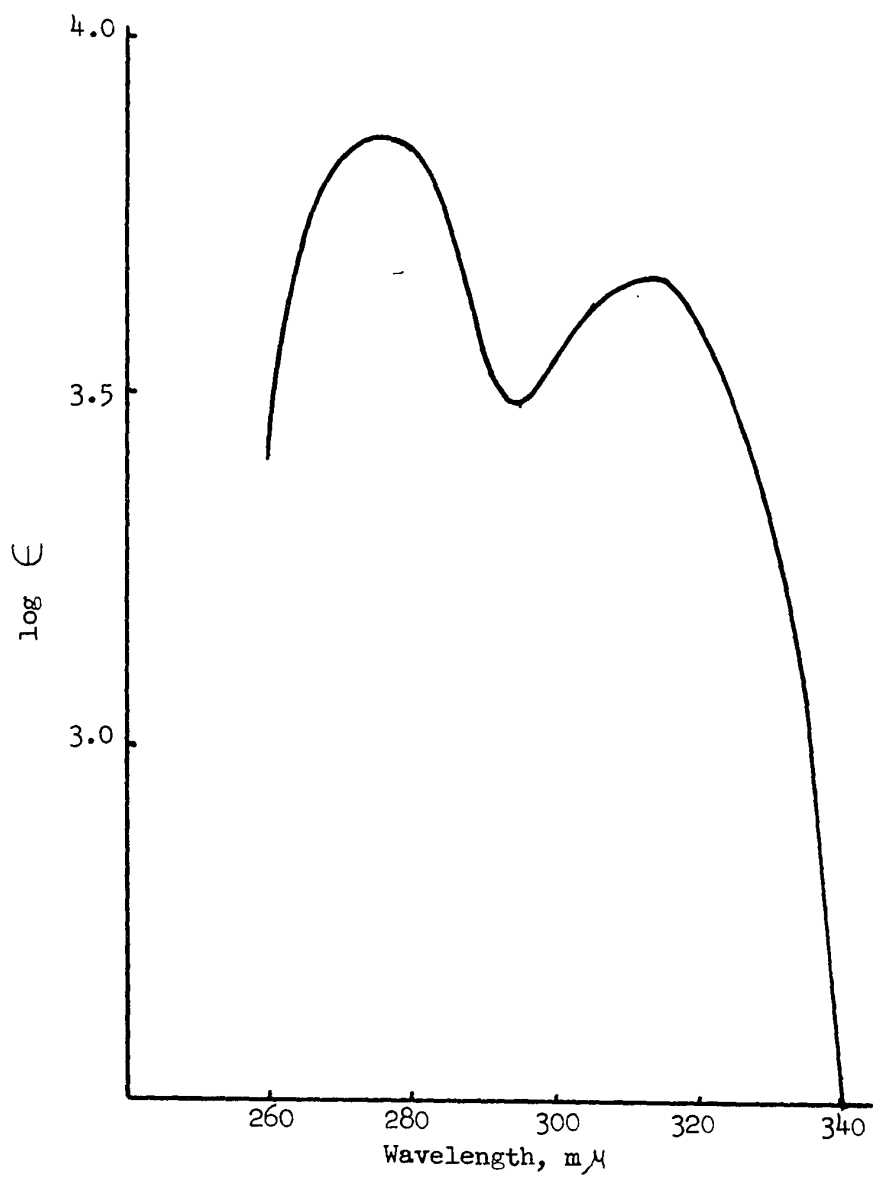


Figure 10. Ultraviolet absorption spectrum of D-1 (coumarin).
(7.19×10^{-5} M in methanol)

Functional group analysis showed no O-methyl groups and 4.11% C-methyl.

The optical rotation was determined using a Rudolph polarimeter and a 2 dm. cell. The specific rotation was $[\alpha]_D^{27.7} = +3.32^\circ$ (1.62% in absolute alcohol).

K. Compound D-2

1. Analysis

Calcd. for $C_{16}H_{20}O_8$: C, 56.47; H, 5.92.

for $C_{16}H_{18}O_7$: C, 59.62; H, 5.63.

for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30.

Found: C, 59.81, 60.16; H, 6.50, 6.43; O, 34.75³³

C, 56.48, 56.56, 56.28; H, 6.17, 6.10, 6.22.³⁴

The ultraviolet and infrared absorption spectra are reproduced in Figures 11 and 12.

Functional group analysis showed 11.94% O-methyl and 1.06% C-methyl. There was 1.53% active hydrogen. A molecular weight determination by the Rast method gave a value of 431.³³

The optical rotation was determined in the same manner as in the case of D-1. The specific rotation was $[\alpha]_D^{27.5} = +33.77^\circ$ (1.84% in absolute ethanol).

2. Solubility Tests on D-2

D-2 was slightly soluble in dilute hydrochloric acid, and only slightly soluble in cold water and in sodium bicarbonate solution. It was soluble in sodium hydroxide solution and in pyridine. The sodium hydroxide solution turned yellow on standing. This action was

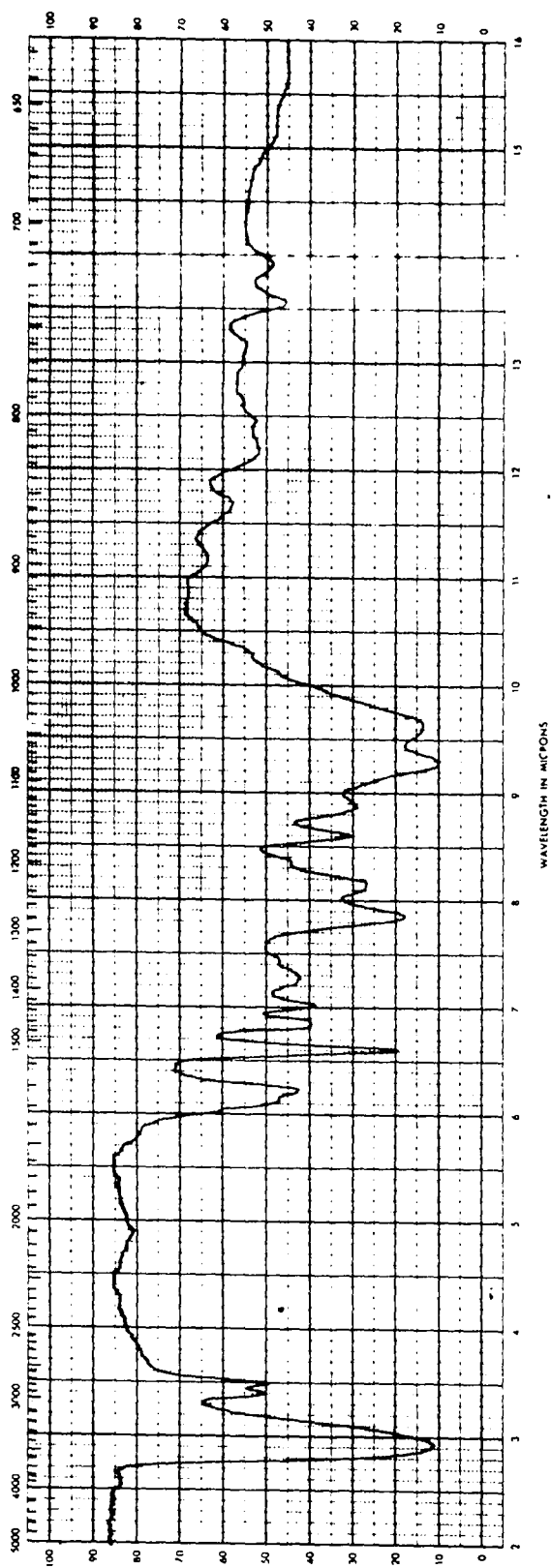


Figure 11. Infrared Spectrum of D-2 in a potassium bromide disc.

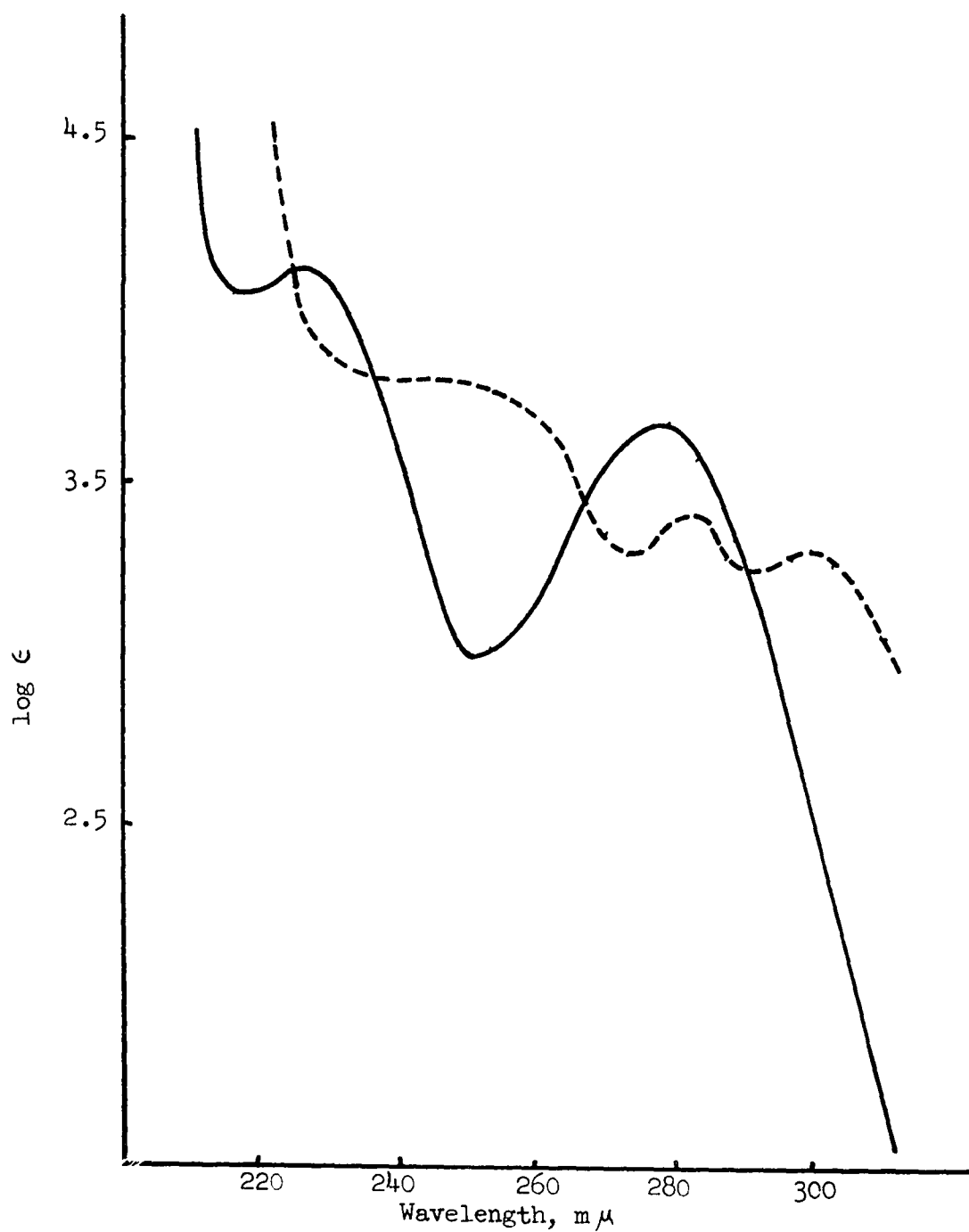


Figure 12. Ultraviolet absorption spectrum of D-2.

(—) 1.47×10^{-4} M in methanol.

(-----) 9.80×10^{-5} M in 0.15 N methanolic sodium hydroxide.

accelerated upon heating.

3. Color Tests on D-2

A test of D-2 with aqueous iron (III) chloride solution showed no development of color. Use of anhydrous iron (III) chloride with pyridine in a chloroform solution prepared according to McElvain²⁸ showed development of a blue-green color.

A series of standard color tests was performed on D-2 according to the methods described by Venkataraman.³⁵ The results, as well as the reactions of a number of relevant plant constituents to those tests, are shown in Table V.

A test with vanillin/hydrochloric acid reagent gave a pale pink color.³⁶

4. Hydrogenation of D-2

Hydrogenation of D-2 was attempted using ethanol as the solvent and Adams catalyst; there was no significant uptake of hydrogen. Infrared spectra of D-2 and the material recovered from the hydrogenation attempt were identical in every respect.

5. Attempted Benzoylation of D-2

D-2 (22.1 mg.) was dissolved in 2 ml. of anhydrous pyridine. Benzoyl Chloride (0.2 ml.) was slowly added. A white solid, soluble in warm pyridine, formed. The mixture was heated in a water bath for one hour, then poured into 10 ml. of water. A white turbidity appeared. The mixture was then placed in the refrigerator for one hour. The yellow solid which separated was washed with 5% sodium carbonate, then with cold water. The pale yellow residue was then taken up in 3 ml. of ethanol and chilled for three hours. White crystals separated and were

filtered off. Two milligram of material was obtained as a pale cream-colored solid melting at 105-106°C.

6. Attempted Acetylation of D-2 with Ketene

Fifty milligram of D-2 was suspended in 25 ml. of dry benzene. Ketene, prepared by the pyrolysis of acetone, was bubbled into the suspension for 2.5 hours. The resulting yellow solution was dried over anhydrous magnesium sulfate and evaporated, yielding 173 mg. of a brown gum. A sample of the gum was redissolved in benzene and washed with aqueous sodium carbonate. The yellow benzene solution was dried and evaporated. Infrared spectral studies of this residue showed it to be impure dehydracetic acid, a polymer of ketene. No acetyl derivative of D-2 was found.

7. Trimethylsilylation of D-2

A trimethylsilyl ether of D-2 was prepared according to the method of Waiss, Lundin, and Stern.³⁷ A white solid (10.5 mg.), m.p. 65-67°C., was isolated from 19 mg. of D-2. This entire sample was dissolved in carbon tetrachloride and an NMR spectrum of the solution was recorded. It is reproduced in Figure 27.

III. DISCUSSION OF RESULTS

A. Alkaloids

A thorough search for alkaloids in Alyxia olivaeformis failed to uncover alkaloids in isolable quantity. Several explanations may account for this failure.

(1) There are no alkaloids present in Alyxia olivaeformis, and the original screening test was a "false positive" test.³⁸

(2) Alkaloids are present in exceedingly small quantities.

(3) The alkaloids of Alyxia olivaeformis are unstable and decompose during work-up.

When one examines these possible explanations in some detail, one does not at first find the absence of alkaloids a plausible rationalization since it will be recalled (Chapter I) that the family Apocynaceae is rich in alkaloid-bearing plants. Examination of the literature, however, reveals that in this family, the genus Alyxia is a rather poor alkaloid producer. Webb reported the results of screening for alkaloids in the Australian Alyxia ruscifolia, A. spicata, A. ilicifolia, and A. sp.^{17,18,39} He found only negative or trace responses. Bisset likewise screened the Indonesian species Alyxia stellata, A. disphaerocarpa, A. gynopogon, and A. pubescens.^{20,21} He, too, reported negative results except for A. stellata for which he indicated the presence of trace amounts.

Finally, Alyxia torresiana from the island of Guam was recently tested and was found to contain no alkaloids.⁴⁰ It would seem, therefore, that the available record of the genus Alyxia as an alkaloid producer is poor. A predominance of negative results on a particular

genus of plants does not, of course, mean that all species of this genus will show similar results, but exceptions to the rule would tend to be infrequent. Webb, for example, observed in his study that almost all Australian species of Ochrosia, another genus in Apocynaceae, have high alkaloid content.^{17,18}

If one assumes that the genus Alyxia would not be expected to be a rich source of alkaloids, one still needs to reconcile the original positive screening results and the failure to isolate alkaloids in a preparative work-up.¹⁵ A recent study of "false positive" test responses to Dragendorff's reagent shows that flavonoids and coumarins are frequently responsible for these misleading test results.³⁸ Erroneous responses to alkaloid screening reagents are apparently more common than had been believed earlier and the present research may well be another case in point.

The second possible explanation, that the quantity of alkaloids was insufficient for isolation, needs no further comment.

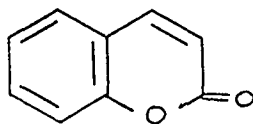
A third possible explanation, that alkaloids which might be present in the plant are too unstable to be isolated by customary procedures, should now be examined briefly. It is well to point out that whenever one deals with the isolation of organic natural products the possibility exists that isolation methods may fail altogether or may produce an artifact. On the other hand, normally encountered "unstable" compounds can generally be recognized and can be transformed into stable derivatives. In the course of this work a number of attempts were made to isolate stable salts of any possibly labile alkaloids, but all attempts were without success. Whether the failure to isolate crystalline

alkaloids from the Mayer's-positive ethyl acetate extract (Chapter II, G-I) was caused by the lability of the compounds, by a false positive Mayer's reaction, or by some undetermined factor cannot be stated with certainty.

In summary, then, the experimental evidence seems to indicate that any alkaloids in Alyxia olivaeformis are present in exceedingly small quantity.

B. Compound D-1

A comparison of the combustion data with the percentage composition of several likely formulas (see page 37) shows that $C_9H_6O_2$ is the most probable empirical formula of compound D-1. A widely occurring plant product of this composition is coumarin (VII), m.p. $70^{\circ}C$. Comparison of D-1 by melting point, ultraviolet and infrared



VII

spectra with an authentic sample of coumarin showed complete identity. For spectral comparisons, see Figures 9 and 10. The only apparent discrepancy lay in the fact that D-1 appeared to possess some slight optical activity which would be impossible in the case of coumarin. The specific rotation calculated for D-1 (see page 40) was computed from an observed rotation of -2.725° . This value was corrected by subtracting

a blank value of -2.833° , giving the corrected value of $+0.018^{\circ}$. It is probable that this apparent trace of optical activity is due to the presence of errors of measurement which were not eliminated even though a total of ten separate determinations of the observed rotation were averaged in the calculation of the specific rotation.

C. The Volatile Component

It will be recalled (Chapter II-J) that the volatile constituent of A. olivaeformis was compound D-1, now identified as coumarin. Coumarin has been isolated from other Alyxia species. Coumarin is mentioned as a constituent of A. reinwardti by van Steenis,¹⁹ and Webb states that coumarin occurs in A. stellata.³⁹ It is therefore not surprising that coumarin has now been found in A. olivaeformis.

Coumarin then is the volatile principle which causes the pleasant odor of maile. It is well known that maile from different locations varies greatly in fragrance. Now that the odoriferous principle has been determined, it would be interesting to study the quantitative occurrence of coumarin in various plant parts from different locations.

D. Compound D-2

Compound D-2 was informally considered to be a bitter principle prior to its structural classification. The term bitter principle is defined as a non-nitrogenous organic natural product possessing a bitter taste. Originally bitter principles were of undetermined structure,⁴¹ but they are now recognized to fall into widely

differing structural types such as tannins, flavonoids, aurones,⁴² and steroidal glycosides.¹⁰

1. Empirical Formula

A total of five separate combustion analyses were carried out on samples of D-2. These analyses were carried out in two separate laboratories. The first series, carried out on samples which were dried in vacuo at 100°C., agreed with a $C_{16}H_{18}O_7$ formula, while the second series, in which the samples were dried in vacuo at room temperature, gave rise to a $C_{16}H_{20}O_8$ formula. These formulas differ from each other by their degree of hydration, and, as might be hoped, rigorous drying of the samples prior to combustion led to loss of water. Whether this behavior represents a change from a hydrated state or whether it is a case of intramolecular dehydration will be considered later.

2. Molecular Formula

The calculated molecular weight for D-2 is 340 if one considers $C_{16}H_{20}O_8$ and 322 for $C_{16}H_{18}O_7$. These values are in poor agreement with the experimental value of 431 determined by the Rast method. This is not a serious problem since Rast values are reliable only when the compound forms an ideal solution with camphor. In fact, the Rast value, poor as it is, does serve to eliminate a possible C-8 or C-32 formulation. A C-24 formula remains a possibility, but it could not account for a mono-dehydro formula.

A mass spectrum of D-2, shown on Figure 13, gave no confirmation of any possible formula. A strong peak at 28 m/e and another at 54 m/e, indicative of CO and C_3H_2O respectively, appear to indicate that the compound is decomposed under the conditions of the determination. No

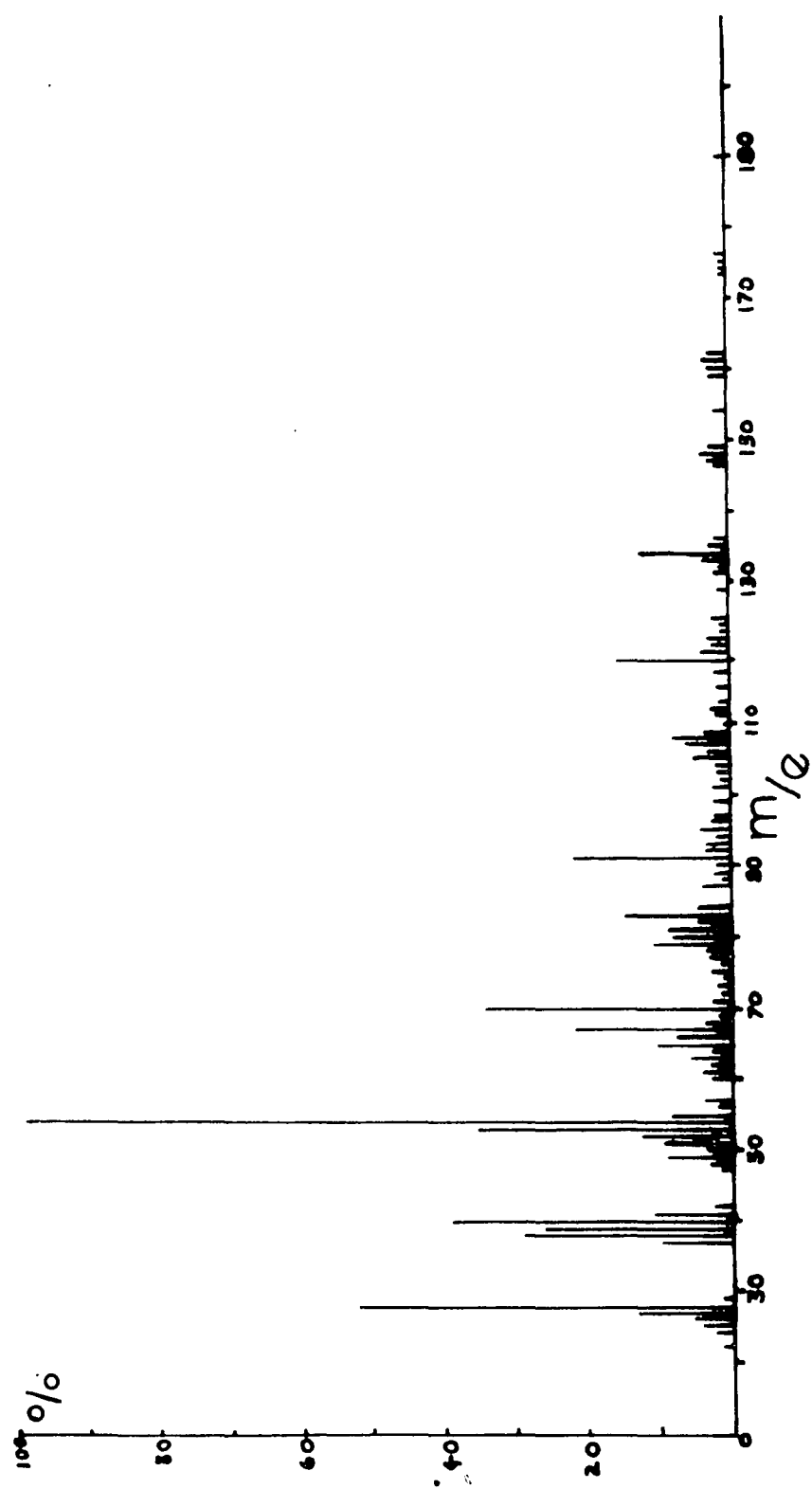


Figure 13. Mass Spectrum of D-2.

M⁺ peak was observed.^{43,44} Further discussion of the mass spectrum of D-2 will be advanced in section 3.

On the basis of these considerations, compound D-2 is best considered to be a C-16 compound.

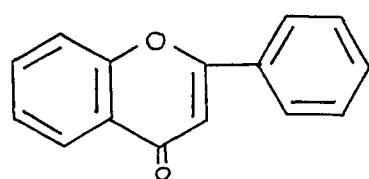
3. Skeletal Structure

There exists in nature a large group of compounds having a molecular structure based on a C-15 system. These compounds are the flavonoids and their derivatives. Figure 14 shows the various members belonging to this class of compounds and lists their structures and generic names.

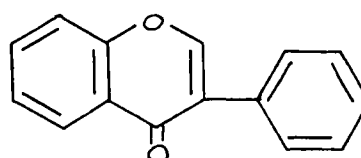
Assuming that D-2 is a member of the flavonoid group of compounds, a single extra-skeletal carbon atom has to be accounted for. According to functional group analysis for methoxyl, D-2 contains one methoxyl group. As the existence of methoxyl-substituted flavonoids is well documented in the literature,^{45,46,47} representation of D-2 as a monomethoxy flavonoid constitutes a reasonable working hypothesis.

As may be seen from Figure 15, only those flavonoids can be optically active which contain no unsaturation at the 2,3-linkage. As compound D-2 is optically active, all flavonoid structures save those having this saturated linkage may be ruled out as possible skeletal structures for D-2.

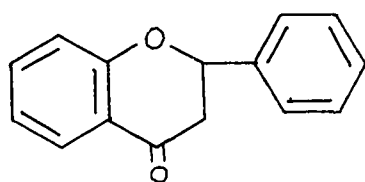
Additional support for the elimination of 2,3-unsaturated flavonoids comes from hydrogenation evidence. D-2 did not take up hydrogen under conditions which would normally hydrogenate a flavonoid having 2,3-unsaturation. The only unsaturation present in D-2 is based on aromatic rings, which would remain intact under the conditions



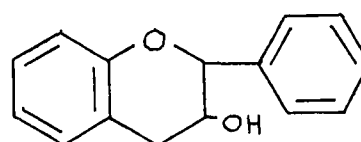
Flavone



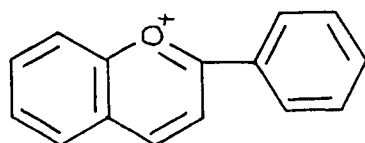
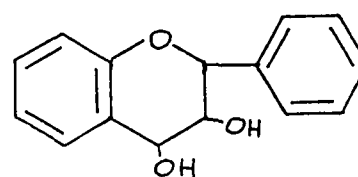
Isoflavone



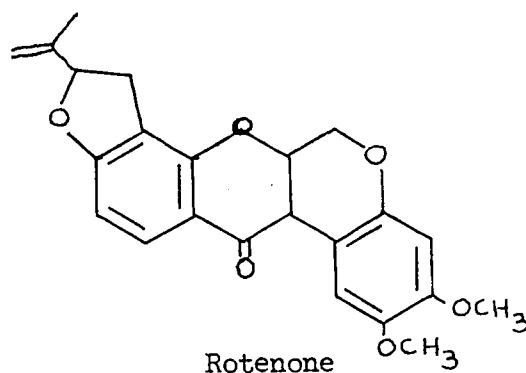
Flavanone



Catechin

Flavylium salt
(Anthocyanin)

Tannin



Rotenone

Figure 14. Structures of Principal Flavonoids

employed.

Further differentiation among the remaining flavonoid types may be made by color tests as described by Venkataraman.³⁵ The results of these tests, which are tabulated in Table V, are good evidence that compound D-2 may be a catechin.

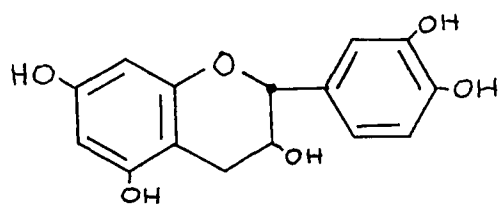
Catechins have been known since 1821, when catechin was first isolated from Acacia catechu by Runge. (+)-catechin has been obtained from several species of Acacia, from the Douglas fir (Abies concolor), and from the ironwood (Casuarina equisetifolia). Other catechins have been obtained from these plants, as well as from species of Eucalyptus and Afzelia, from the barks of oak and sweet chestnut, and from green tea.^{48,49,50,51} The catechins do not appear to be rare in nature, but variations in the structure are relatively uncommon, resulting in the same catechin being isolated from a number of different genera. In addition, catechins do not appear to be physiologically active.⁴² As a result, this class of flavonoids, although not an uncommon one, has not received much attention in the literature.^{52,53}

Samples of the following catechins were obtained for comparison with D-2: (+)-catechin,⁵⁴ (-)-epicatechin,⁵⁴ (-)-robinetinidol,⁵⁴ (+)-afzelechin,⁵⁵ and (-)-epiafzelechin.⁵⁶ A summary of the principal physical properties of these and other catechins is found in Table VI. The structures of these compounds are illustrated on Figure 15.

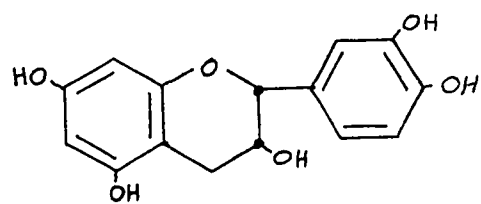
Comparison of the infrared spectra of authentic catechins and samples of D-2 were made. These spectra are shown in Figures 16-20. From these spectra, it may be seen that D-2 is not identical with any of the known catechins, but that there are a number of similarities

TABLE V
COMPARISON OF FLAVONOID COLOR TESTS

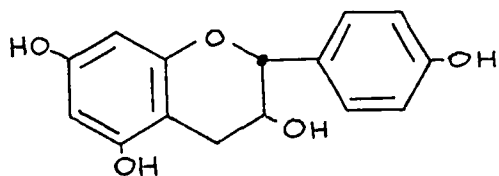
Flavonoid	Reagent		
	Aq. NaOH	Conc. H ₂ SO ₄	Mg/HCl
Anthocyanins and Anthocyanidins	Blue to violet	Yellowish orange	Red (fades to pink)
Catechins	Yellow to red-brown	Red	None
Chalcones	Orange to red	Orange to red	None
Flavanones	Yellow to orange	Orange to crimson	Red
Flavones	Yellow	Yellow with fluorescence	Red to magenta
Isoflavanones	Yellow	Yellow	None
Isoflavones	Yellow	Yellow	Yellow
Leucoanthocyanins	Yellow	Crimson	Pink to red
<u>D-2</u>	Yellow to red-brown	Red	None



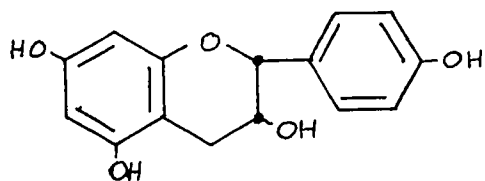
(+)-catechin



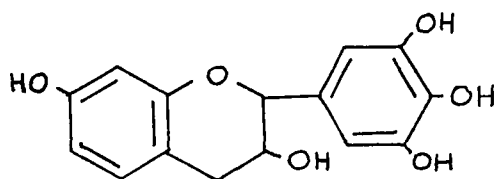
(-)-epicatechin



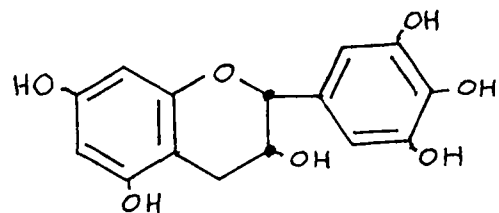
(+)-afzelechin



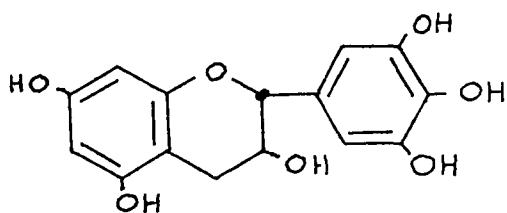
(-)-epiafzelechin



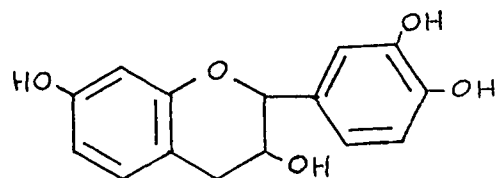
(-)-robinetinidol



(-)-epigallocatechin



(+)-gallocatechin



(-)-fisetinidol

Figure 15. Structures of Known Catechins

TABLE VI
PHYSICAL PROPERTIES OF KNOWN CATECHINS AND OF D-2

Compound	m.p., °C.	$[\alpha]_D$	$\lambda_{\max.}$	Ref.
(+)-catechin	96 (hyd.) 177 (anhyd.)	18.4 (H ₂ O)	280 (3980)	42
(-)-epicatechin	245	-69 (EtOH)	280 (3300)	42
(+)-gallocatechin	188	14.7 (aq. acetone)		42
(-)-epigallo- catechin	218	-67.5 (EtOH)	271 (1240)	42
(-)-epigallo- catechin gallate	254	-190 (EtOH)	280 (13600)	42
(-)-epiafzelechin	243	-59 (EtOH)	276 (2200)	42
(+)-afzelechin	221	20.6 (aq. acetone)	277 (--)	50
(-)-robinetinidol	207	-10.7	281 (--)	42
(-)-fisetinidol	214	--	-- (--)	42
(+)- <u>D-2</u>	143-145(hyd.) 138-140 (dehyd.)	33.77 (MeOH)	279 (3890)	

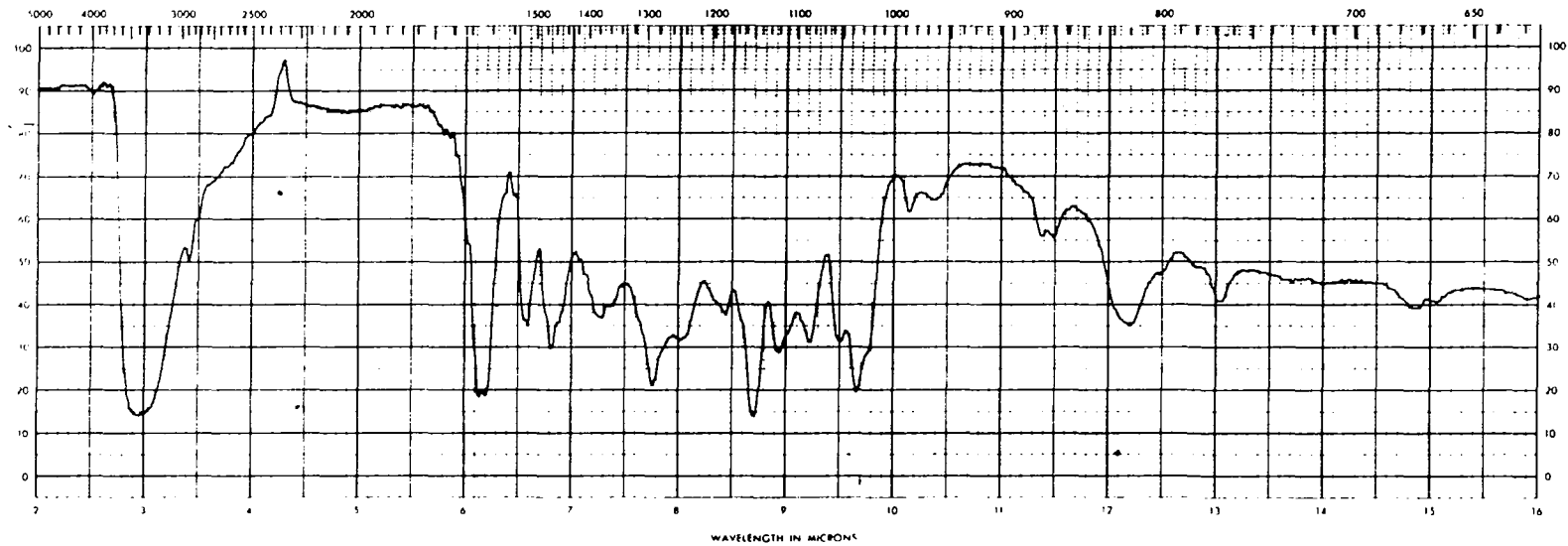


Figure 16. Infrared Spectrum of (+)-Catechin
in a Potassium Bromide Disc.

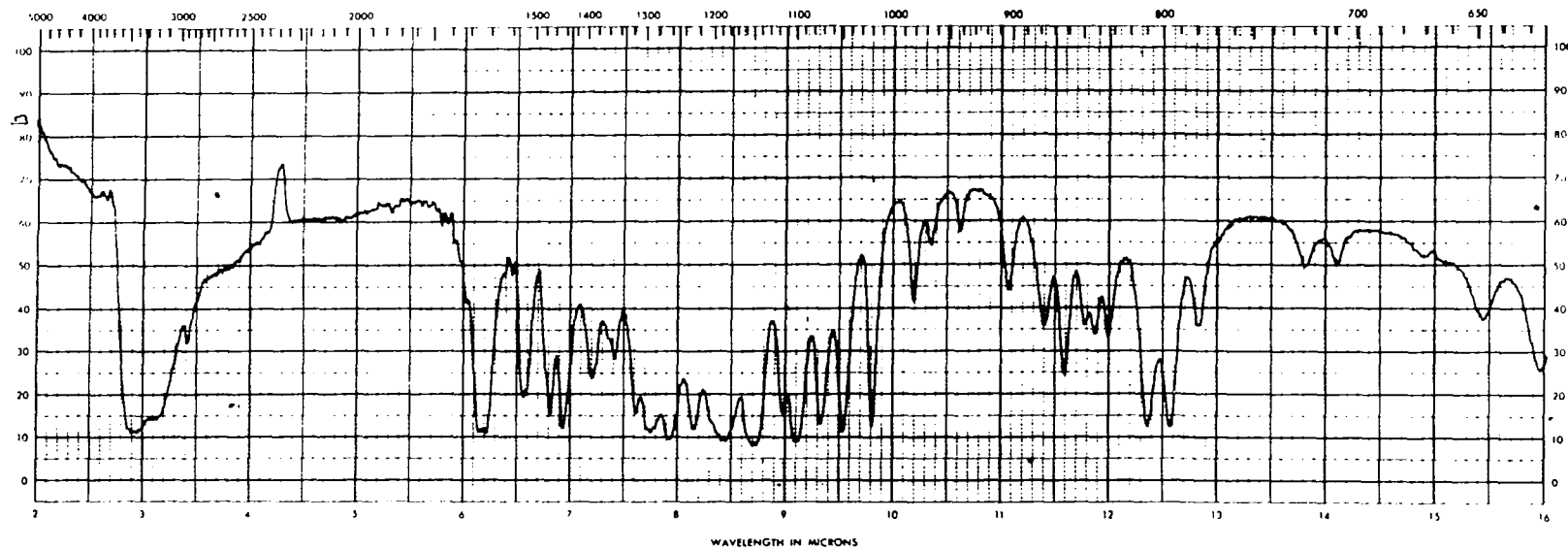


Figure 17. Infrared Spectrum of (-)-Epicatechin
in a Potassium Bromide Disc.

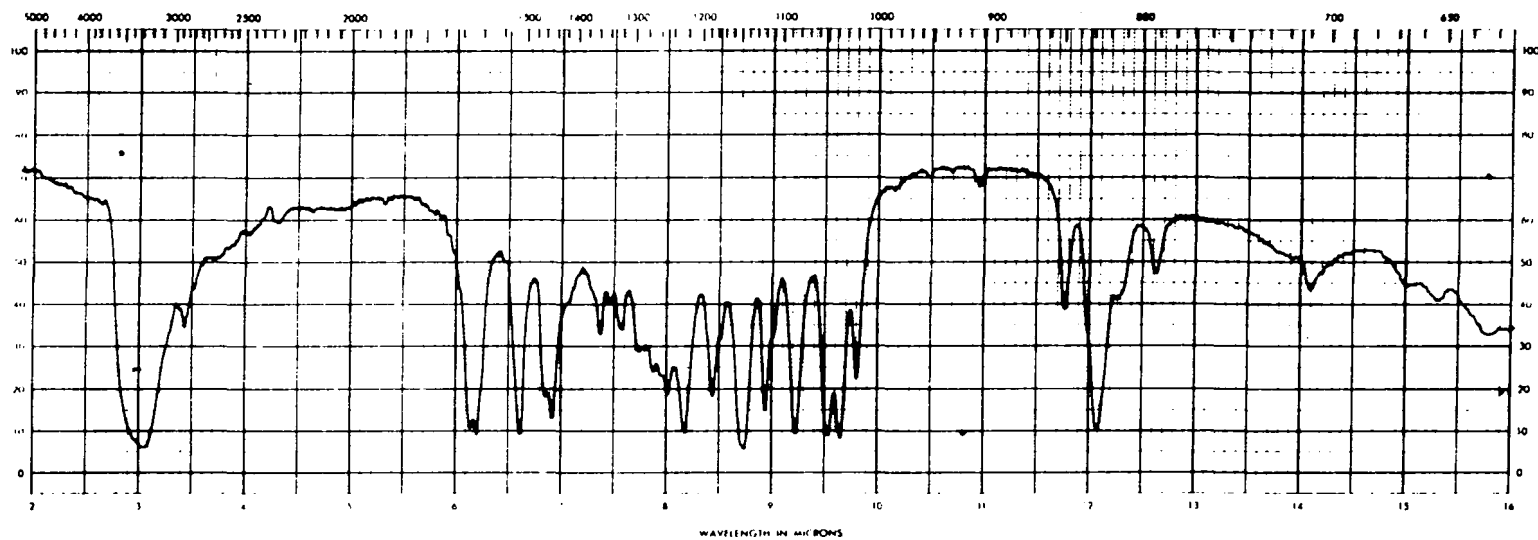


Figure 18. Infrared Spectrum of (+)-Afzelechin
in a Potassium Bromide Disc.

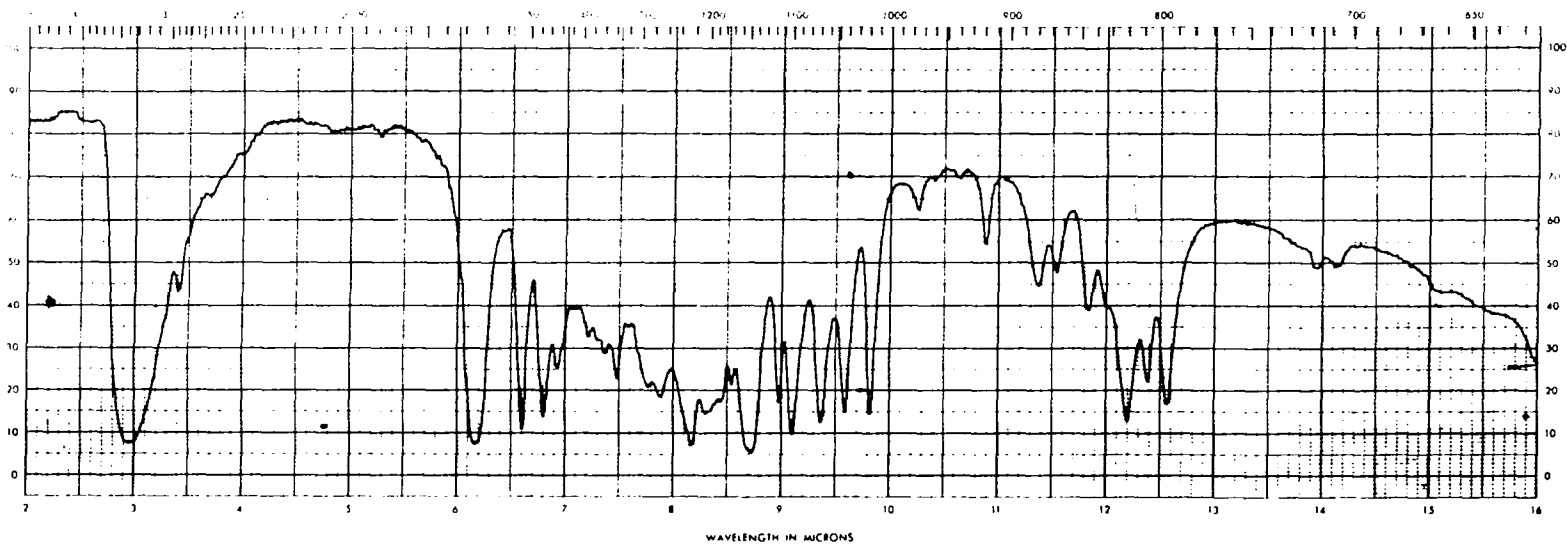


Figure 19. Infrared Spectrum of (-)-Epiarzelechin
in a Potassium Bromide Disc.

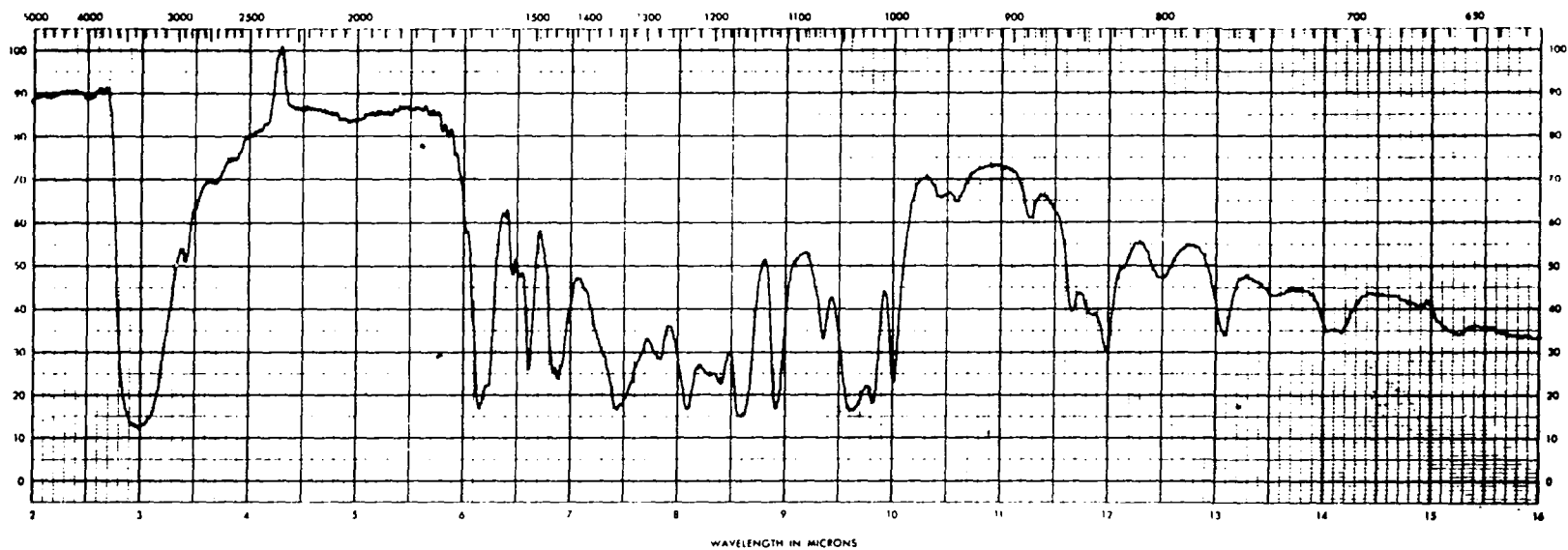


Figure 20. Infrared Spectrum of (-)-Robinetinidol
in a Potassium Bromide Disc.

among all the spectra. All show a strong peak at $2.9-3.1\mu$, indicative of hydroxyl groups. In addition, there are peaks at $6.1-6.2\mu$, 6.6μ , and $6.8-6.9\mu$. Finally, the spectra of (+)-catechin and D-2 are similar to each other in the $7-10\mu$ region. This similarity, however, is less pronounced than are the overall resemblances in the region below 7μ .

The ultraviolet spectra of D-2, (+)-catechin, and (-)-epicatechin, in both neutral and basic solutions, may be compared in Figures 12, 21, and 22. It will be seen that the principal λ_{\max} common to D-2, (+)-catechin, and (-)-epicatechin lies in the $280\text{ m}\mu$ region. This band is shifted approximately $10-15\text{ m}\mu$ in basic solution. This type of shift is characteristic of phenolic hydroxyl groups, which in alkaline solution are converted to strongly chromophoric phenylate anions. The remaining $283\text{ m}\mu$ band in the spectrum of D-2 in base is felt to be a remnant of the original hydroxyl peak resulting from the existence of an equilibrium between the two forms. The basis for this assumption is the fact that the hydroxide ion concentration in the spectra of the two catechins was $1.25\text{ meq. NaOH/ml.}$, while in the D-2 spectrum the concentration was 0.15 meq./ml. This assumption was tested by running spectra of (+)-catechin in gradually increasing concentrations of sodium hydroxide solution. By this procedure a gradual diminution of the $280\text{ m}\mu$ absorption area was observed.

The band in the $220\text{ m}\mu$ region of D-2 is more difficult to interpret. Jurd⁵⁷ reports only the $275-280\text{ m}\mu$ band as characteristic of the ultraviolet spectra of the catechins. King, et al., however, report the existence of a λ_{\max} of $207\text{ m}\mu$ for afzelechin,⁵⁰ and Hillis

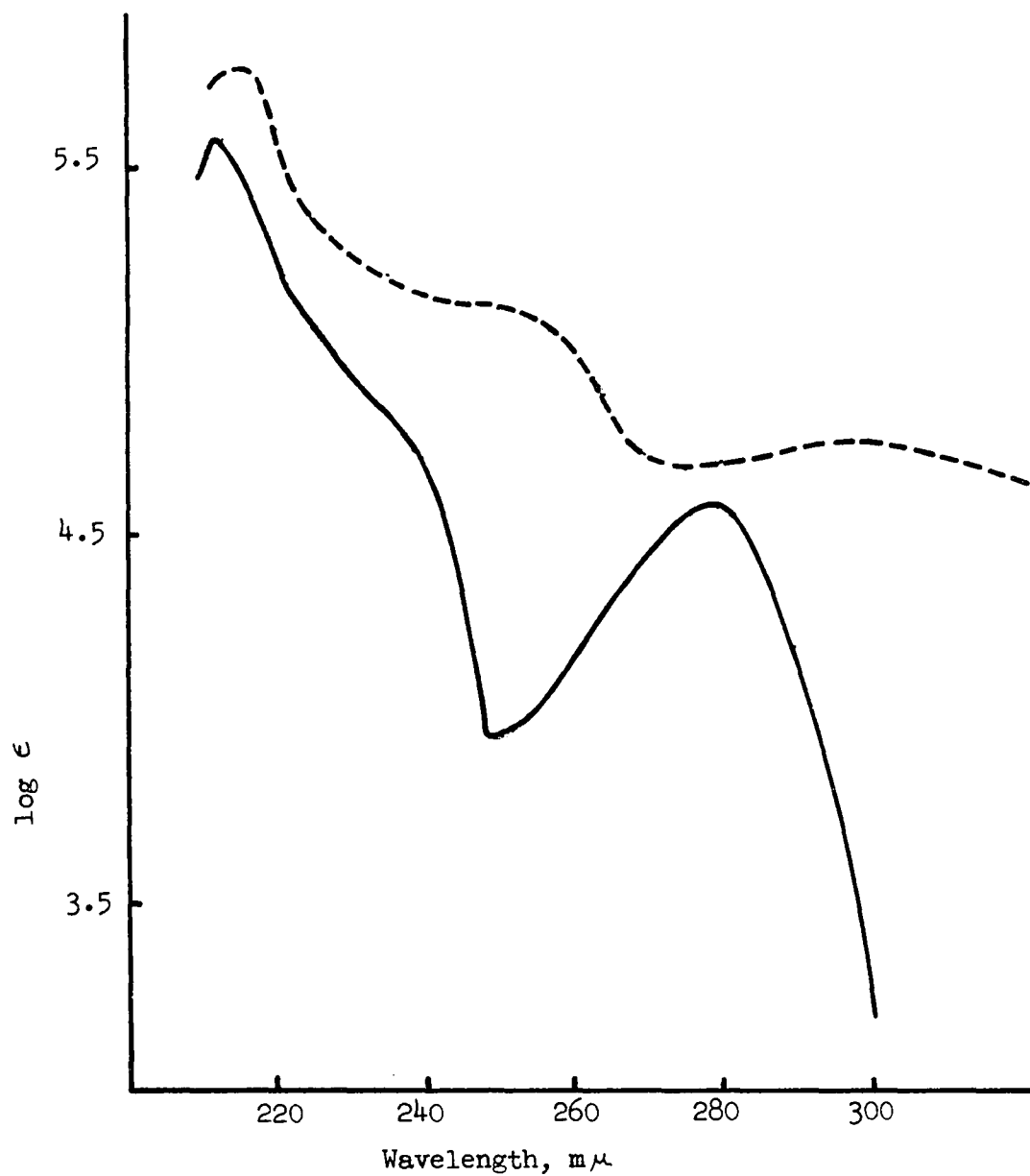


Figure 21. Ultraviolet absorption spectrum of (+)-catechin.

(—) 3.4×10^{-6} M in methanol.

(-----) 3.4×10^{-6} M in 1.25 N methanolic sodium hydroxide.

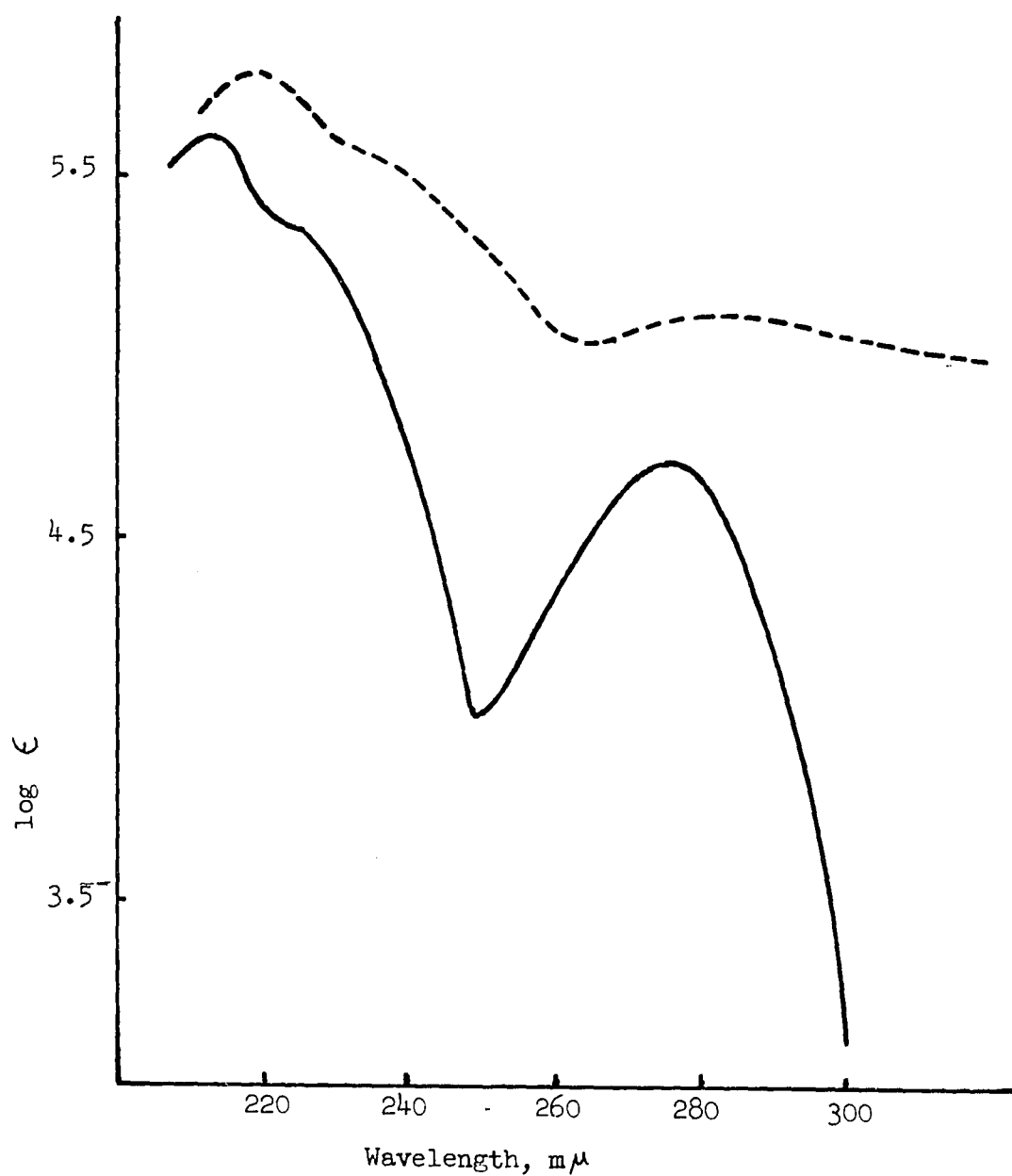


Figure 22. Ultraviolet absorption spectrum of (-)-epicatechin.

(—) 3.4×10^{-6} M in methanol.

(-----) 3.4×10^{-6} M in 1.25 N methanolic sodium hydroxide.

and Carle report 214 m μ as a λ_{max} for both (+)-afzelechin and (+)-catechin.⁴⁹ The spectra shown in this work show 212 and 213 m μ for values of the short wavelength λ_{max} for (+)-catechin and (-)-epicatechin respectively. It should be pointed out that these values are uncertain as this region of the spectrum is on the borderline both of instrumental sensitivity and solvent transmission.

Scott interprets this short wavelength absorption in phenolic systems to the interaction of the p-orbitals of the oxygen atom with the π -orbital system of the aromatic ring.⁵⁸ Such an interpretation could be advanced for the 220 m μ absorption in the case of D-2, but this explanation is questionable, first because this region lies well above the range of absorption mentioned by Scott for this type of interaction, and second, because there is no definite proof in the case of D-2 that another absorption exists at a wavelength below that of 227 m μ , which would fall within the range of an Ar-O interaction of this type.

A more plausible interpretation of the 227 m μ band is that it is produced by an interaction of the methoxyl oxygen with the aromatic system. Scott mentions a maximum of 217 m μ for anisole which is accounted for in this manner.

In Figures 21 and 22, the catechins exhibit an inflection at the 225-230 m μ region. This inflection could be produced by the interaction of the hetero oxygen atom with ring A in the manner mentioned. The definite presence of a methoxyl group in D-2, together with the hetero oxygen, could produce an actual peak in this region rather than an inflection.

In summary, the ultraviolet spectrum of D-2, while not identical with the spectrum of a catechin, is nevertheless felt to be consistent with a possible methoxyl substituted molecule of this structural type.

Returning to the mass spectrum of D-2 (Figure 13), it is possible to reconcile a hypothetical structure of D-2 based on the catechin skeleton with the results given by this spectrum. A cleavage of the catechin structure which gives rise to propynal, C_3H_2O , is shown on Figure 23. It will be recalled that the peak corresponding to this fragment is the one possessing the greatest relative intensity.

The peak at $m/e = 28$ can, as has been stated earlier, be interpreted as carbon monoxide. This compound has been reported as a decomposition product of phenols⁴³ and is therefore consistent with the known existence of phenolic hydroxyl groups in D-2. Such a decomposition is illustrated in Figure 24. The dihydroxybenzonium ion arises from the initial cleavage shown in Figure 23. Both $m/e = 81$ and $m/e = 53$ are peaks found in the mass spectrum of D-2.

The fact that there is no M^+ peak in the mass spectrum of D-2, together with the absence of other information concerning the mass spectra of the catechins makes a complete interpretation of the mass spectrum of D-2 of highly questionable value. The complexity of the spectrum would appear to indicate that other cleavages of the D-2 molecule may occur simultaneously. This would explain the multiplicity of peaks in the spectrum. Two such possible cleavage paths are illustrated on Figure 25.

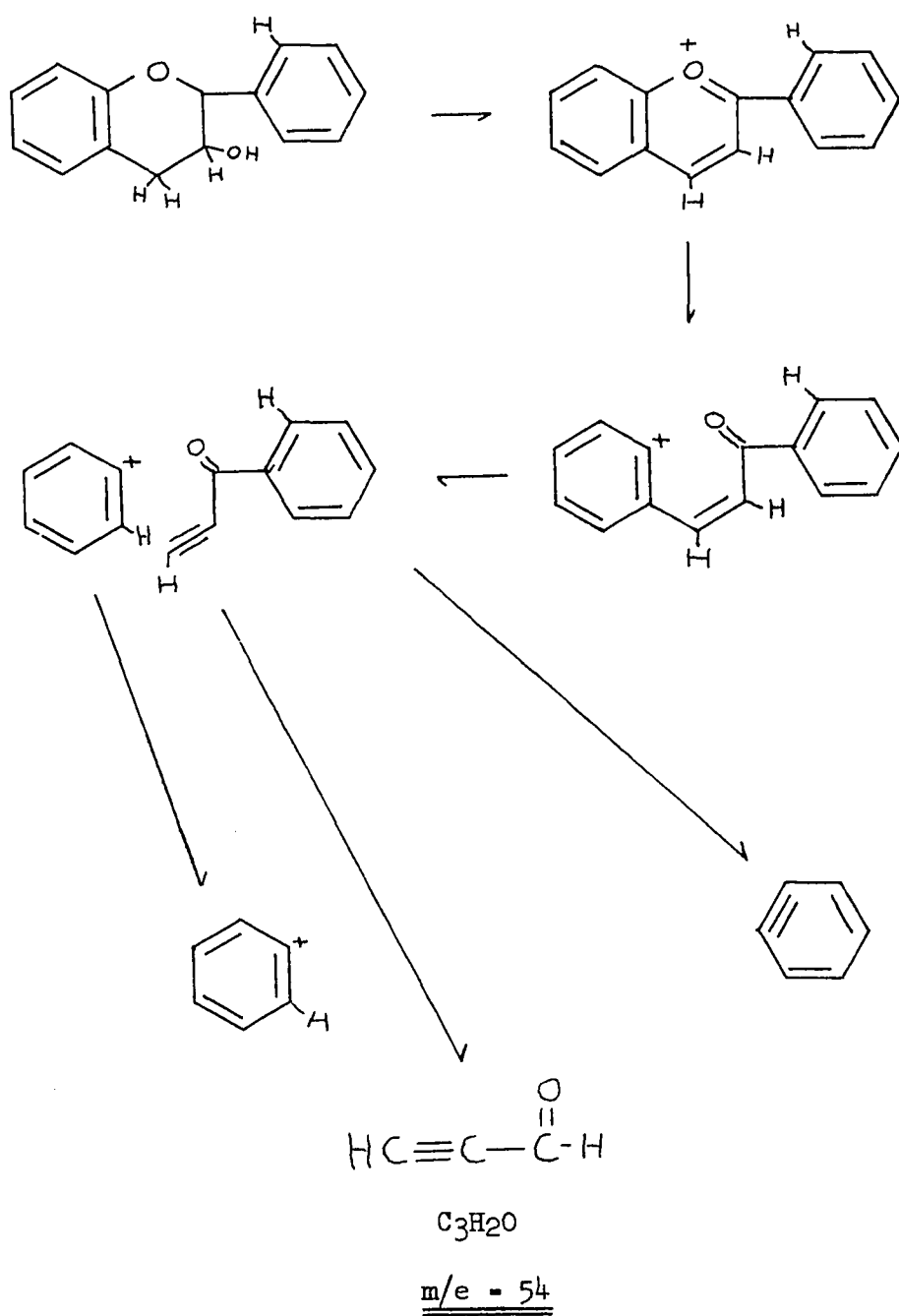


Figure 23. A possible cleavage of the catechin skeleton in mass spectroscopy.

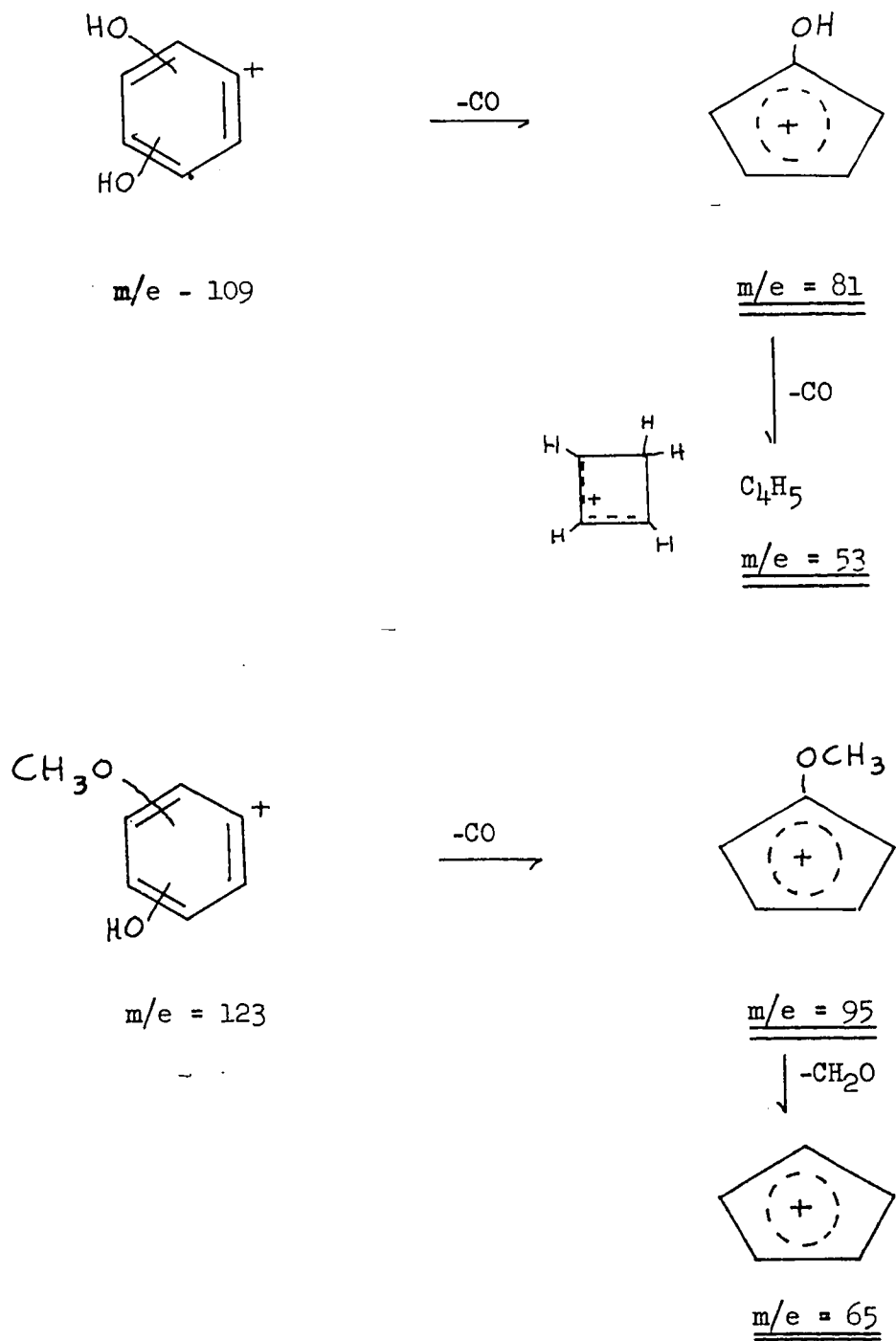


Figure 24. Possible interpretation of some peaks in the mass spectrum of D-2.

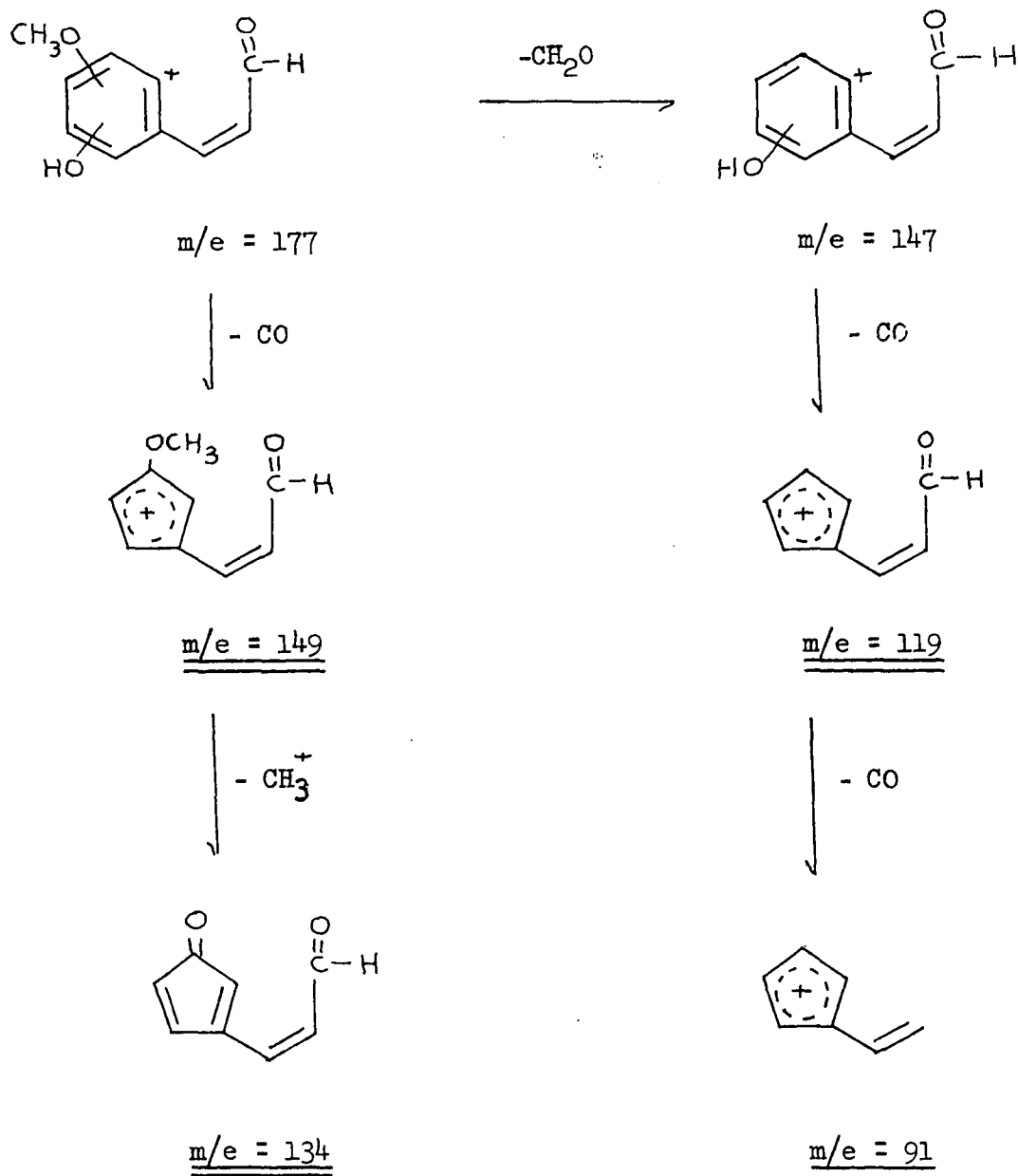
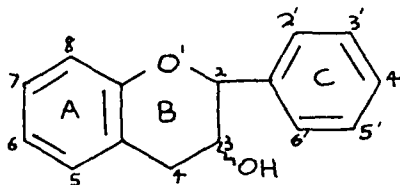


Figure 25. Possible interpretation of some peaks in the mass spectrum of D-2.

This partial interpretation of the mass spectrum of D-2 serves to illustrate that a hypothetical structure based on a flavan-3-ol system containing hydroxyl and methoxyl substituents is not unreasonable.

The results of the experimental evidence thus far allows postulation of part structure VIII for compound D-2.



VIII

Still to be established are the following features:

- (1) Whether or not the molecule is a hydrate.
- (2) The number and location of hydroxyl groups.
- (3) Location of the methoxyl group.
- (4) Stereochemical relationship of C-2 to C-3.

These points will now be examined in order.

4. Hydration of D-2.

The question whether D-2 is a hydrate should now be re-examined. Color tests and spectral evidence indicate that D-2 is probably a catechin. Yet, molecular formulae for monomethoxycatechins should be $C_{16}H_{16}O_3$, $C_{16}H_{16}O_4$, $C_{16}H_{16}O_5$, etc., depending on the number of hydroxyl groups present. Formulas for D-2 based on combustion data differ from these by one or two molecules of water. It is known that (+)-catechin is obtained as a hydrate, and that the degree of hydration varies with the conditions of drying.⁴⁹ Hence there is a possibility

that D-2 and catechin are similar in this respect.

The question whether D-2 is a hydrate was resolved by the use of nuclear magnetic resonance (NMR) spectroscopy. An NMR spectrum of D-2 in deuteriated dimethyl sulfoxide, shown in Figure 26, illustrated two points about the compound. One was the confirmation of a methoxyl group, and the other was the existence of water of hydration in crystalline D-2. The NMR spectrum had a signal at 3.4-3.5 δ , which was absent in the solvent. This signal was proven to be caused by water by running a second NMR spectrum on the same sample of D-2, which had been contaminated by the addition of a few drops of water. The second spectrum was identical in all respects with the first, except that the 3.4-3.5 δ peak had shifted to a value of 3.5-3.6 δ . This shift is probably caused by the small change in the quantity of protons in the system which are hydrogen-bonded.⁵⁹

An explanation of the differing combustion data of D-2 can now be made. D-2, when crystallized from water, is a dihydrate, having the formula $C_{16}H_{16}O_6 \cdot 2H_2O$, m.p. 143-145 $^{\circ}C$. Upon drying in vacuo at 100 $^{\circ}$, this molecule is converted in large part to a monohydrate which may be written as $C_{16}H_{16}O_6 \cdot H_2O$. The melting point of this material is unknown as only samples which were being prepared for analysis were subjected to this drying procedure. It should be pointed out that a similar drying procedure was employed by the analysts of these samples.

In order to test this hypothesis, a sample of D-2 was dried in vacuo at 100 $^{\circ}C$. for 36 hours. This sample showed a melting point of 138-140 $^{\circ}$. This represents a 5 degree difference from the sample

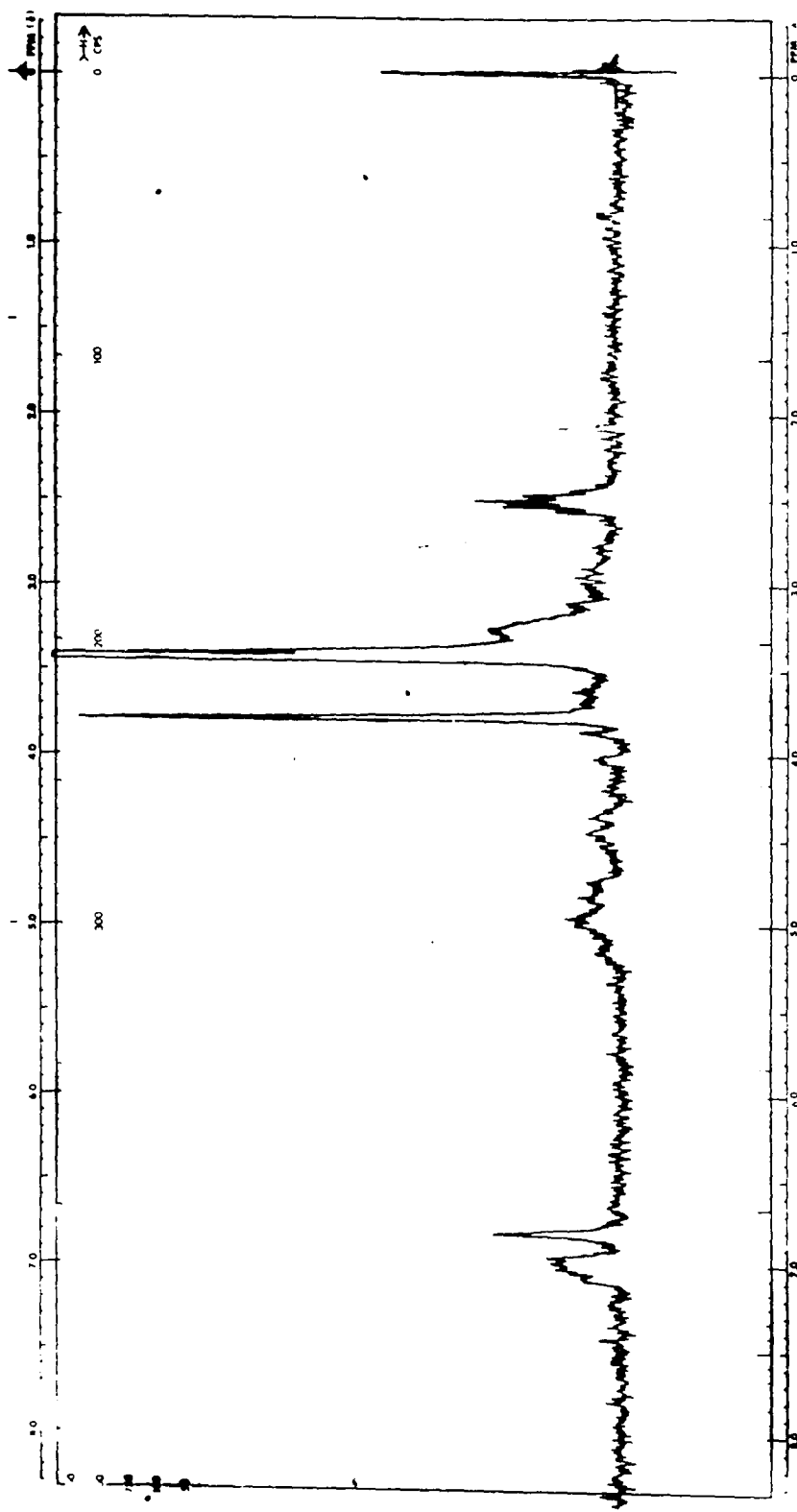


Figure 26. NMR Spectrum of D-2
in Deuteriated Dimethyl Sulfoxide.

which had been dried under milder conditions, and supports the hypothesis explaining the differing molecular formulas.

It has been reported that (+)-catechin exists as a hydrate from which it is exceedingly difficult to remove the water.⁴⁹ This gives rise to different melting points for this compound. It would appear that this is also the case for D-2, although from the elemental analyses, it is likely that it is easier for D-2.2H₂O to be converted to D-2.H₂O than in the case of (+)-catechin itself.

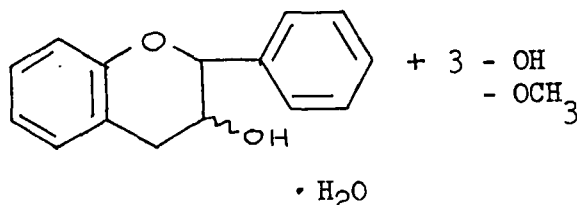
The dihydrate, which was preserved during drying in vacuo at room temperature gave rise to the second series of combustion analyses shown in Section K-1 of Chapter II. These samples were not rigorously dried by the analysts before combustion. The samples which had been thoroughly dried before combustion produced the first series of combustion analyses.

5. Number and Location of the Hydroxyl Groups

Since the functional group analyses were performed upon a thoroughly dried sample of D-2, the molecular formula which will be employed in this discussion will be C₁₆H₁₆O₆.H₂O. Assuming D-2 to be a catechin, it can be seen that two of the six oxygen atoms in the molecular formula are taken up by the hetero atom and the 3-hydroxyl (see structure VIII). A third oxygen is contained in the methoxyl group, leaving three oxygen atoms which must be phenolic hydroxyl groups.

The analysis for active hydrogen in D-2 monohydrate supports this hypothesis. For a compound of this formula, 1.53% active hydrogen would result in 5 active hydrogen atoms. Two of these are accounted for by the single water molecule and the 3-hydroxyl group, leaving the other

three as phenolic hydroxyls. This enables the part structure of D-2 to be expanded to part structure IX.



IX

This leaves the location of the methoxyl group and of the three phenolic hydroxyl groups to be determined. NMR studies of D-2 itself are not helpful on these points, as the water peak is sufficiently strong to cover a considerable portion of the spectrum. It was, therefore, of vital importance that a derivative of D-2 be obtained. However, attempts to prepare acylated D-2 derivatives resulted in only traces of products. It would appear that D-2 is sufficiently unstable under the conditions in which these derivatives are formed that it decomposes faster than the derivative can be formed. Methylation was considered, but since the existence of a natural methoxyl has already been shown for D-2 itself, it would be advantageous to employ a different derivative, thereby enabling distinction between natural and introduced substituents to be made.

At this point, the possibility of preparing a trimethylsilyl ether of D-2 was considered. These compounds are readily soluble in carbon tetrachloride, rendering them excellent subjects for NMR study. In addition, the trimethylsilyl hydrogens appear in an NMR spectrum almost along with the tetramethylsilane standard peak. Hence, there is

practically no interference with the section of the NMR spectrum above 2δ . Finally, the trimethylsilyl ethers of a number of flavonoid compounds, including (+)-catechin, have been prepared and subjected to NMR investigation, thereby furnishing a source for comparison.³⁷

A trimethylsilyl ether of D-2 was prepared and an NMR spectrum of the compound is shown in Figure 27. Comparison of this spectrum with the chart published by Waiss et al., which is reproduced in Figure 28, shows that there may be substitution on the 6 and 8 positions of D-2. These authors state that protons in these positions give signals exclusively in the $5.8-6.7\delta$ region of the spectrum. As there are no discernible signals in this region of the spectrum of D-2 trimethylsilyl ether, these positions may well be substituted in D-2.

It will be seen from the NMR spectra of D-2 and its trimethylsilyl ether (Figures 26 and 27) that there is a pronounced set of signals between 6.8 and 7.00δ . It is in this region that the protons of Ring C of catechin absorb (Figure 28). In the spectrum of D-2, this region is resolved into two signals, the larger of 6.8δ and the smaller at 7.0δ .

Using a planimeter, the 3.8δ peak of the D-2 spectrum, attributed to methoxyl, was integrated. Setting the area obtained as equivalent to three protons, the 6.8δ and the 7.0δ signals were likewise integrated. The result appeared to indicate that the 6.8δ signal was equivalent to one proton and the 7.0δ signal was equivalent to two protons. This type of signal would be expected from an unsymmetrical substitution such as 2', 4'- or 3', 4'- in Ring C. Catechin itself possesses the 3', 4'- substitution, and thus it is possible that D-2

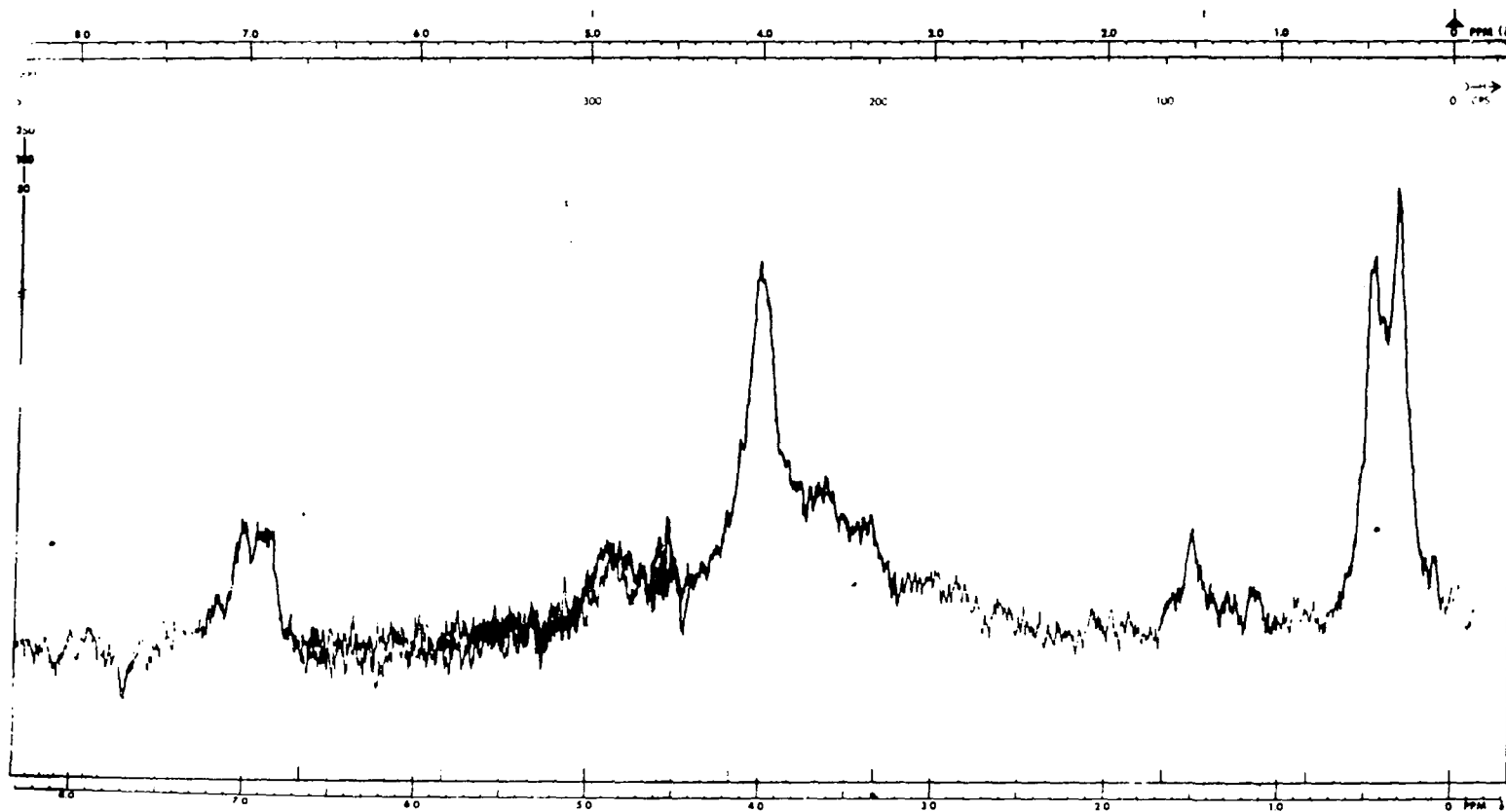


Figure 27. NMR Spectrum of D-2 Trimethylsilyl Ether
in Carbon Tetrachloride.

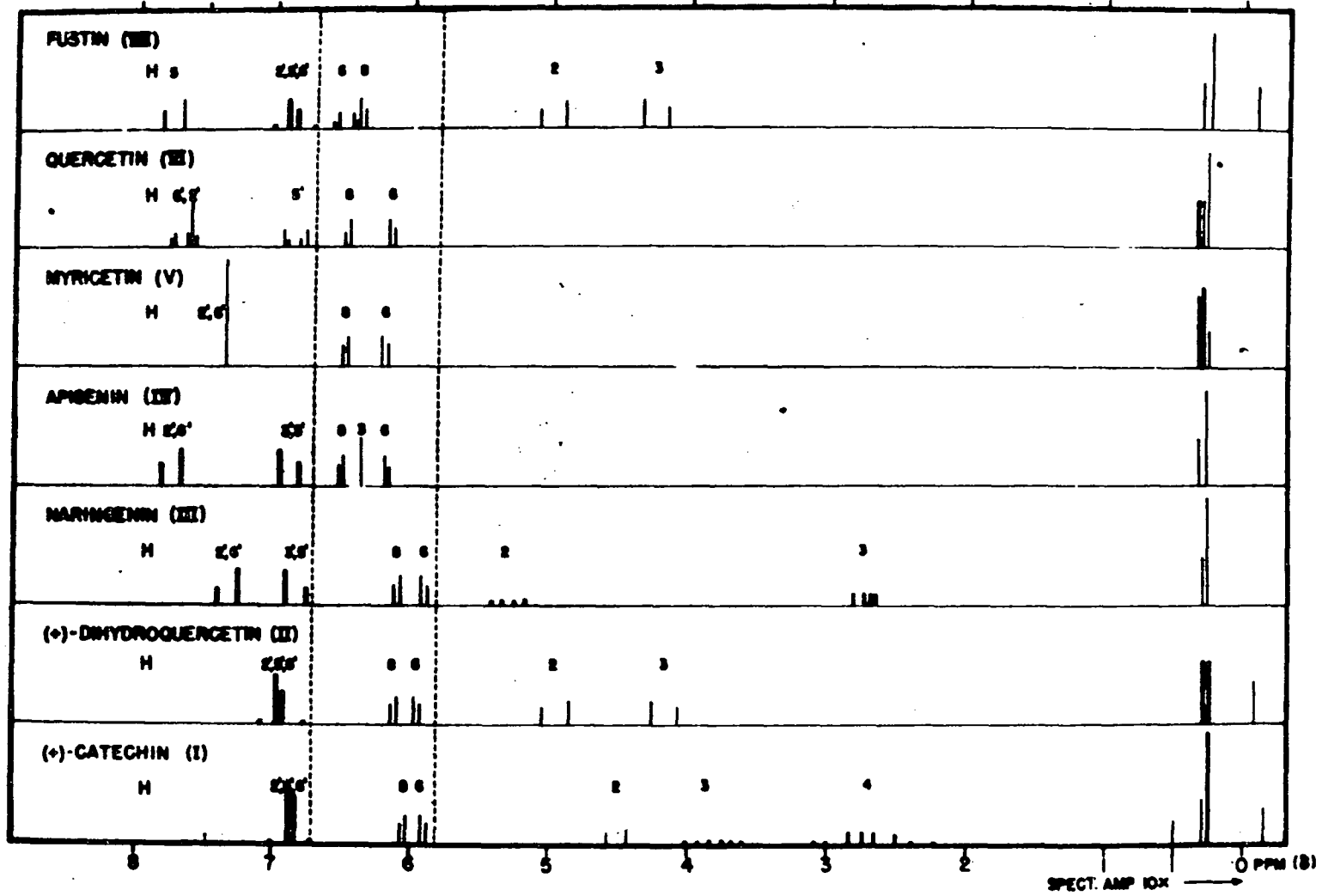
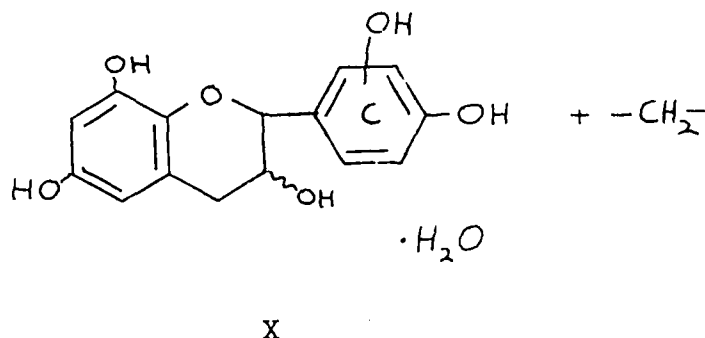


Figure 28. NMR Spectra of Some Trimethylsilyl Ethers of Some Flavonoids.³⁹

may have the 2', 4'- or 3', 4'- arrangement.

NMR data, then, favor a structural formula proposed for D-2 based on part structure X.

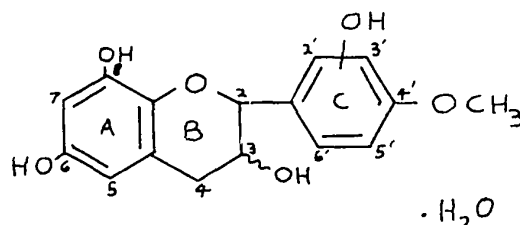


6. Placement of the Methoxyl Group

A definite location of the methoxyl group of D-2 cannot be made in this study. Such a location can only be accomplished by degradation of the molecule and identification of the fragments, or by a total synthesis. Degradation of D-2 is theoretically possible. Catechin, when treated with alkali, is decomposed into phloroglucinol and 3,4-dihydroxybenzoic acid.⁴⁶ Phloroglucinol arises from ring A and benzoic acid from ring C. Hence, degradation of D-2, and the identification of the phenol and the benzoic acid arising from this degradation would both locate the position of the methoxyl group and prove the hypothetical structure which has been advanced for D-2. Unfortunately, the quantity of D-2 available at any given time was never large enough to permit performance of such a degradation study which requires starting amounts of flavonoid in excess of several grams in order to obtain enough degradation products for positive identification. For the purpose of simplicity in this discussion, a tentative placement of the

methoxyl group will be made. A survey of 100 different flavonoids of known structure was carried out.^{46,47} In these compounds there were twenty cases of methoxyl substitution in both the 4'- and the 7- position, an intensity of substitution far greater than any other position in the flavonoid skeleton.

If the information suggested by the NMR spectra is assumed to be correct, this would suggest part structure XI as the most probable for D-2.



XI

This structure has, however, certain highly questionable points. First, it represents the first 6,8-dihydroxyflavonoid to be found in nature. All of the other flavonoids of known structure having a dihydroxy substitution in ring A, have other arrangements than 6,8-. In fact, there is a preponderance of 5,7-dihydroxy substitution.^{46,47}

Second, structure XI is difficult to reconcile with the information given from the vanillin/hydrochloric acid test. This test is supposed to give a red condensation product with a phloroglucinol structure. Such a structure is present in catechin itself (Figure 16). The test is positive, according to Bate-Smith, when a red tint of any intensity is obtained in comparison with a blank.³⁶ D-2, when tested

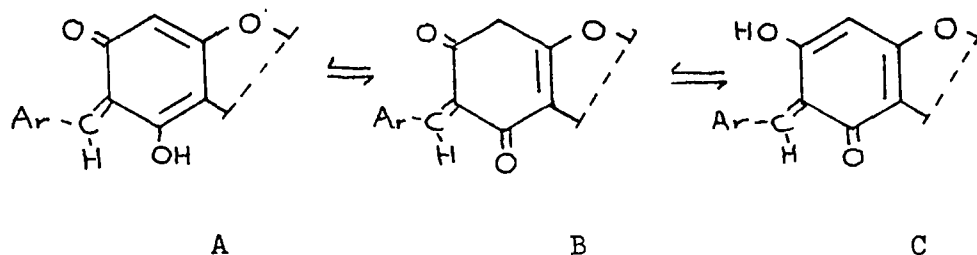
with this reagent, gave a pale pink color. Similar tests were performed on the authentic catechins, pyrogallol, resorcinol and hydroquinone.

The results of these tests are shown in Table VII.

It now becomes necessary to decide between the evidence of NMR spectra, and the evidence of the vanillin/hydrochloric acid test coupled with statistical evidence. The former evidence indicates a 6,8-disubstitution in ring A, while the latter would suggest a 5,7-arrangement.

The NMR spectrum of D-2 trimethylsilyl ether, on examination, has a considerable amount of noise in the region where signals from 6- and 8-protons would be expected. Hence this spectrum cannot be regarded as sufficiently conclusive evidence of the absence of protons at these positions in D-2. This fact, together with the overwhelming precedent against a naturally occurring 6,8-dihydroxy flavonoid, makes structure XI an improbable one.

The vanillin/hydrochloric acid test may well result in a condensation between the aldehyde and a keto form of the phenol, resulting in an equilibrium between several tautomeric forms, three of which are shown as part structures A, B, and C. These structures would

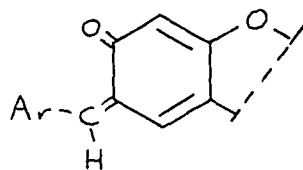


be expected to be highly colored.

TABLE VII
RESPONSES OF COMPARISON COMPOUNDS AND OF D-2
TO VANILLIN-HYDROCHLORIC ACID REAGENT

<u>Compound</u>	<u>Color</u>
(+)-catechin	Red
afzelechin	Red
epiafzelechin	Red
epicatechin	Red
robinetinidol	Pink
hydroquinone	Blue-black
pyrogallol	Purple
phloroglucinol	Red
resorcinol	Pink
<u>D-2</u>	Pink

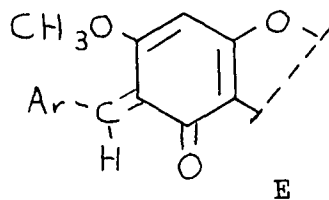
Resorcinol and robinetinidol, on the other hand, would be expected to be less reactive than phloroglucinol and the 5,7-dihydroxyflavonoids. Moreover, the principal structure which might be expected to be common to both of these compounds would be represented by part structure D.



D

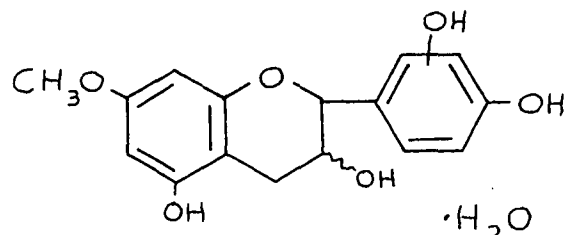
Such a structure should be less highly colored than would be expected in the case of A - C. This explanation would account for the behavior of both resorcinol and robinetinidol shown in Table VII.

If the 5- and 7- positions in D-2 are substituted by hydroxyl groups, then the results of the vanillin/hydrochloric acid test should be the same as that observed for the catechins other than robinetinidol. However, if one of these positions is occupied by a methoxyl group, it is quite possible that a less intense color would result since there would be only one possible keto form. Placement of the methoxyl group at position 7, for the reasons mentioned earlier in this section, would produce a condensation product of a type shown in structure E.



E

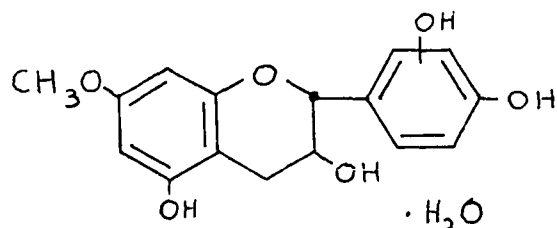
This would result in part structure XII as the most probable structure of D-2 after all the evidence is considered.



XII

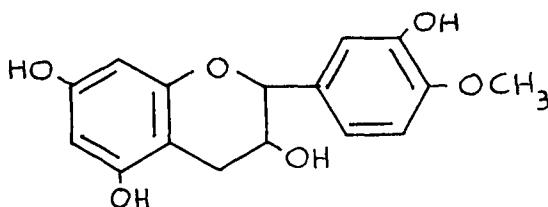
7. Stereochemical Relationships in Ring B

The stereochemical relationship of C-2 to C-3 remains to be assigned in this structure. On the basis of the similarities between the infrared spectra of D-2 and (+)-catechin, the hydrogens of C-2 and C-3 have been tentatively assigned a trans configuration. Use of molecular models seems to suggest that such a configuration, with the hydrogens occupying the axial positions, would result in a minimum of interference between the various ring systems of the molecule. This assignment is, however, purely hypothetical, as configurational analysis cannot be accomplished by infrared comparisons, and the existence of (-)-epicatechin, which was shown to have a cis configuration at C-2 and C-3,^{60,61} indicates that such a cis arrangement for D-2 cannot be ruled out by studies with models. A configurational relationship with another system would have to be made before a stereochemical configuration can be definitely established. The tentative assignment, however, would result in part structure XIII for D-2. A structure of the type proposed here has a relatively uncommon feature in that it represents a naturally methoxylated catechin. Only one other naturally methoxylated catechin



XIII

has been reported in the literature. This was the aglycone of a glycoside extracted from Arachis hypogaea by Teyeau and Masquelier.⁶² Structure XIV was advanced for this compound. However, no properties



XIV

of the catechin were reported, and no evidence for the choice of the structure was furnished.

There are, however, a number of naturally methoxylated flavonoid compounds which are well characterized,^{46,47} and thus the presence of such a substituent on D-2 is not unreasonable.

Since D-2 appears to represent a new member of the catechin series of flavonoids, the name mailein is proposed for this compound.

8. Summary

Compound D-2, hereafter referred to as mailein, was shown by elemental analysis to be a C-16 compound having a single methoxyl group and five active hydrogen atoms. Mailein was shown by application of

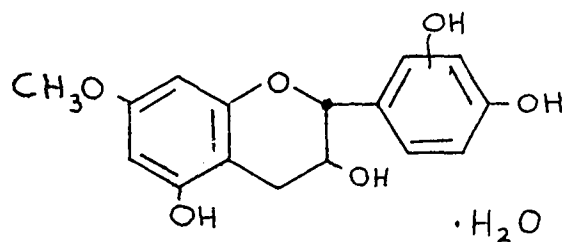
classification tests to be, in all probability, a catechin. Both infrared and ultraviolet spectra could be interpreted in support of this hypothesis.

Mailein is believed to crystallize from water as a dihydrate, which may be converted under drastic drying to a monohydrate. This hypothesis was confirmed both by elemental analyses and by melting point comparison.

NMR spectra of mailein and its trimethylsilyl ether confirmed the existence of water of hydration, and suggested either a 2', 4'- or a 3', 4'- arrangement of ring C. There was also a possibility that ring A might be substituted in the 6- and 8- positions, suggested by the NMR spectrum of the trimethylsilyl ether of mailein.

The evidence furnished by the NMR spectra regarding the substitution of ring A was felt to be inconclusive in comparison with the substitution patterns of known flavonoids. Alternatively, a 5-hydroxy-7-methoxyflavan was proposed. This structure was felt to account for the observed behavior of mailein with vanillin in hydrochloric acid.

Tentative assignment of a trans configuration to the C-2 and C-3 positions of ring B leads to the postulation of structure XIII as a hypothetical structure of mailein.



XIII

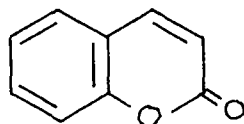
IV. CONCLUSIONS

A. Alkaloids

An exhaustive search was made for alkaloids in Alyxia olivaeformis. Although different isolation schemes were employed, which had previously been successful, no alkaloids were obtained. The apparent positive response of the plant to the initial screening tests appears to be an example of a "false-positive" test.

B. Coumarin

During the attempts to obtain alkaloids from the plant, a compound was isolated which was found to be coumarin (VII). This compound was also shown to be the principal volatile constituent of A. olivaeformis. The odor of this plant is therefore due to the presence of coumarin in its tissues. The location of the odoriferous portion of the plant could not be precisely determined, but it is believed to be concentrated in the leaves and bark of the vine.



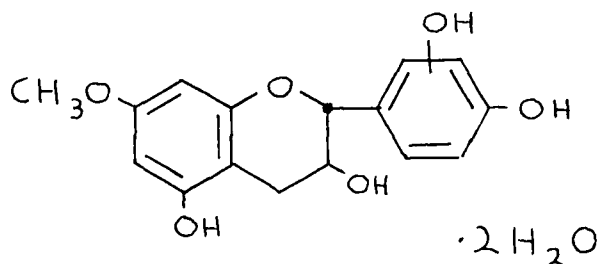
VII

C. Mailein

A second non-nitrogenous compound was isolated from A. olivaeformis during the unsuccessful alkaloid isolation schemes. This

compound was shown to have a probable structure based on a C-16 formula. From this assumption it was shown that the compound was probably a flavonoid, and possibly a catechin.

Based on the reasonable assumption that the compound is a catechin, interpretation of various molecular spectra and of analytical data resulted in a proposed molecular formula of $C_{16}H_{16}O_6 \cdot 2 H_2O$ for D-2, and a tentative structure XIII. This compound represents a new member of the catechin series of flavonoids and the name mailein is proposed.



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