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A CHEMICAL STUDY OF THE BITTER PRINCIPLE OF PIA

"

(Tacca Leontopetaloides (L.) O. Ktze)

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I. INTRODUCTION

Pia belongs to the plant family Taccaceae, which consists of approximately thirty species (1), widely distributed over the warmer regions of the globe (2). Taccaceae contains only two genera, Schizocapsa and Tacca. The former consists of only two species located in the South China-Malaya area, while Tacca, which is sub-divided into three sections - Ataccia, Palmotacca, and Eutacca - is more widely distributed (3).

Limpricht (4) accepted the name Tacca pinnatifida J. & G. Forst. for pia, but the presently accepted name is Tacca Leontopetaloides (L.) O. Ktze (5)*.

Pia is probably native to tropical Asia and of aboriginal introduction to Hawaii (6), although the exact origin of this species is not known (7).

Pia is an herb consisting of tuberous underground stems similar to the potato, with leaves that rise directly from the ground and which resemble papaya leaves. The stem, which may reach a height of four feet or more, is topped by a striking inflorescence of small green and purplish flowers (8) (see Fig. 1).

The pia plant was cultivated for its starch content and was produced in Hawaii in sizable quantities in the 1850's for export to Europe under the name "arrowroot" (9). The plant is rarely cultivated now, but small

* Another common synonym of pia is Tacca hawaiiensis. However, cf. reference (1), p. 30.

quantities can still be found growing wild near Hilo in the Puna district.

The pure starch from the pia tubers was combined with coconut cream, bananas or ti root juice to yield food staples eaten by most Polynesians. It was well known to the Hawaiians, however, that a bitter material had to be removed from the raw tuber in order to make the starch edible. The raw tuber has been reported to be toxic (10) and only the Easter Islanders are reputed to eat the unwashed tuber, but only after baking (11).

The Hawaiians grated the tubers and washed them in running water for prolonged periods. The resultant bitter extract was used in this dilute state as a medicine to combat diarrhea and dysentery, particularly in infants (12).

In 1857, Lépine carried out a superficial chemical analysis of pia and reported 2.2% of a bitter extract (13). This report is the sole published record of a chemical nature concerning pia. Except for a similar paper dealing with the bitterness and toxicity of Tacca umbrarum* (14), no chemical study has been made of any species of this plant family.

Since no chemical compounds have been isolated from Taccaceae, a prediction of the general nature of the bitter principle cannot be made. It was hoped that a search of the literature for bitter principles from other plants might indicate some relationship from which the bitter principle of pia could be categorized as to type.

Some representative bitter principles of current interest, some

* Tacca umbrarum is synonymous with T. madagascariensis (15), which is endemic to Madagascar where pia is found in abundance. Pia is closely related to T. madagascariensis taxonomically, which indicates that their bitterness might arise from the same (or a similar) chemical substance.

TABLE I

SOME NON-NITROGENOUS BITTER PRINCIPLES ISOLATED FROM PLANTS					
Name	Source	Formula	Chemical Type or Functional Groups	Pharmacological Action	Reference
Pikrosalvin	<u>Salvia officinalis</u>	$C_{20}H_{26}O_4$	Diphenolic lactone	Bacteriostat	16
Limonin	<u>Citrus aurantium</u> <u>var. Natsudaikai</u>	$C_{26}H_{30}O_8$	"Furano dilactone"	Not reported	17
Lactucin	<u>Lactuca virosa</u>	$C_{15}H_{16}O_5$	Dihydroxy-alpha, beta-unsaturated ketone-guaianolide	Not reported	18
Tenulin	<u>Helenium</u> <u>tenuifolium</u>	$C_{16}H_{20}O_5$	Lactone-hemiacetal-alpha, beta-unsaturated ketone-guaiane skeleton	Not reported	19
Geigerin	<u>Geigeria aspera</u>	$C_{15}H_{20}O_4 \cdot H_2O$	"Monolactone"	"Pharmacologically active"	20
Absinthin	<u>Artemisia</u> <u>absinthium</u>	$C_{30}H_{40}O_6$	Dihydroxy-dimeric guaianolide	Inconclusive	21
Darutoside	<u>Siegesbeckia</u> <u>orientalis</u>	$C_{26}H_{44}O_3$	Hydrolysis yields glucose, a glycol and a trihydroxy diterpenoid compound	Not reported	22
Alantopicrin	<u>Inula helenium</u>	$C_{17}H_{24}O_4$	Lactone ?	Fish poison	23

TABLE I (cont'd)

Picrolichenic acid	<u>Pertusaria amara</u> (crustose lichens)	$C_{25}H_{30}O_7$	Depsidone	Anti-malarial	24
Marrubiin	<u>Marrubium vulgare</u>	$C_{17}H_{22}O_6$	Lactone-carboxylic, carbonic acid anhydride containing spiro linkage	?	25
Nimbin	<u>Melia indica</u>	$C_{28}H_{40}O_8$	Steroidal lactone	Cardiac	26
Kondurangin	<u>Marsdenia</u> <u>condurango</u>	?	Glycoside. Aglycon is dodecahydrofluorenone derivative	Carcinogen	27
Elaterin	<u>Ecballium</u> <u>elaterium</u>	$C_{32}H_{44}O_8$	Diosphenol grouping	Anti-tumor activity	28
Acetylandromedol	<u>Rhododendron</u> <u>maximum</u>	$C_{22}H_{36}O_7$	Acetate of hexahydroxy compound	Hypotensive agent	29

of which are still incompletely characterized, are listed in Table I. Only those containing carbon, hydrogen and oxygen are recorded, since they are more pertinent to this investigation, as will be seen later.

It is apparent from Table I that non-nitrogenous bitter principles occur in widely diverse plant types - flowering plants and even lichens. Chemically, several skeletal types can be distinguished: steroid, terpenoid, aromatic and depsidone, any of which may be glycosidically bound to a sugar moiety. Although most of the compounds are highly oxygenated, the nature of the oxygen functions vary widely and any relationship which might exist is not immediately apparent. Due to this great diversity of chemical types found in plants, little a priori information can be surmised about the bitter principle of pia. It is interesting to note that many of these bitter principles possess pharmacological activity of great diversity.

Because of the very small amount of the bitter principle that was obtained by extraction of the tubers, only a limited quantity was available for structural studies. Although micro methods were used, checking of results and duplicate runs frequently were impossible.

The object of this investigation was to isolate, purify and characterize the bitter principle found in pia.

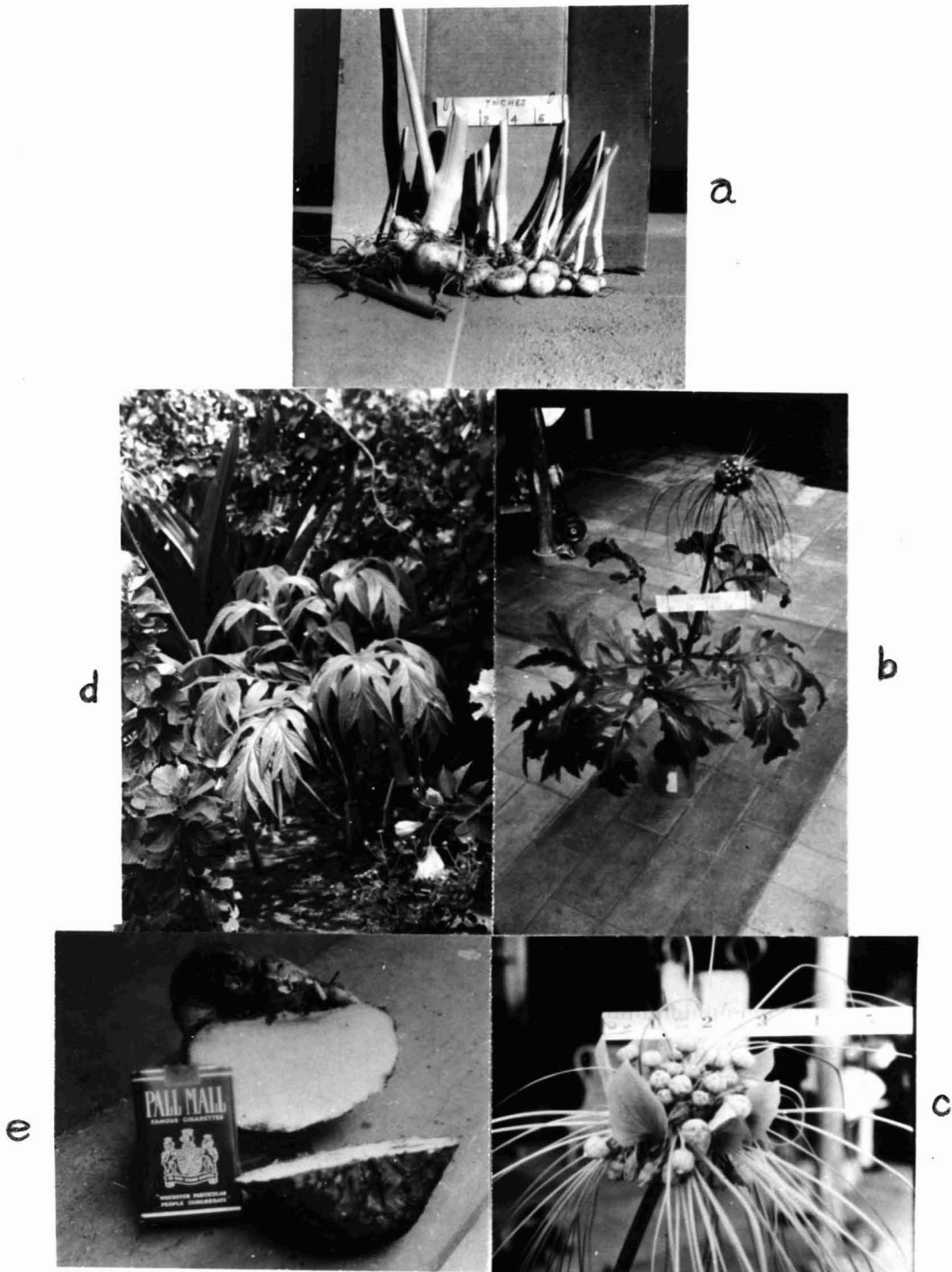


FIG. 1. THE PIA PLANT, *Tacca Leontopetaloides* (L.) O. Ktze.
 (a) tubers, (b) flowering plant, (c) inflorescence,
 (d) cross-section of tuber, (e) growing plant.

II. EXPERIMENTS AND RESULTS*

A. Extraction Procedure

The pia tubers used in the preliminary stages of this investigation were obtained from the Puna and Kau districts on the island of Hawaii. The large quantities required in the later stages of the study were obtained from the Carolines and Marshall Islands.** Botanical identification of all pia used in this investigation was made by Dr. Harold St. John of this University's Botany Department. It was noted that the tubers obtained from Micronesia were generally larger and less bitter than those from Hawaii. It became quite evident during this study that the more mature tubers were sweeter and distinctly less bitter than the younger tubers. This difference was apparently due to age and not to geographical distribution.

One method of preparing the tubers for extraction involved careful washing with water, slicing each tuber in half and drying at 55°C. in a forced-convection oven for 36-54 hours. In processing 100 pounds or more of tubers, it was necessary to grind the tubers in a meat grinder

* Melting points were taken on a Fisher-Johns block and were uncorrected unless stated otherwise. Ultraviolet spectra were determined on a Beckman Model DU spectrophotometer unless otherwise noted. Infrared spectra were obtained with a Beckman Model IR-2A spectrophotometer equipped with sodium chloride optics unless otherwise noted. Microanalyses were carried out by Dr. Alfred Bernhardt, Mikroanalytisches Laboratorium, Muhlheim, Germany, unless otherwise noted. Optical rotations were determined with a Rudolph polarimeter (scale, 0.01°). $\log E = \log (\text{Absorbancy/concentration in mols per liter})$.

** The author is grateful to Mrs. Mary K. Hamili and Mr. Lester W. Bryan of the island of Hawaii and to Mr. Benjamin C. Stone of this University for supplying pia for this study.

and dry the resulting pulp in a stream of hot air.* The latter method was less satisfactory since some fermentation was noted, but was unavoidable on account of the limited size of drying facilities at the time.

The oven-dried tubers were ground in a Wiley mill to pass 10 mesh before extraction. The wet-processed tubers were suitable for extraction without further grinding.

Preliminary studies showed that refluxing methanol, 95% ethanol or acetone removed the bitterness from the tubers completely and relatively quickly. However, difficulties were invariably experienced in the following separation scheme, but could be averted by a prior petroleum ether extraction which did not remove the bitter principle. A petroleum ether extraction followed by acetone proved to minimize the difficulties experienced in the isolation stage and was adopted for all subsequent extractions.

B. Isolation and Purification of the Bitter Principle (Taccalin)

The acetone extract of 1.1 kg. (dry) of "defatted" tubers was stripped in vacuo on a water-bath (50°C.) and the resulting reddish-brown gum dried at 40°C./30 mm for 48 hours. Yield, 22g (2.0% based on dry weight)** The gummy solid was dissolved in a minimum amount (ca.

* The author is indebted to Mrs. Eleanor D. Tyau for making this equipment available and for her assistance in this tedious chore.

** This value compares favorably with the 2.3% obtained by Lépine (13) by water and alcohol extractions.

125 ml.) of refluxing acetone* and adsorbed on 450 g. of Florisil, previously prepared by wet-packing with acetone in a 5-cm.-diameter column. The column was developed and eluted with acetone, the first eluate being yellow, bitter and practically unadsorbed. A narrow yellow band was eluted next, followed closely by a light brown band. Further elution with acetone failed to elute any additional bands. By gradually increasing the amount of methanol in acetone as the developer, a yellow band followed by a narrow light purple band were eluted separately. With pure methanol, an additional dark purple band was eluted, leaving a brownish-purple material adsorbed at the top of the column. A small amount of pink material could be eluted with dioxane: water (1:2), but the majority of the strongly bound colored substances could not be eluted with any solvent. Extrusion of this colored band and extraction with boiling dioxane, water or nitrobenzene failed to remove any significant amount of material. The various colored substances which were removed - generally in good purity - were not investigated further as none were bitter.

The yellow, bitter eluate from the column (ca. 300 ml.) was concentrated in vacuo to 200 ml. 50 ml. of water was added and the solution was cooled in the refrigerator. The yellow, amorphous precipitate which formed was collected and dried, giving 910 mg. (0.08%) of crude, bitter powder. This yellow powder was rechromatographed over Florisil with chloroform as developer. The first 75 ml. of eluate was bright yellow and non-bitter; the next fraction of 300 ml. was light yellow and

* 5.6 g (0.4%) of a brown, non-bitter residue failed to dissolve. This residue yielded 110 mg. of sucrose (m.p., mixed m.p.) by trituration with water and slow evaporation of the aqueous solution.

bitter. Further elution of the column with acetone, followed by methanol, failed to produce any bitterness in the eluate. The first eluate, free of bitterness, was evaporated to dryness in vacuo and yielded 10 mg. of a yellow viscous oil possessing a characteristic odor reminiscent of fats. A preliminary investigation of this oil indicated the presence of a complex ester, the properties of which are described in the Appendix (see ester (I)).

The light yellow, bitter eluate from the column was stripped and dried in vacuo, dissolved in 60 ml. of hot acetone and an equal volume of water added. The light yellow precipitate that formed was collected and air-dried to give 35 mg. of a light yellow powder. The mother liquor yielded an additional 9 mg. to give a total yield of 44 mg. (0.004%)* of a fairly pure bitter principle.

Adsorption chromatography over alumina or Florisil with various solvents failed to remove the last traces of the yellow component from the bitter principle. Purification was ultimately achieved by fractional precipitation from acetone-water and ether-petroleum ether mixtures, employed alternately. The crude bitter material treated in this way afforded 31 mg. (0.003%) of a white, amorphous powder which was named taccalin. Taccalin tasted intensely bitter, not unlike strychnine.

Attempts to induce taccalin to crystallize failed. The following solvents and solvent pairs were tried, without success, to obtain a

* The yields varied from 0.010% for some small batches of tubers from Hawaii, to 0.003% using large quantities from Micronesia. A total of 132 kg. of tubers (field weight) weighing 30.5 kg. dry, were used for this study.

crystalline product: n-butanol, methyl ethyl ketone, benzene, ethyl ether, cyclohexanone, chloroform, water: ethyl ether (1:1), water: isopropyl alcohol (1:1), benzene: acetone (2:1), chloroform: acetone (1:1) and dimethylformamide with and without water. Small colorless plates were obtained with wet ethyl ether, but the crystals collapsed upon removal of solvent. A sample of taccalin in dimethylformamide - one of the most encouraging solvents in preliminary tests - failed to crystallize, even after two years in the refrigerator.

One attempt was made to sublime taccalin under reduced pressure. As the temperature was raised at a pressure of 1 mm, sintering took place at about 120°C. and melting occurred at ca. 180°C. No indication of sublimation was apparent up to the decomposition point of ca. 200°C.

C. Criteria for Homogeneity

Since taccalin could not be obtained crystalline, some criterion of purity was needed in order to demonstrate its homogeneity. The melting point was useless, being broad and variable. Constancy of repeated carbon and hydrogen analyses, frequently used in the natural products field, was not feasible due to the small quantity of taccalin isolated. However, paper chromatography and absorption in the ultra-violet region provided good evidence of homogeneity.

The yellow oil, shown to be an^{*} ester (I) (see Appendix), was a known contaminant of taccalin by its behavior in the work-up. Ester (I) absorbed quite strongly in the 300-400 μ region ($\log E = 2.0-2.7$; see Fig. 2), while taccalin was essentially transparent in this region see Fig. 3). A small amount of ester (I) contaminated with taccalin

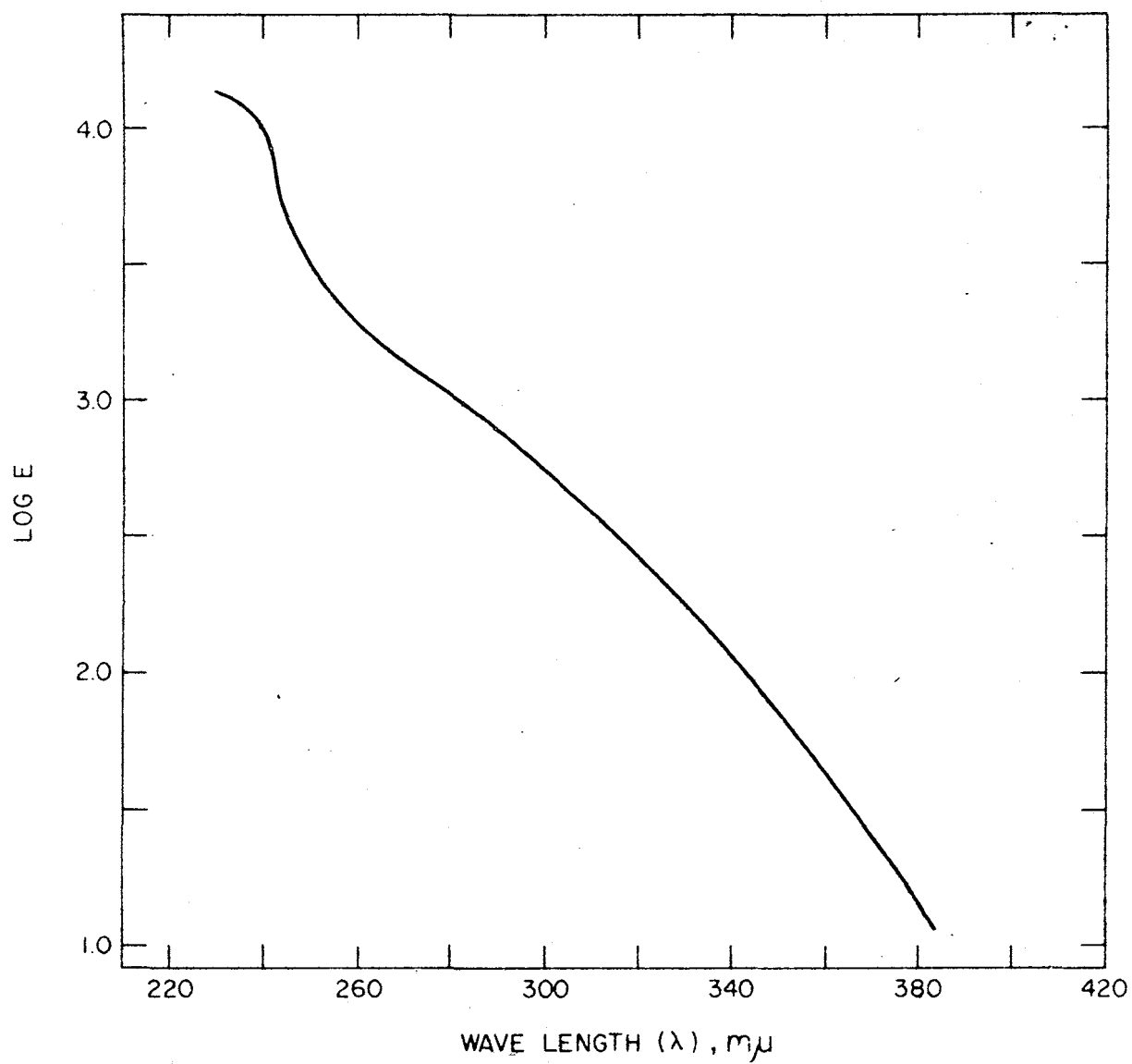


FIG. 2UV ABSORPTION SPECTRUM OF ESTER (I) IN ETHYL ETHER
($C = 5.0 \times 10^{-1}$ g/L)

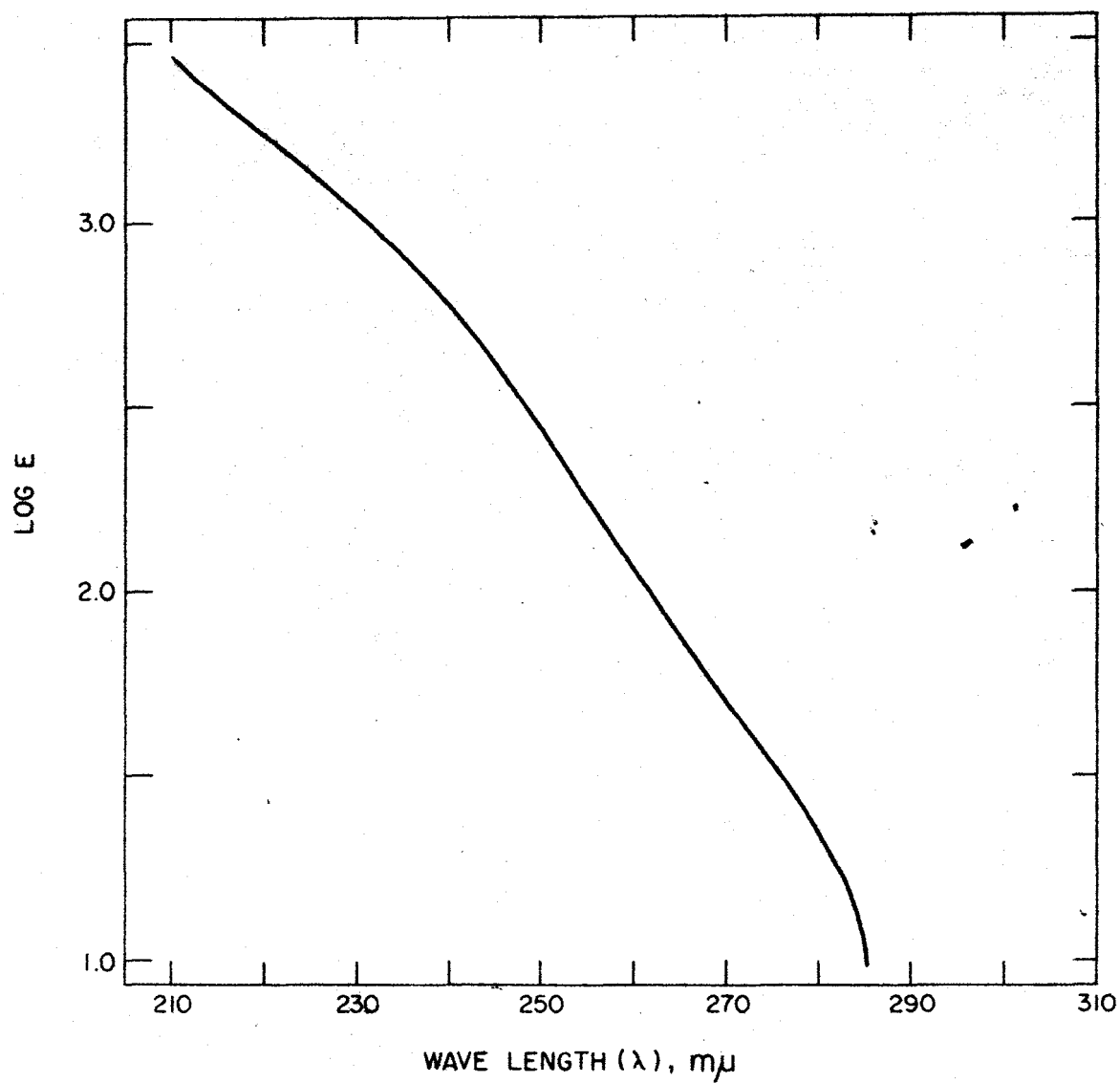


FIG. 3 UV ABSORPTION SPECTRUM OF TACCALIN IN METHANOL
($C = 4.42 \times 10^{-2}$ g/L).

would readily reveal itself and the extent of its removal could be systematically followed by measuring the UV absorption of taccalin after each precipitation. Although this technique was successful in demonstrating the absence of ester (I), what about possible impurities which were transparent in the UV?

Ascending paper chromatography of taccalin on Whatman #1 paper with n-butanol: acetic acid: water (40:10:50, organic phase) produced only one spot with a short wavelength UV light (white non-fluorescent spot against a purple fluorescent background), $R_f = 0.28$. The above experiment was a good indication that only one component was present, but this demonstration did not preclude the existence of isomers or closely related compounds. A chromatogram produced in at least one other solvent system would have supported the above experiment, but two other solvent systems failed to yield any information. These were heptane: n-butanol: acetic acid: water (various concentrations) and heptane saturated with isopropyl alcohol.

Evidence was presented above which indicated that taccalin was homogeneous, but the evidence was not conclusive.

D. Characterization Studies

1. Physical and Chemical Properties.

(a) Molecular weight: The molecular weight of taccalin was determined by isothermal distillation in a commercial apparatus slightly modified from that described by White and Morris (30). Ca. 12 mg. of taccalin was equilibrated against azobenzene as the standard, with acetone as solvent. After 21 days at room temperature, as equilibrium was nearly attained, erratic readings developed. The calculated molecular weight at this

point was 321 and was increasing slowly.

(b) Optical rotation: The optical rotation of taccalin was determined in a one-dm. glass tube at 27°C. using sodium D illumination at a concentration of 2.2 g./l. in methanol. The observed rotation of -0.17° was just within the experimental error of the blank. Unfortunately, more taccalin was not available at the time nor was a micropolarimeter tube. The specific rotation was calculated using a deviation of 0.03°

$$\left[\alpha\right]_D^{27} = 0 \pm 45^\circ \text{ (c = 2.2 g./l. in methanol)}.$$

(c) Spectral data: Taccalin showed only end absorption in the UV region; $\lambda_{210} \text{ m}\mu$, $\log E = 3.46$ in methanol (see Fig.3).

IR max. = 3400 (M)*, 1824 (M), 1756(S), 1252(S) cm.^{-1} (see Fig. 4).

(d) Chemical tests and solubility: A sodium fusion indicated only carbon, hydrogen and oxygen to be present. Taccalin was very soluble in methanol and acetone, moderately soluble in chloroform, slightly soluble in benzene and ethyl ether, very slightly soluble in water, and insoluble in hydrocarbons and carbon tetrachloride.

Taccalin gave positive tests with ceric ammonium nitrate (hydroxyl) and with chromic acid anhydride (tertiary hydroxyl) after Houben-Weyl (31). Negative tests were obtained with ferric chloride (enol), 2,4-dinitrophenylhydrazine (ketone or aldehyde), and tetranitromethane (isolated double bond). Bromine in carbon tetrachloride (dioxane

* IR absorption intensity abbreviations: S = strong, M = medium, W = weak, sh = shoulder and Br = broad.

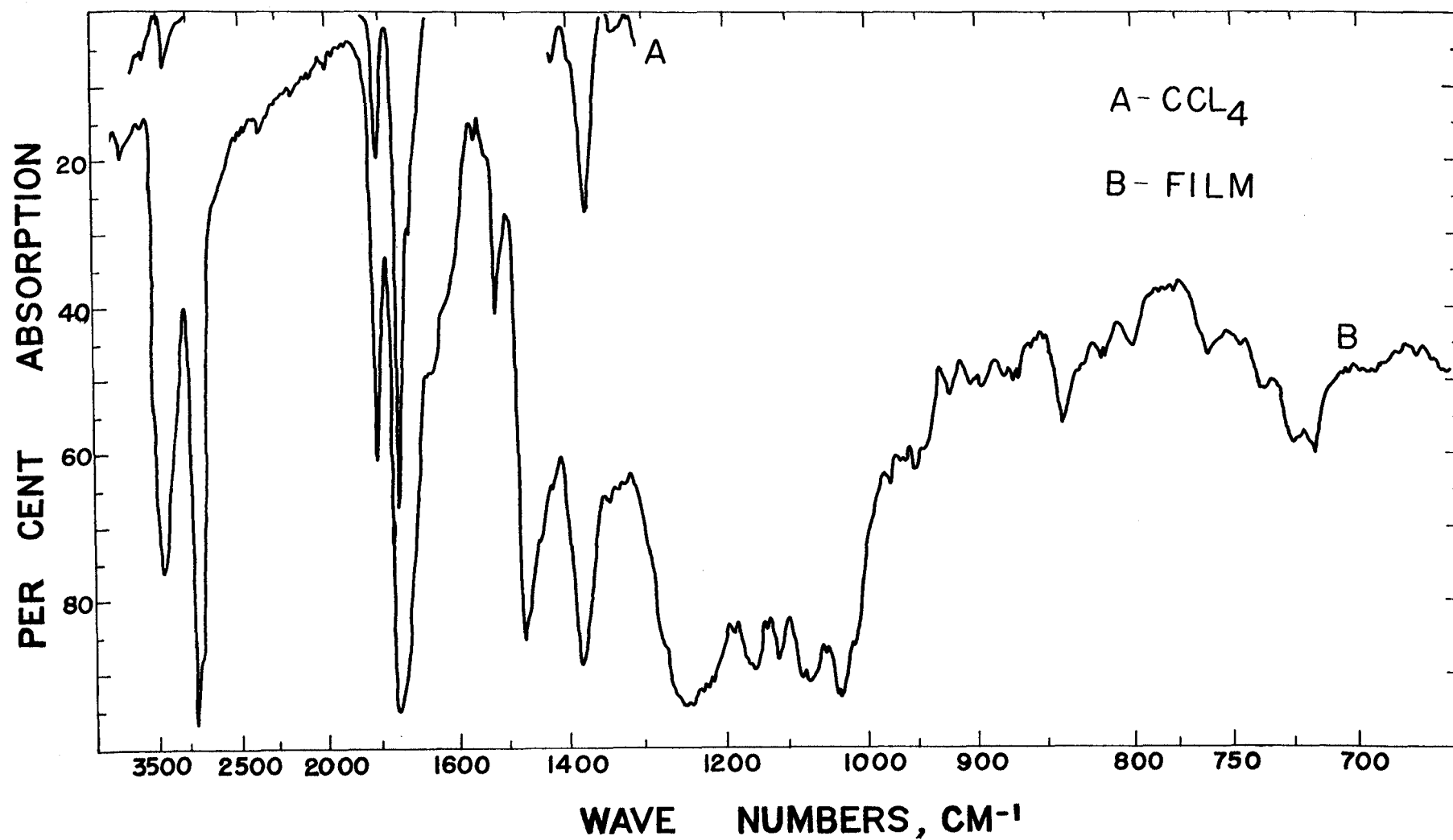


FIG. 4 IR SPECTRUM OF TACCALIN

solvent) was discharged very slowly (no HBr) and a ferric hydroxamate which formed appeared unstable, the color fading in 5 - 30 minutes.

(e) Elemental analyses: The samples for analysis were purified according to the scheme given previously and dried at 80°C./25 mm. for six hours.

	<u>% C</u>	<u>% H</u>	<u>% O</u>
Sample #1*	61.0	7.0	- -
Sample #2	62.3	7.1	30.5
	62.4	7.1	- -

Sample #2 was of doubtful purity and sample #1 was based on an ignition weight of only 1.2 mg. These data will be discussed in Section III A.

(f) Functional group analyses:

(1) Active hydrogen: A single Zerewitinoff determination gave 1.1% active hydrogen, as measured by methane evolution, plus 1.1% addition of Grignard reagent without the formation of methane. This unexpected, but informative, result will be discussed in Section III A.

(2) C-CH₃ determination: It is well known that certain groupings give less than the theoretical amount of acetic acid in the Kuhn-Roth oxidation for C-CH₃ groups. Since taccalin was thought to have an isopropyl or gem-dimethyl grouping (vide infra), which are known to give only 40% of theory of acetic acid in the Kuhn-Roth oxidation (32), a C-CH₃ determination by quantitative measurement of the 1380 cm.⁻¹

* Analysis by Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England.

C-CH₃ band in the IR region was used. The method of Barton and Elad (33) was followed except that an external standard was used in lieu of an internal standard. Procedure: A solution of 3.0 g./l of taccalin in reagent-grade carbon tetrachloride was placed in a 1.0 mm. sodium chloride cell and its absorption recorded several times in the 1380 cm.⁻¹ region with several slit schedules. A standard solution of cholesteryl acetate was treated in a similar way. A base line was established by measuring the absorption of carbon tetrachloride of 1.0 mm. thickness at 1380 cm.⁻¹. The relative areas under the 1380 cm.⁻¹ absorption bands were measured with a Leitz planimeter and the areas averaged for each slit schedule. Using 5 C-CH₃ groups for cholesteryl acetate, the relative area / C-CH₃ was calculated for the standard and the number of C-CH₃ groups calculated for taccalin for each slit schedule. At the two optimal slit schedules of 1.00 and 1.10 mm. the number of C-CH₃ groups computed for taccalin was 3.1 ± 0.1 and 4.2 ± 0.1 respectively.

Measurements of menthol and borneol indicated an expected accuracy of $\pm 10\%$ at a given slit schedule.

(g) Microhydrogenation: The uptake of hydrogen over Adams' catalyst in methanol was obtained with a Swiss-made apparatus, slightly modified from the one described by Clauson-Kaas and Limborg (34). Uptake of hydrogen amounted to 0.05 mols/mol of taccalin after three days and 0.07 mols/mol of taccalin after five days.

2. Structural Studies

(a) Acetylation Experiments: Attempts to acetylate taccalin using acetic anhydride and dry pyridine at reflux for twelve hours resulted in

recovery of starting material in fair yield. No other products were discernible (IR). Refluxing for twenty-four hours with acetic anhydride and catalytic amounts of 85% phosphoric acid led to extensive decomposition - only a small amount of starting material remained (IR).

Acid (II): Fifteen milligrams (0.042 mmol) of taccalin, which was recovered from the acetylation attempt described above, was dissolved in 5 ml. of freshly distilled acetic anhydride and 5 mg. of fused sodium acetate was added. The mixture was refluxed for two hours, cooled and allowed to stand at room temperature for two months protected from moisture. The mixture was made distinctly acid with 6 N hydrochloric acid and extracted with 5 X 5 ml. of ethyl ether. The volume was reduced to half in vacuo and petroleum ether (40-60°) added drop by drop until a cloudiness appeared. After standing in the refrigerator for two months, small colorless platelets were found on the surface of the solution. The solution was decanted and the crystals washed with water and dried at 80°C./30 mm. for two hours. Yield, 5 mg.; m.p. 111-26°C., Acid (II).

IR max. = 3165-3356 (Br), 2530-2604 (Br), 1712(S), 1420(W),
1234-1261 (Br) cm.⁻¹ (see Fig. 5).

(b) Opening of the Anhydride: Since an anhydride was indicated by the IR spectrum (vide supra) (35), attempts were made to open the anhydride with various nucleophiles with the aim of obtaining a crystalline derivative.

Taccalin was stable to water, even at the boiling point for short periods. A slightly stronger base - methanol - was used next. Refluxing methanol for 48 hours failed to yield the expected half-ester (36).

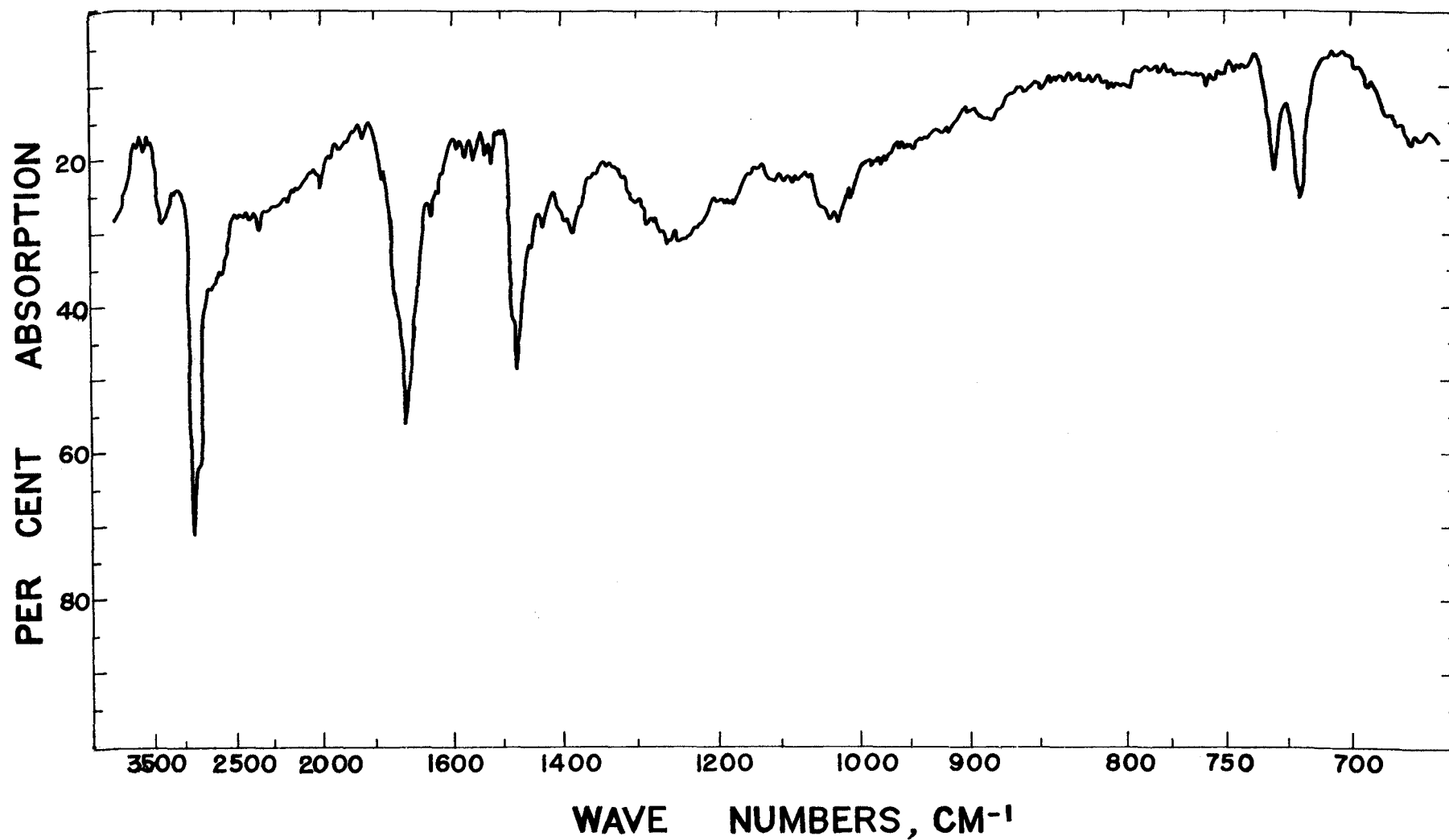


FIG.5 IR SPECTRUM OF PRODUCT FROM "ACETYLATION" OF TACCALIN-
ACID (II); FILM FROM CHLOROFORM

Only unchanged starting material was recovered in good yield (IR).

Attack by amines was equally disappointing, but for different reasons.

Both aniline and o-phenylenediamine gave dark colored gums which failed to crystallize. No taccalin was recovered.

A series of hydrolyses using aqueous potassium hydroxide and sodium carbonate at various temperatures were conducted in order to establish the conditions necessary for acid formation from the anhydride. Taccalin dissolved in both bases at 100°C. to give yellow solutions. Upon acidification, a sweet odor was noted, but a crystalline product could not be obtained by extraction of the aqueous solution. At room temperature, sodium carbonate produced only starting material (IR), even after long standing. Hydrolysis with aqueous potassium hydroxide at room temperature proved more fruitful.

Acid (III): Four milligrams (0.011 mmol) of taccalin was suspended in 5 ml. of aqueous 6 N potassium hydroxide and shaken intermittently for one week at room temperature. The solution was neutralized with 6 N hydrochloric acid to pH 6 with cooling (sweet odor noted) and extracted with 10 X 5 ml. of ethyl ether, the ether dried over sodium sulfate and the ether stripped in vacuo. The white solid was collected, washed with water and dissolved in ca. 5 ml. of 95% ethanol. The solution was evaporated slowly at room temperature until a cloudiness appeared. A drop of absolute ethanol was added and the clear solution was allowed to stand in the refrigerator overnight. The white precipitate which formed was filtered, washed with water, air-dried and crystallized from absolute ethanol by slow evaporation at room temperature. Small, transparent hexagonal plates were produced after long standing. Yield, less than

1 mg., m.p. 153-154°C. The substance dissolved in sodium bicarbonate solution with evolution of carbon dioxide.

A repetition of the above experiment designed to produce large amounts of the acid led to a semi-crystalline white solid, m.p. 130-41°C., which resisted further crystallization. Its properties were the same as that of the acid above, however. Labeled acid (III).

IR max. = 3333 (Br), 2857 - 2500 (Br), 1733 (S), 1706 (S) cm.⁻¹
(see Fig. 6).

Volatile fragments: A hydrolysis similar to the above was conducted in order to investigate the volatile fragment noted upon acidification. Eleven milligrams (0.031 mmol) of taccalin was placed in a test tube and heated with 2 N aqueous sodium hydroxide at 85°C. for 1 hour. The cooled, yellow solution was neutralized carefully to pH 3 with 6 N hydrochloric acid. The solution was cooled to 5°C. and saturated with sodium chloride. The solution was decanted from the yellow gummy solid and extracted continuously with ethyl ether for 72 hours employing a cold-finger type condenser at 5°C. The ether was dried over sodium sulfate and the ether stripped carefully at ca. 15°C. in vacuo. The residual liquid had a peppermint-like odor. Yield, ca. 6 mg.

A GLPC* was obtained on a small amount of the liquid and compared with mesityl oxide and acetone under the same conditions (see Fig. 7). The eluate corresponding to peak C (Fig. 7) was trapped on a salt plate

* The author is indebted to Mr. Dawes N. Hiu for the gas-liquid-partition chromatograms (GLPC).

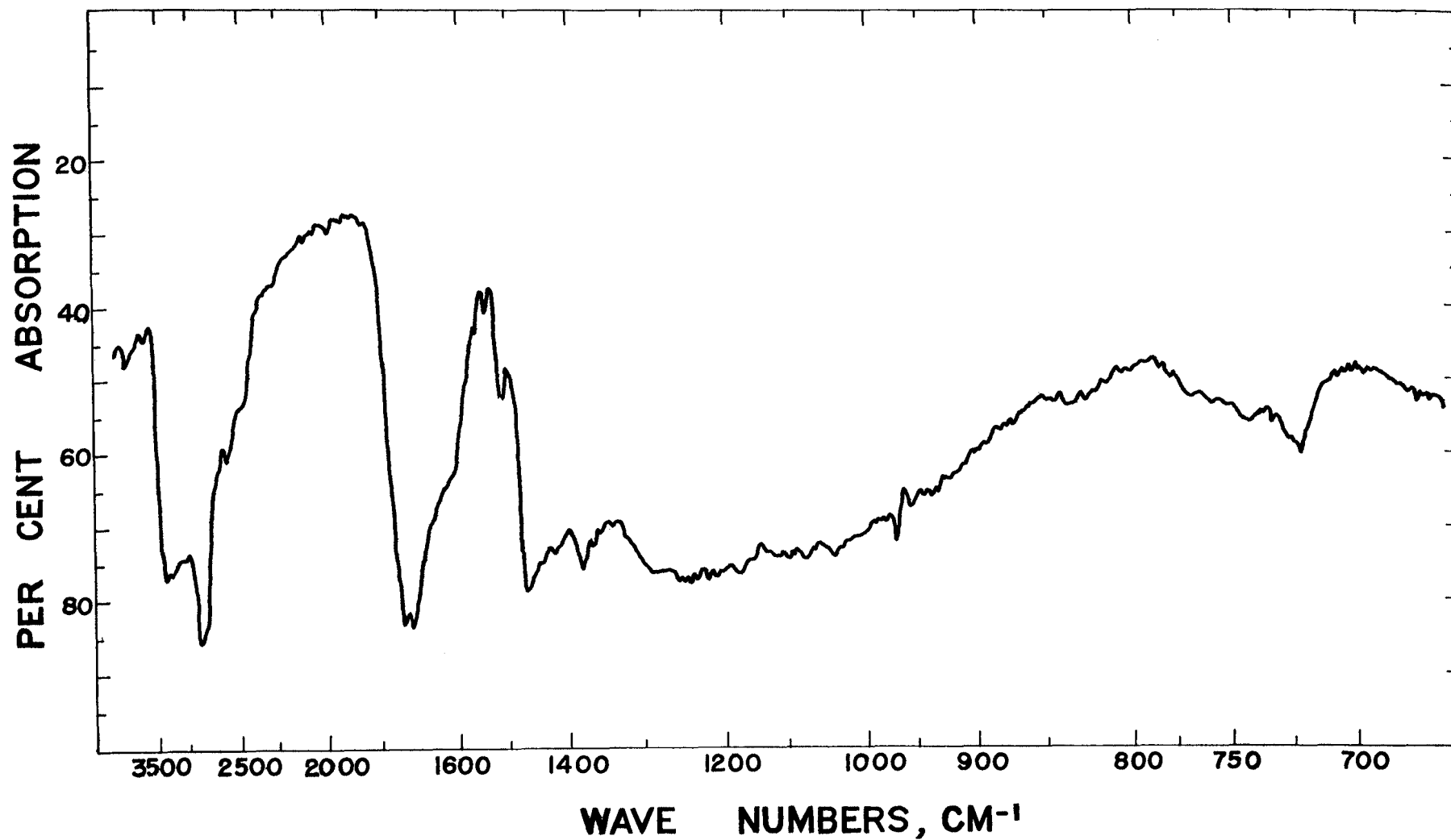


FIG. 6 IR SPECTRUM OF ACID (III); FILM FROM CHLOROFORM

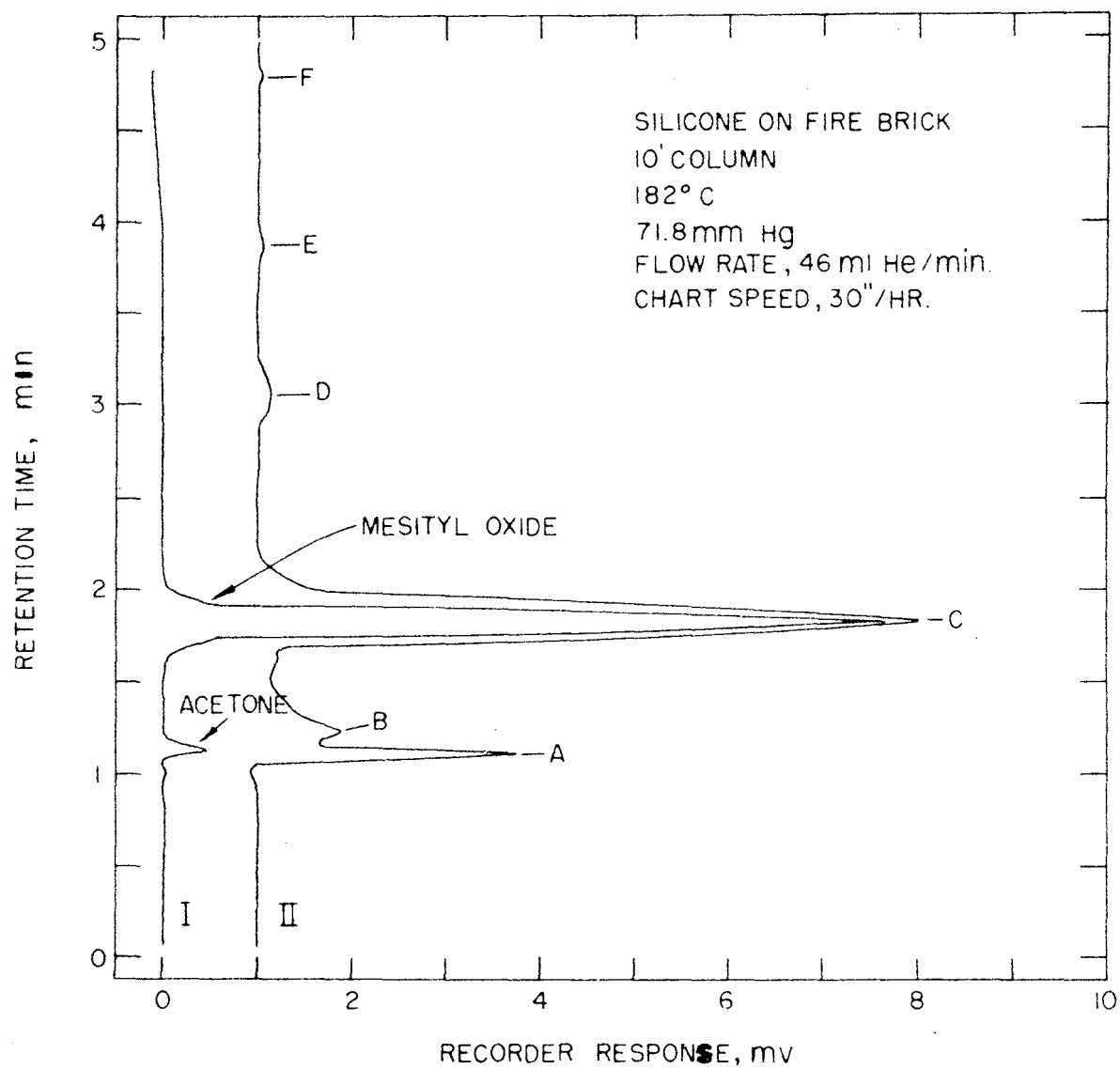


FIG. 7. GLPC OF:

I. MESITYL OXIDE / ACETONE (99/1)

II. VOLATILE FRAGMENTS FROM THE BASIC HYDROLYSIS OF TACCALIN

and an IR spectrum obtained and compared with that of mesityl oxide (see Fig. 8).

One milliliter of absolute alcohol was added to the remaining liquid (ca. 3 mg.) and 2 drops of 2,4-dinitrophenylhydrazine in phosphoric acid was added. One milliliter of 95% ethanol was added and the solution heated for 15 minutes on the water bath. A cloudiness appeared and the solution was allowed to cool slowly. Carmine prisms formed and were recrystallized once from 95% ethanol to give carmine prisms, m.p. 201-202°C., ca. 4 mg. A mixed melting point with authentic mesityl oxide 2,4-dinitrophenylhydrazone (m.p. 198-200°C.) melted at 199-200°C. A sample dried at 100°C./30 mm. for analysis was sufficient for only one determination.

Analysis. Calc'd. for $C_{12}H_{14}O_4N_4$: N, 20.1%

Found : N, 19.7%

(c) Degradation Experiments.

(1) beta-Diketone (IV): In attempts to remove the yellow ester (I) from taccalin, several solvents were used. Acetic anhydride appeared promising on a small scale as the ester (I) was more soluble than taccalin. However, when a large amount of taccalin-ester (I) mixture was dissolved, taccalin reacted to produce a new substance.

The crude yellow solid (ca. 1.5 g.) from the Florisil column (vide supra) was dissolved in 300 ml. of freshly distilled acetic anhydride and heated to 65-70°C. for several minutes. The resulting yellow-orange solution was cooled rapidly to ca. 5°C., whereupon a voluminous cream-colored precipitate formed. This solid was filtered by suction

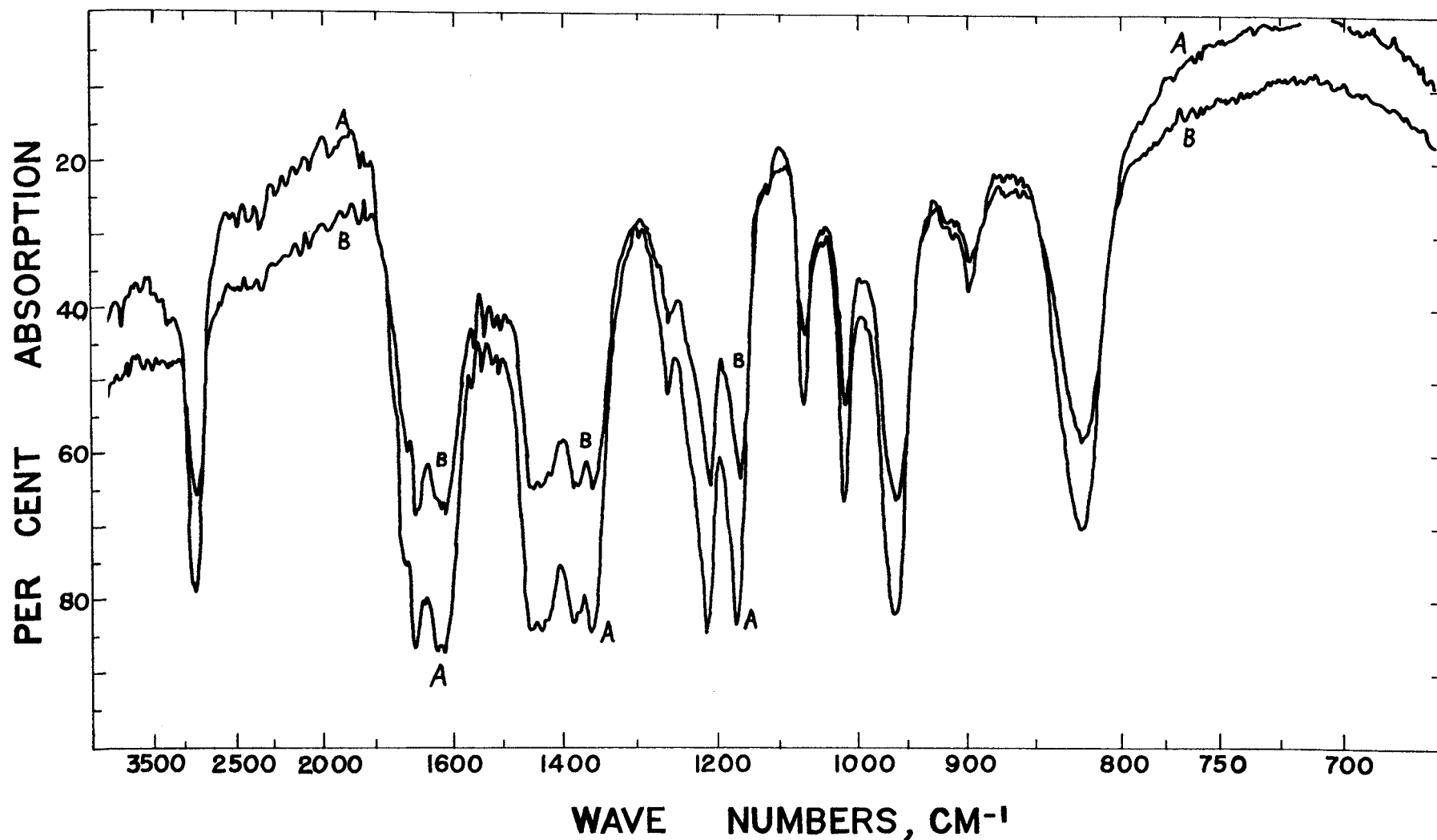


FIG. 8 IR SPECTRA OF MESITYL OXIDE (CURVE A) AND VOLATILE FRAGMENT FROM BASIC HYDROLYSIS OF TACCALIN (CURVE B); LIQUID FILMS

and dried. The mother liquor yielded additional solid after concentrating. The light brown solid was not bitter and an IR spectrum lacked the bands (1824 and 1756 cm^{-1}) characteristic of the anhydride grouping. Yield, 620 mg. of low-melting solid.

The dark-red mother liquor from the above solid was chromatographed directly over Florisil. The following components were discernible and were eluted in the following order: 30 mg. of crude taccalin, 55 mg. of crude ester (I), 60 mg. of a dark-red oil, 70 mg. of red-purple solid, 100 mg. of a reddish, viscous oil, 15 mg. of a purple liquid and 25 mg. of a purple solid. An additional purple band could not be eluted. All the fractions were incompletely separated and were discarded except for the first two.

The light brown solid from the acetic anhydride was recrystallized five times from methanol to give an analytical sample of off-white crystals, m.p. 59-60°C., which tended to turn pink upon exposure to air. Dried at room temperature / 30 mm. for six hours.

Analysis. Calc'd. for $\text{C}_{16}\text{H}_{28}\text{O}_2$: C, 76.1% H, 11.2%

Found	:	C, 76.9	H, 11.5
		76.7	11.5

Although this sample was thought to be pure on account of its sharp melting point, further recrystallization from chloroform yielded colorless crystals, m.p. 92-94°C., which also tended to turn pink upon exposure to air. The IR spectra of both melting varieties contained the same absorptions, but of varying intensities. Paper chromatography of the lower melting variety gave only one spot. The higher melting substance was dried at 56°C./30 mm. for 3 hours for analysis and labeled beta-diketone (IV).

Analysis. Calc'd. for $C_{16}H_{26}O_2$: C, 76.8% H, 10.5 M. Wt., 250
 Found : C, 77.0 H, 10.5 M. Wt., 285*
 76.8 10.4

$$[\alpha]_D^{27} = 0 \pm 45^c (C = 1.0 \text{ g./l. in chloroform}).$$

$\lambda_{\text{max.}} = 228, 250, 255, 297 \text{ m}\mu$ ($\log E = 3.60, 3.63, 3.61, 3.37$ respectively) in isopropyl alcohol.**

$\lambda_{\text{max.}} = 222, 265, 340 \text{ m}\mu$ ($\log E = 3.72, 3.64, 3.42$ respectively) in 0.1 N sodium hydroxide (for UV spectra, see Fig. 9).

IR max. = 3300 (W), 1735 (S), 1659 (S), 1630 (S) cm.^{-1}
 (see Fig. 10).

beta-Diketone (IV) failed to react with o-phenylenediamine, gave negative tests with Tollen's reagent, ceric ammonium nitrate and with tetranitromethane. It failed to dissolve in 5% sodium bicarbonate solution, but gave a deep red-violet color with ferric chloride which was lasting (vide infra).

Copper (II) chelate of beta-diketone (IV): Twenty-two milligrams (0.088 mmol) of beta-diketone (IV) was dissolved in ca. 10 ml. of absolute ethanol and heated to 50°C. One milliliter of warm copper acetate monohydrate (114 mg./ml. in water) was added. An immediate green precipitate was formed, which, after standing in the refrigerator for 24 hours, was collected and washed three times with water. The crude green solid weighed 94 mg. (95%).

* Isothermal distillation.

** Beckman Model DK-2 recording spectrophotometer.

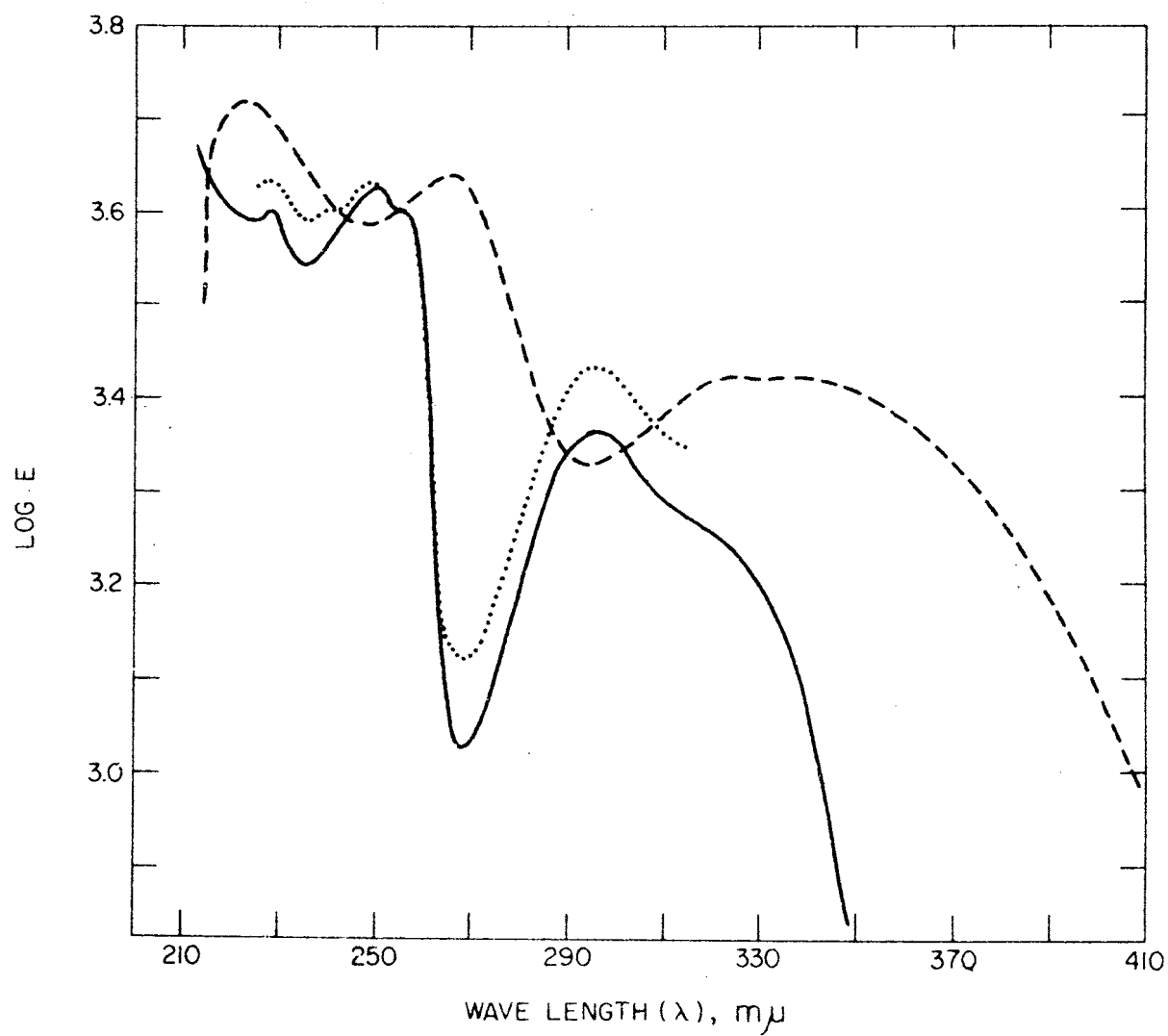


FIG. 9 UV ABSORPTION SPECTRUM OF β -DIKETONE (IV), — IN ISOPROPANOL ($C = 2.5 \times 10^{-2}$ g/L), IN ISOPROPANOL ($C = 12.4 \times 10^{-2}$ g/L), — — — IN 0.1 N NaOH ($C = 6.8 \times 10^{-2}$ g/L).

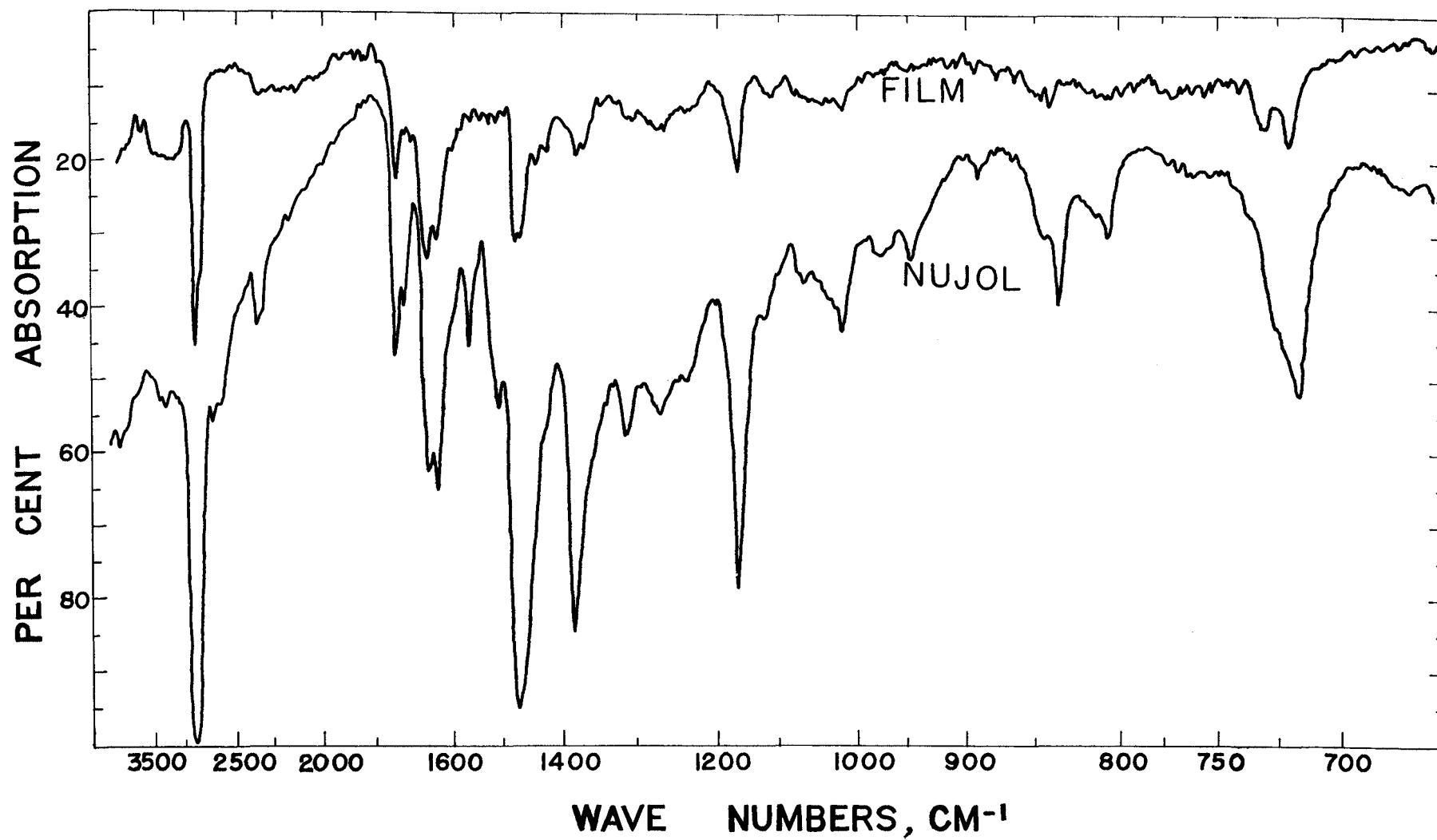


FIG.10 IR SPECTRUM OF BETA-DIKETONE (IV)

The chelate was insoluble in water, tended to decompose in hydroxylic solvents and was practically insoluble in most other solvents. Recrystallization was finally achieved with dimethylformamide: dioxane (1:1). Three such recrystallizations produced small, light green crystals which darkened at 152°C. and decomposed at 210°C. Vacuum sublimation at 1 mm. pressure failed. An analytical sample was dried at 100°C./30 mm. for six hours.

Analysis. Calc'd. for $C_{32}H_{50}O_4Cu$:

C, 68.4%	H, 9.0%	Cu, 11.3%	2 C-CH ₃ , 5.4%
Found: C, 72.0 71.8	H, 9.3 9.3	Cu, 3.9	C-CH ₃ , 1.3 1.7

IR max. = 1645 (M), 1609 (M), 1545 (S), 1524 (W) cm.⁻¹ (see Fig. 11).

Reaction of beta-diketone (IV) with 2,4-dinitrophenylhydrazine, even after 15 hours refluxing, yielded starting material in good yield (IR). The substance also failed to produce a benzylidene derivative after three hours refluxing with benzaldehyde and a basic catalyst.

Iron (III) chelate of beta-diketone (IV): Aqueous ferric chloride was added in excess to beta-diketone (IV) dissolved in isopropyl alcohol and the visible spectrum of the iron (III) chelate was measured against ferric chloride in isopropyl alcohol.

$$\lambda_{\text{max.}} = 533 \text{ m}\mu \text{ (log } E = 2.88)$$

$$\lambda_{\text{min.}} = 445 \text{ m}\mu \text{ (log } E = 2.81) \text{ (see Fig. 12).}$$

Since it was necessary to recover unreacted beta-diketone (IV) from various reaction mixtures, a simple purification procedure was devised based on the ability of the beta-diketone to form an iron (III) chelate. Procedure: The impure beta-diketone (IV) was dissolved in dioxane:

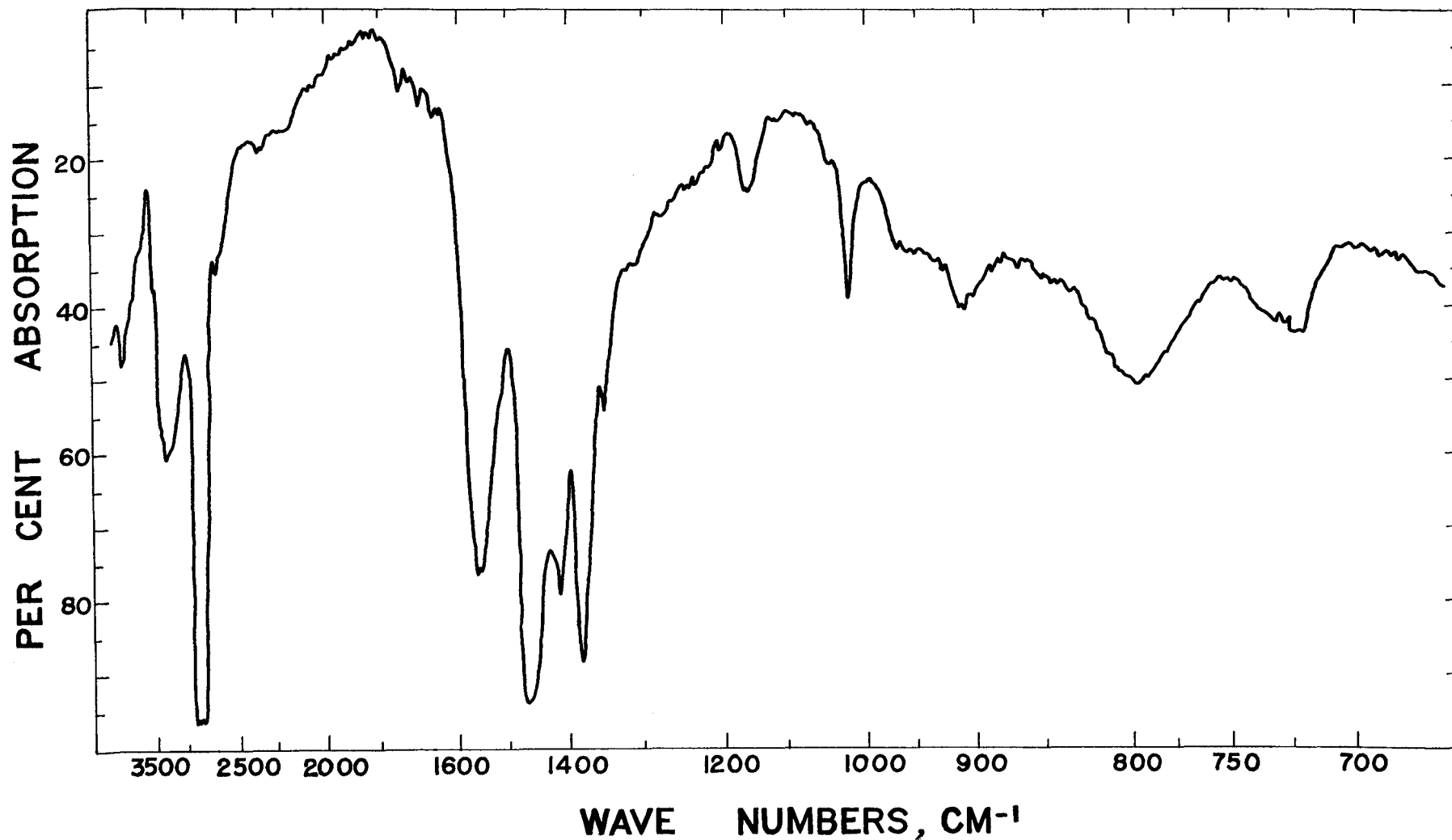


FIG.II IR SPECTRUM OF COPPER (II) CHELATE OF BETA-DIKETONE (IV);
MINERAL OIL MULL

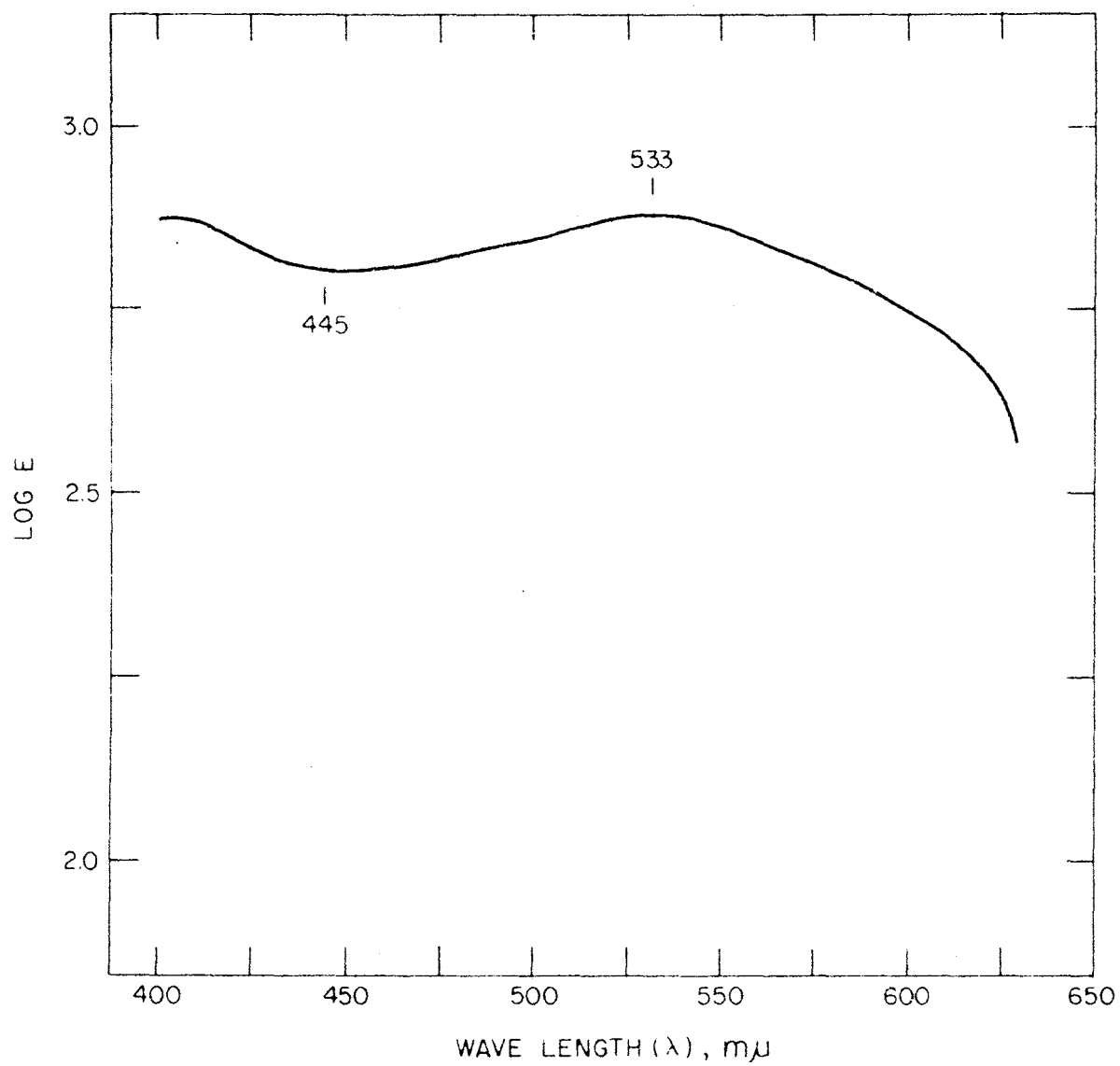


FIG. 12... ABSORPTION SPECTRUM OF IRON (III) CHELATE OF β -
DIKETONE (IV) IN ISOPROPANOL ($C = 1.05 \times 10^{-1}$ g/L)

water (7:3) and poured on the top of a cation exchange column (IRC-50) which had been regenerated in the iron (III) form. The beta-diketone (IV) was held in the resin as the iron (III) chelate while non-exchangeable impurities were eluted with 95% ethanol. After the resin had been washed free of impurities, the beta-diketone could be eluted successfully with 70% ethanol containing 3% hydrochloric acid. The beta-diketone (IV) could then be recovered in high purity by extraction into ether.

Acid (V): Ten milligrams of beta-diketone (IV) was added to 5 ml. of aqueous sodium hydroxide, heated briefly on a water bath and cooled. A tan solid coagulated slowly which was collected, washed with water and dried. The solid was washed with cold ethanol, leaving a white, mushy solid. The solid was dissolved in 10 ml. of ethanol and 1 ml. of 2,4-dinitrophenylhydrazine was added. A yellow precipitate formed after addition of several drops of water. The yellow solid was collected, washed with ether and dried to yield 3 mg. of a yellow 2,4-dinitrophenylhydrazone. The derivative was dissolved in methanol and chromatographed by the ascending method with heptane saturated with methanol. Two yellow, circular spots were observed at $R_f = 0.78$ and 0.82 (the reagent remained at the origin).

The basic filtrate from the treatment with aqueous base was neutralized with 6 N hydrochloric acid to pH 4. A white, cloudy precipitate formed which coagulated upon standing in the cold. The solid was collected and dried to give 2 mg. of a fragrant acid (V) whose melting point was low and broad. The substance was acidic to sodium bicarbonate and failed to give a positive test with 2,4-dinitrophenylhydrazine.

IR max. = $1661 \text{ (S) cm.}^{-1}$ (see Fig. 13).

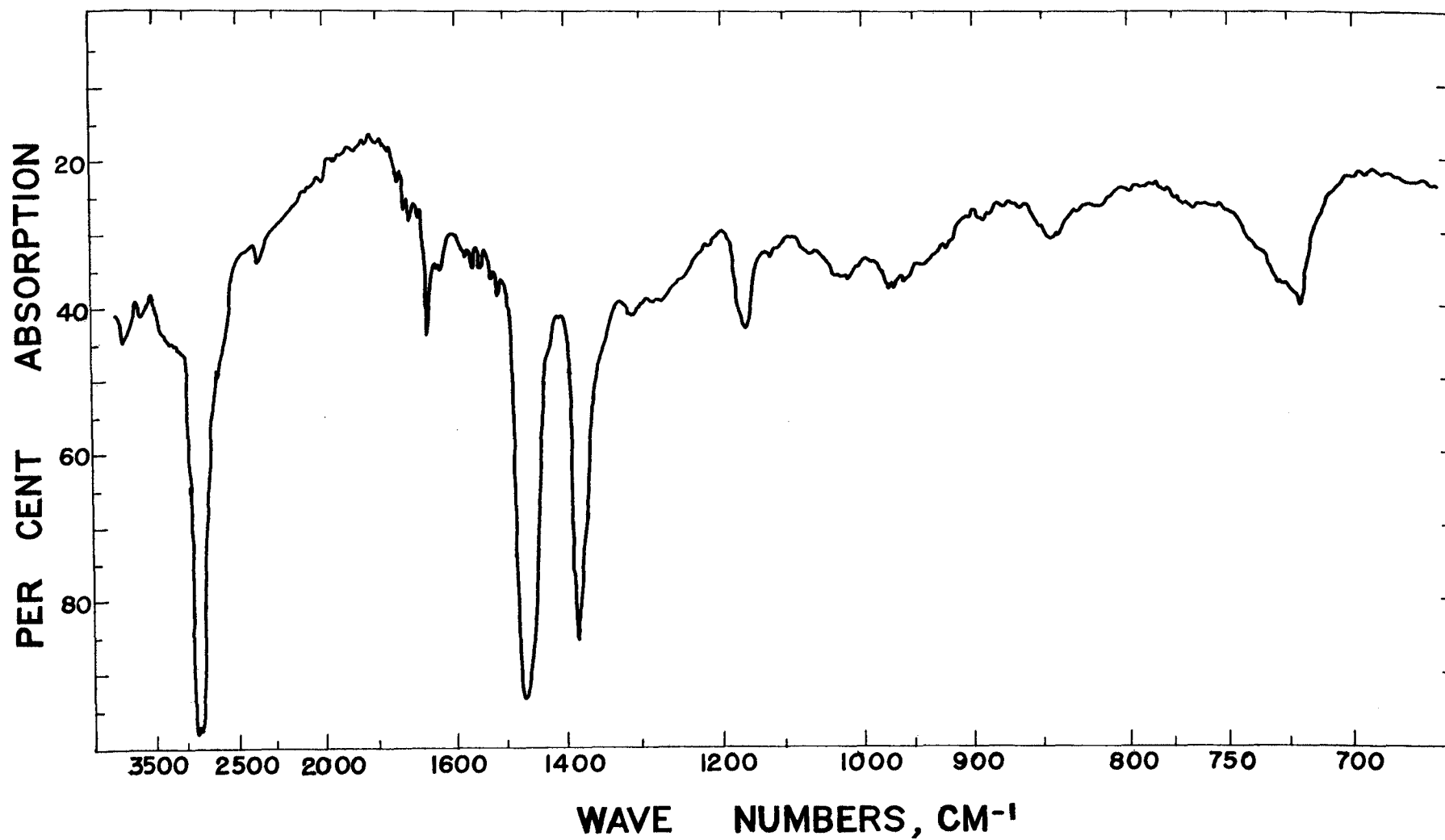


FIG.13 IR SPECTRUM OF ACID (V); MINERAL OIL MULL

(2) Ketone (VI): Microhydrogenation of beta-diketone (IV) over Adams' catalyst in isopropyl alcohol at room temperature resulted in an uptake of 1 mol of hydrogen in nine hours and leveled off at 1.5 mols in 80 hours.

Hydrogenation on a preparative scale under slightly different conditions is described below: 83 mg. (0.33 mmol) of twice-recrystallized beta-diketone (IV) was hydrogenated over 29 mg. of Adams' catalyst in 25 ml. of p-dioxane containing 1 drop of glacial acetic acid. At room temperature, the uptake was complete in 82 hours (1.9 mols) and the product isolated by evaporating to dryness. Yield, 79 mg. (95%), low-melting white solid. Three recrystallizations from ethanol produced colorless micro-crystals which turned off-white upon drying, m.p. 59-66°C., but when the crystals were heated to 100°C., the m.p. was 64-66°C. consistently and reproducibly. Labeled ketone (VI).

$$[\alpha]_D^{19} = -4 \pm 2^\circ \text{ (c = 23.0 g./l. in chloroform).}$$

Analysis. Calc'd. for $C_{16}H_{28}O_2$:

C, 76.1% H, 11.2% 1 C-CH₃, 6.0% 2 C-CH₃, 11.9%

Found: C, 76.3 H, 11.5 C-CH₃, 7.8%

$\lambda_{\text{max.}}$ = 296 m μ (log E = 1.98) in ethanol (see Fig. 14).

IR max. = 1740 (S) cm.⁻¹ (see Fig. 15).

Ketone (VI) failed to form a benzylidene derivative after three hours' refluxing with benzaldehyde and sodium hydroxide. Neither an oxime nor a 2,4-dinitrophenylhydrazone were produced, even under forcing conditions of long reflux times (starting material recovered, IR).

(3) Alcohol (VII): A 3-neck flask containing 50 mg. of lithium

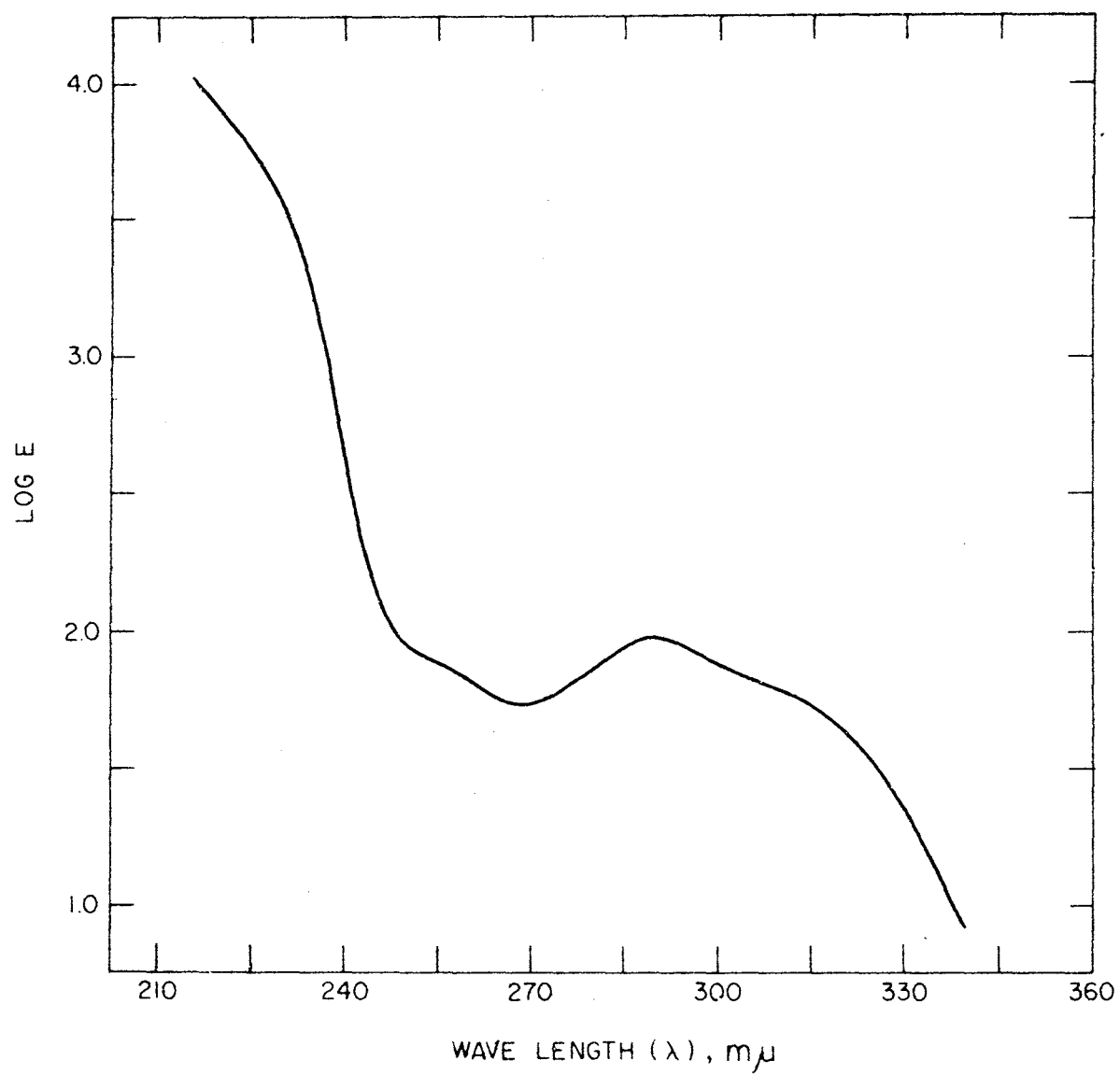


FIG. 14. UV ABSORPTION SPECTRUM OF KETONE (VI) IN ETHANOL
($C = 3.0 \times 10^{-1}$ g/L).

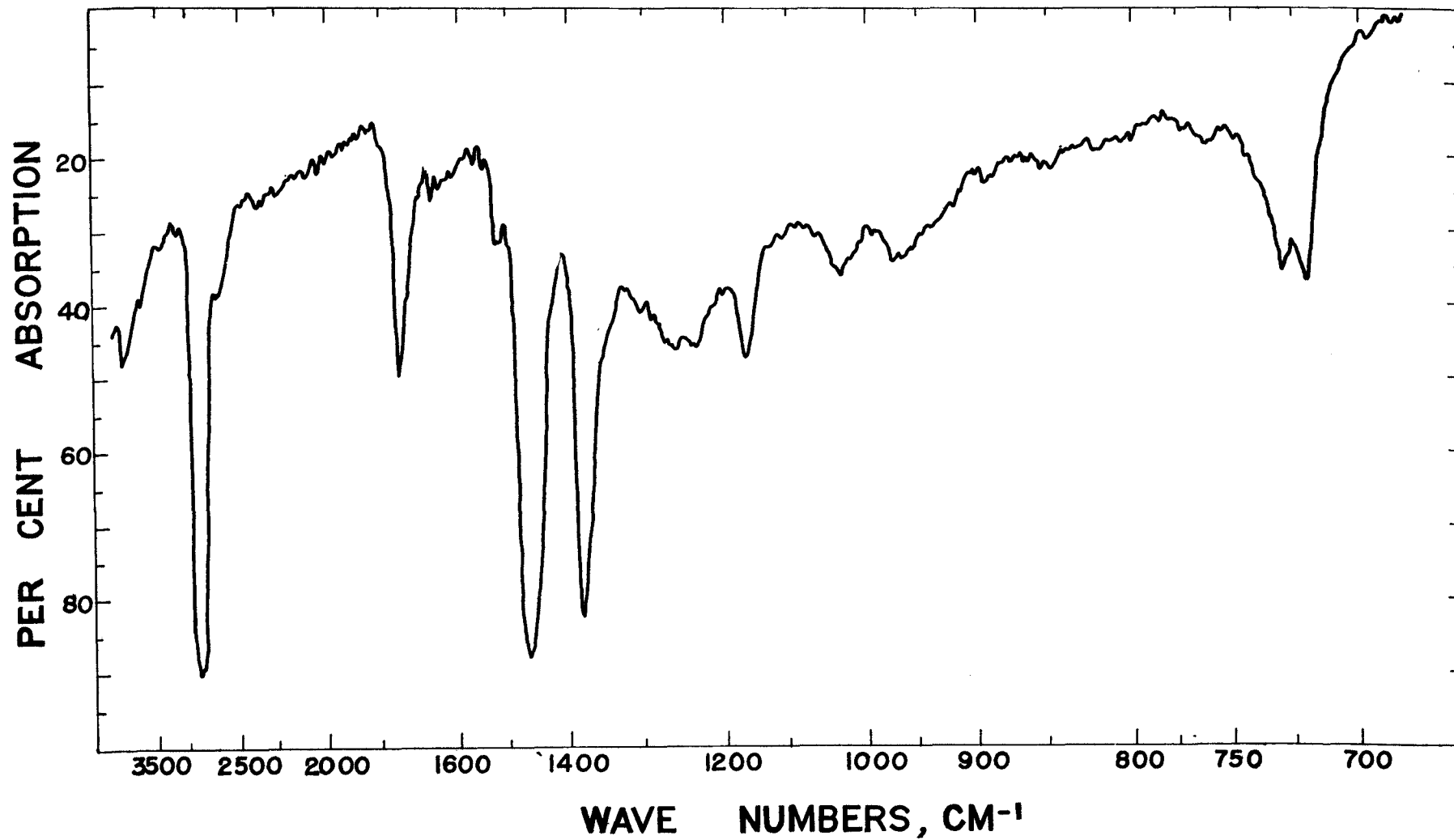


FIG.15 IR SPECTRUM OF KETONE (VI); MINERAL OIL MULL

aluminum hydride in 50 ml. of ethyl ether was equipped with a Soxhlet extractor in which was placed 10 mg. (0.040 mmol) of ketone (VI). After four hours at reflux, all of the ketone (VI) had been extracted into the reaction flask. The mixture was refluxed for an additional four hours, cooled and poured into several grams of ice. One milliliter of concentrated hydrochloric acid was added with stirring and the ether phase separated. The aqueous phase was extracted with 3 X 20 ml. of ethyl ether, the ether fractions combined, washed with water and dried over sodium sulfate. The ether was stripped in vacuo to give 9 mg. (89%) of a white solid, labeled alcohol (VII). The IR spectrum was obtained without purification as a solid film (3268 cm^{-1} , see Fig. 16) and recovered. The solid was recrystallized once from acetone: methanol (1:1) to give micro-platelets, m.p. $71-75^{\circ}\text{C}$.

Analysis. Calc'd. for $\text{C}_{16}\text{H}_{28}\text{O}$: Active H, 0.42%

Found : Active H, 0.52%

Insufficient material prevented a C and H determination.

(4) Lactone (VIII): Twenty milligrams (0.080 mmol) of ketone (VI) was placed in a test tube fitted with a reflux condenser and 5 ml. of freshly distilled acetic anhydride was added. To this mixture was added two ml. of conc. sulfuric acid with cooling, followed by three ml. of 30% hydrogen peroxide over 15 minutes. A rise in temperature and vigorous evolution of oxygen were noted. The mixture was slowly heated to 45°C . and another three ml. of 30% hydrogen peroxide was added over two hours. The mixture was cooled to room temperature and kept in the dark for 70 hours with occasional swirling. A white precipitate slowly formed during this period. The mixture was poured onto several grams of ice, and then

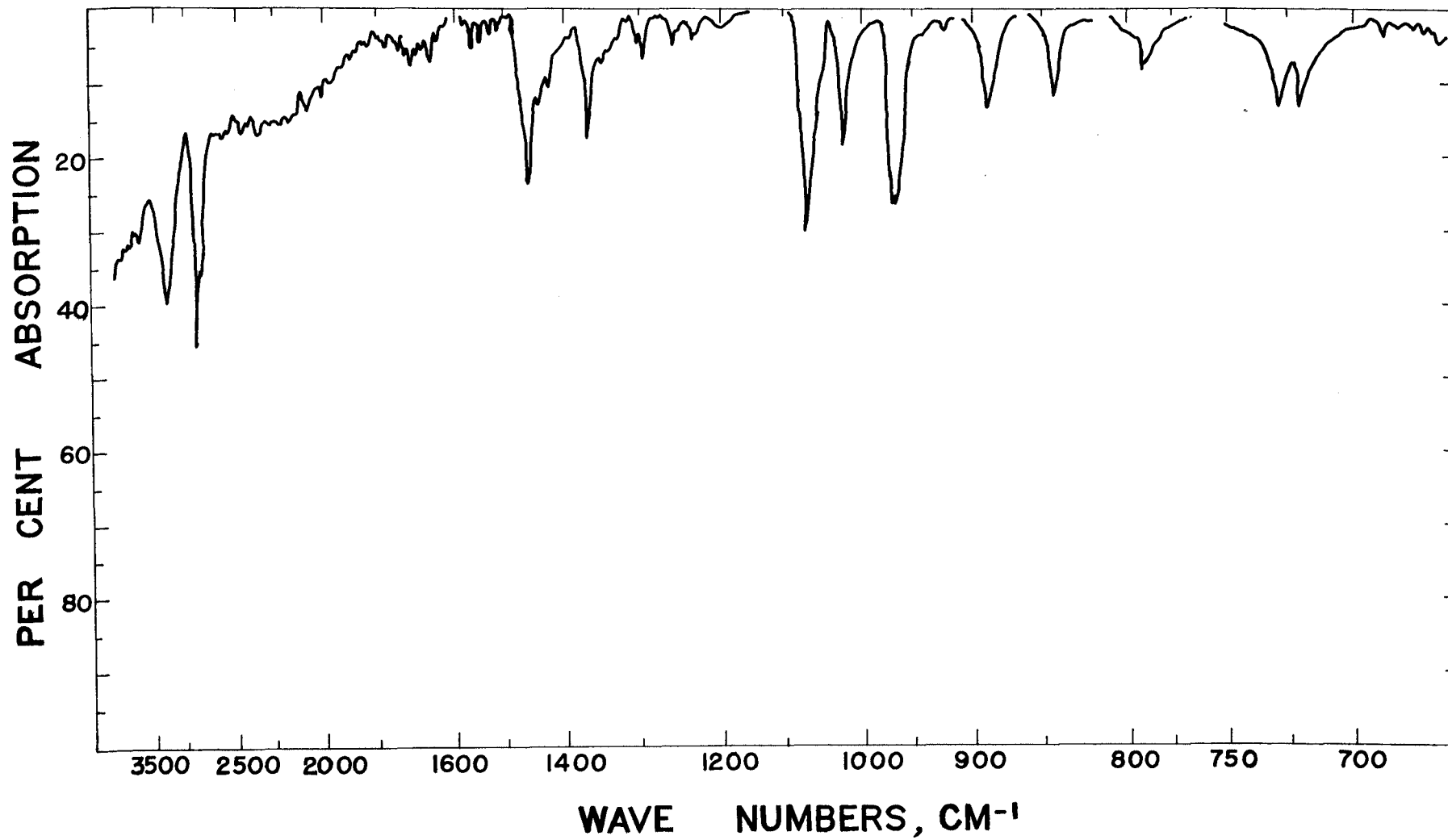


FIG.16 IR SPECTRUM OF ALCOHOL (VII); SOLID FILM

was neutralized with sodium carbonate solution (2M) containing 1% sodium bisulfite. A slight cloudiness was observed. The solution was extracted with 5 X 12 ml. of ethyl ether, the ether dried over sodium sulfate and the ether stripped in vacuo. The white, slushy solid was dried thoroughly in vacuo to give 16 mg. (80%) of lactone (VIII).

IR max. = 1740 cm.^{-1} (solid film; recovered).

(5) Hydroxy ester (IX): The recovered crude lactone (VIII) was added, without purification, to 10 ml. of a sodium ethoxide solution (ca. 3 mg. sodium/ml. ethanol). The mixture was heated slowly and the solid dissolved. The temperature was increased gradually and refluxed for three hours. The solution was cooled, neutralized carefully with 6 N sulfuric acid and extracted with 6 X 5 ml. of ethyl ether. The ether was washed with water, dried over sodium sulfate and stripped in vacuo. The white, low-melting solid was washed with water and dried to give 6 mg. (32%) of hydroxy ester (IX). Recrystallization once from chloroform produced colorless crystals, freezing point $31-29^{\circ}\text{C}$.

IR max. = $3448\text{ (M)}, 1731\text{ (S)}, 1249\text{ (S)}, 1179\text{ (M)}\text{ cm.}^{-1}$ (see Fig. 17).

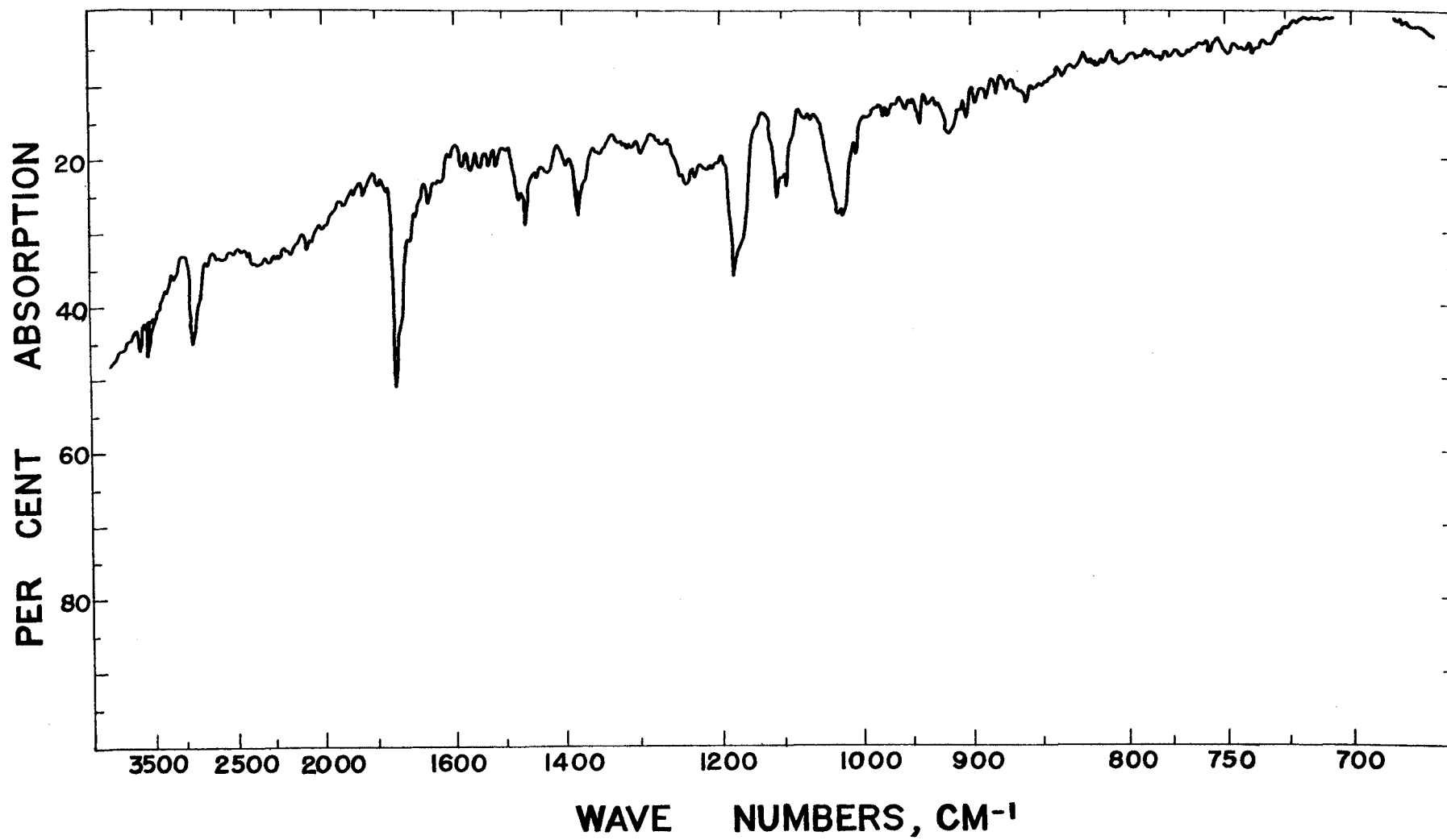


FIG.17 IR SPECTRUM OF HYDROXY ESTER (IX); SOLID FILM

III. DISCUSSION OF RESULTS

A. Determination of the Molecular Formula

Chemical tests and elemental analyses revealed that taccalin contained only carbon, hydrogen and oxygen. The IR spectrum (Fig. 4) showed prominent absorption bands at 3400 (hydroxyl), 1824 and 1756 (anhydride) and 1252 cm^{-1} (ether attached to unsaturated carbon)(37). Chemical tests failed to indicate any other functional groups so that taccalin must contain a minimum of four oxygens, and probably more, as the hydroxyl band was of medium intensity.

The elemental analyses failed to agree, but the purity of one analytical sample was questionable. Little reliance can be placed in the other analysis as the sample used was less than two milligrams, but coupled with the active hydrogen determination, a reasonable argument can be presented for the proposed molecular formula.

The Zerewitinoff determination gave 1.1% active hydrogen, as measured by methane evolution, in addition to an exactly equal amount of Grignard reagent addition. The only grouping present in the molecule which could conceivably enter into Grignard addition is the anhydride group. Then, the number of mols of Grignard consumed by the anhydride must equal the number of hydroxyl groups present in the molecule. The number of hydroxyl groups can be ascertained by knowing the consumption of Grignard by an anhydride, under the conditions of the Zerewitinoff determination. A search of the literature revealed only one pertinent study, but this was concerned with the slightly high values given by anhydrides in methane evolution only. No mention was made of addition reactions (38).

However, if one assumes a consumption of one or two mols of Grignard addition by the anhydride, then the only possible formulas that are consistent with the analyses are $C_{10}H_{14}O_4$ and $C_{13}H_{18}O_5$. These can be excluded immediately as they would require 0.5% and 0.8% active hydrogen respectively, whereas 1.1% was actually found. Apparently, more than two mols of Grignard reagent was consumed by the anhydride.

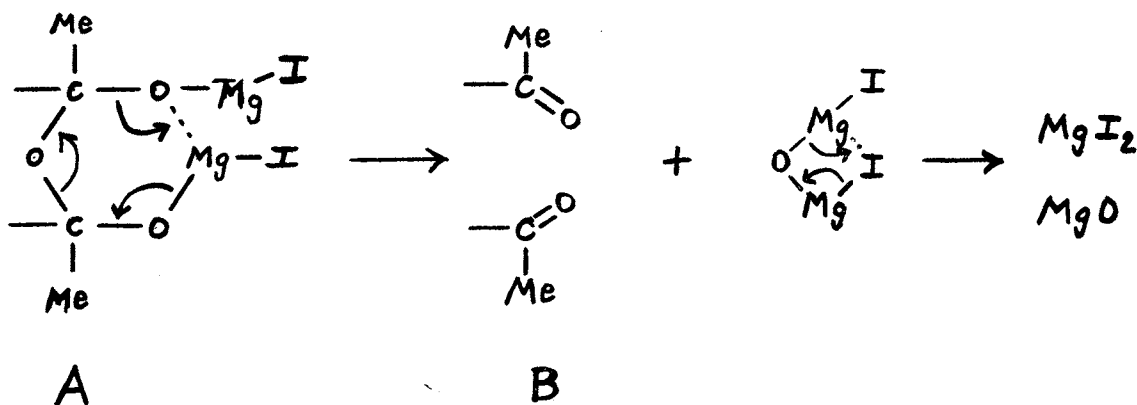
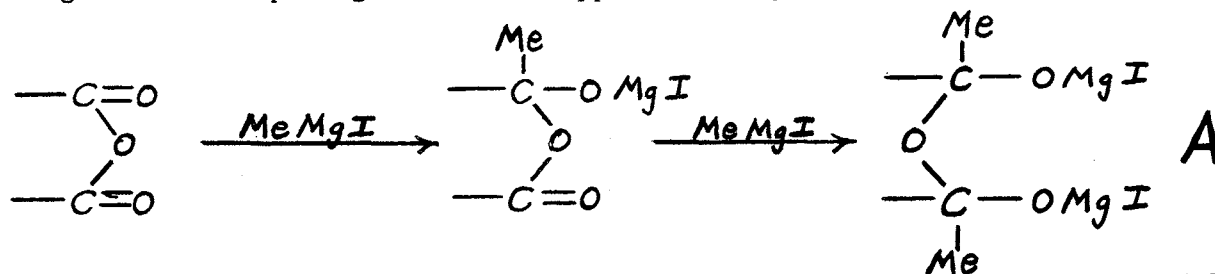
Table II lists the four remaining possible formulas - through C_{20} - which fall into the range of $61.0 \pm 1.3\%$ C and $7.0 \pm 0.5\%$ H, and which are consistent with the active hydrogen found.

TABLE II
COMPARISON OF POSSIBLE MOLECULAR FORMULAS OF TACCALIN

	1	2	3	4
	$C_{15}H_{20}O_6$	$C_{18}H_{26}O_7$	$C_{19}H_{26}O_7$	$C_{20}H_{28}O_8$
Grignard Addition, mols	3	4	4	4
% Carbon	Calc'd.	60.8	61.0	62.3
	Found	61.0	61.0	61.0
% Hydrogen	Calc'd.	6.8	7.4	7.2
	Found	7.0	7.0	7.0
% Active Hydrogen	Calc'd.	1.0	1.1	1.1
	Found	1.1	1.1	1.1
# Hydroxyls Calc'd. for -	3	4	4	4
# Ether Oxygens Required	0	0	0	1
Molecular Weight	Calc'd.	296	354	366
	Found	321	321	321

If the anhydride consumed more than two mols of Grignard reagent,

then there appears to be no a priori reason why the uptake should stop at 3 mols. If two mols of Grignard reagent add to the anhydride to produce part structure A and further reaction takes place, then ring-opening analogous to the opening of a ketal appears likely;



A compound containing part structure B is free to add two more mols of Grignard reagent, since both carbonyls are of equal reactivity. Thus the addition of three mols of Grignard appears unlikely and Formula 1 can be excluded from serious consideration. The conventional method of determining the number of hydroxyl groups in the molecule - obtained by multiplying the active hydrogen determined by the molecular weight - yields 3.5 hydroxyl groups.

If we accept the four mol hypothesis, then three formulas remain to be considered. Formula 4 is less likely than the other two on the basis of the following considerations: (1) The molecular weight deviates by 20% from the experimental value, (which is a larger deviation than either formula 2 or 3), (2) and ether link is not in accord with data to be

presented later, and (3) formula 4 requires one more ring than formula 2. This is difficult to reconcile with the results of degradation studies (vide infra).

Differentiation between formulas 2 and 3 is difficult as both are consistent with the observed properties of taccalin. However, formula 2, $C_{18}H_{26}O_7$, is favored since the molecular weight and percent composition are more closely in accord with the observed data. In addition, a tetracyclic system ($C_{18}H_{26}O_7$) is preferred over formula 3 ($C_{19}H_{26}O_7$), which requires 5 rings (vide supra). The $C_{18}H_{26}O_7$ formulation will be used in subsequent discussions, but is subject to change if additional data become available. It should be remembered that the formula is tentative and subsequent arguments should be viewed with this in mind.

B. Nature of the Oxygen Functions

With the molecular formula tentatively established as $C_{18}H_{26}O_7$ the nature of the seven oxygen atoms can be discussed. From the above presentation it is apparent that three oxygen atoms are involved in an anhydride grouping and four in hydroxyl groups.

It will be instructive at this point to consider data which shed some light on the nature of the four hydroxyls. The chromic oxide test after Houben-Weyl (31) indicated a tertiary hydroxyl group to be present in taccalin.

Several attempts to acetylate taccalin failed and in only one case was a product isolated. This product will be shown below to be an acid and not an ester. Since no trace of an ester was ever observed, it must be concluded that taccalin contains four tertiary hydroxyl groups.

The product referred to above resulted from treatment of taccalin with acetic anhydride and sodium acetate, a common acetylating mixture. The product showed broad absorption near 3250 cm.^{-1} (hydroxyl; see Fig. 5), supported by absorption at 1060 cm.^{-1} . Very weak inflections at 1825 and 1759 cm.^{-1} must have arisen from starting material (anhydride), but a strong carbonyl absorption was evident at 1712 cm.^{-1} , too low for an acetate carbonyl, which normally absorbs near 1735 cm.^{-1} (39). Since the reaction must have led to cleavage of the anhydride, the possibility exists that lactonization occurred between one of the carboxyl groups and a suitably placed hydroxyl group. However, a lactone can be excluded on the basis of the IR spectrum also. A gamma-lactone would be expected to absorb near 1775 cm.^{-1} and a delta-lactone near 1740 cm.^{-1} (40), which is incompatible with the observed 1712 cm.^{-1} band. This band at 1712 cm.^{-1} is probably due to a carboxylic acid dimer (41). An acid assignment is supported by broad absorption near 2830 cm.^{-1} , the strong band at 1250 cm.^{-1} and the inflection at 1730 cm.^{-1} (42).

The above argument lends credence to the hypothesis that although the anhydride was opened to an acid (II), no acetate was formed. This in turn supports the contention that taccalin contains four tertiary hydroxyl groups.

The other three oxygen atoms must be involved in either a cyclic or an acyclic anhydride grouping. Since chemical tests and basic hydrolyses (vide infra) failed to shed any light on the nature of this grouping, recourse must be made to the IR spectrum of taccalin. The two strong carbonyl absorptions at 1824 and 1759 cm.^{-1} have been assigned to the anhydride grouping (see Fig. 4). These two bands arise from resonance

coupling of the two carbonyl groups (43), whose $\Delta\bar{\nu} = 65 \text{ cm.}^{-1}$, in good agreement with the observation that all anhydrides, regardless of type or environment, invariably have two carbonyl bands 60 cm.^{-1} (44) or 65 cm.^{-1} (43) apart.

The carbonyl frequencies of taccalin correspond closely to those of saturated acyclic types (1826 and 1758 cm.^{-1} (44-46)), but from the general chemical properties and reactivity of taccalin, an acyclic structure is unlikely. This assumption is supported by arguments to be presented in Section III D.

The generalization by Stork and Breslow (47) that 5- and 6-membered cyclic anhydrides absorb at 1776 and 1852 cm.^{-1} and at 1764 and 1795 cm.^{-1} , respectively; and, further, that the lower frequency band is diagnostically more important, has been cited frequently in structural work (*e.g.* 48). The lower frequency band of taccalin (1759 cm.^{-1}) is consistent with a 6-membered cycle, but the high frequency band has been shifted considerably to higher frequencies. While the frequency of the lower band is relatively unaffected by substitution on the ring carbons (47), the high frequency band is susceptible to shifts due to ring strain (49), which may account for its shift to higher frequencies.

Since complicating conjugation effects* with the carbonyls are not involved (UV transparency), the anhydride grouping of taccalin is best represented by a 6-membered ring containing sufficient ring strain to account

* Conjugation causes the usual bathochromic shift. It is not possible to distinguish between an alpha, beta-unsaturated 5-membered cyclic anhydride and a saturated 6-membered cyclic (or acyclic) anhydride (50).

for the hypsochromic shift of the high frequency carbonyl absorption.

In the above analysis, two types of anhydrides were not included - cyclic anhydrides containing more than 6 members and those of the mixed carboxylic-carbonic acid type. The former was excluded since (1) very few 7-membered cyclic anhydrides are known (none are known containing more than 7 members) and (2) the 7-membered cyclic anhydrides have been reported to be unstable (51). These macrocyclic anhydrides have not been excluded from consideration on the basis of experimental evidence. They have been neglected due to their apparent rarity.

Anhydrides of the second type - the mixed carboxylic-carbonic acid anhydrides - have been synthesized and were found to be more stable than was formerly thought (52). In contrast to the macrocyclic type, at least one of the mixed anhydrides has been found in nature- marrubiin, a bitter principle from horehound (see Table I), which absorbs at 1827 and 1766 cm^{-1} , very much like taccalin (25). However, 1.5 mols of carbon dioxide are lost upon heating of marrubiin (53), whereas taccalin is stable up to its melting point. The ready loss of carbon dioxide upon melting is a general phenomenon of the mixed acid anhydrides. Taccalin is therefore excluded from this class of compounds.

In summary, evidence has been presented to show that taccalin, $\text{C}_{18}\text{H}_{26}\text{O}_7$, contains four tertiary hydroxyl groups and a 6-membered cyclic anhydride grouping.

C. Establishment of the Number of Rings

With the molecular formula tentatively assigned and the nature of the oxygen functions established, the carbon skeleton of taccalin can be considered.

Catalytic hydrogenation over Adams' catalyst resulted in a negligible uptake of hydrogen. This result indicates that taccalin contains no center of unsaturation. This conclusion is supported by the negative test with tetranitromethane.

Since an aromatic character for taccalin is excluded by spectral considerations, the number of alicyclic rings can be established by formulating the hypothetical parent hydrocarbon of taccalin. The parent hydrocarbon, $C_{18}H_{30}$, differs from the corresponding saturated acyclic hydrocarbon, $C_{18}H_{38}$, by eight hydrogen atoms. Taccalin must therefore be tetracyclic; it must contain three homocyclic rings and an anhydride ring.

It will be remembered that taccalin contained three or four $C-CH_3$ groups as determined by quantitative measurement in the IR region. This result necessitates that the tricyclic skeleton is composed of 13 or fewer carbon atoms. Additional data bearing on the ring structure will be considered later.

D. Structural Studies

1. Basic Hydrolyses. An attempt to form the half-ester of taccalin by refluxing with methanol without a catalyst resulted in recovery of starting material (54) thus indicating that methanol was not sufficiently basic to effect ring opening (55). However, aniline or o-phenylenediamine produced only intractable gums, which indicates that ring opening occurred but degradation followed.

Nucleophilic attack by aqueous base produced a crystalline acid (III) in low yield and several volatile fragments, two of which were identified as mesityl oxide and acetone. Acid (III) exhibited broad absorption in

the 2500-3300 cm^{-1} region and a strong band at 1712 cm^{-1} , both consistent with a carboxylic acid (see Fig. 6 and reference 37, p. 162). The second strong carbonyl absorption at 1733 cm^{-1} is best interpreted as a delta-lactone (reference 37, pp 185-86), possibly arising from lactonization of one of the carboxyl groups and a suitably located hydroxyl group.

Acid (III) appears to be the only product isolated in this study in which both anhydride carbonyls are intact. We will return to consideration of this acid after examining the structural implication of the two identified volatile fragments.

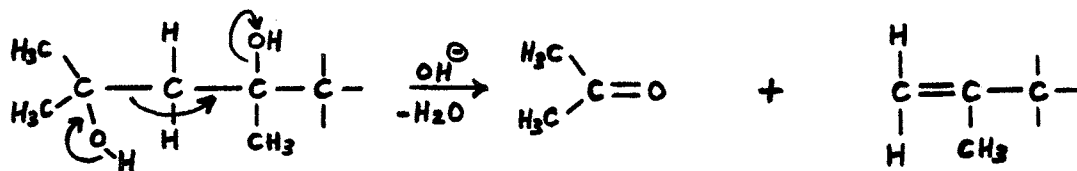
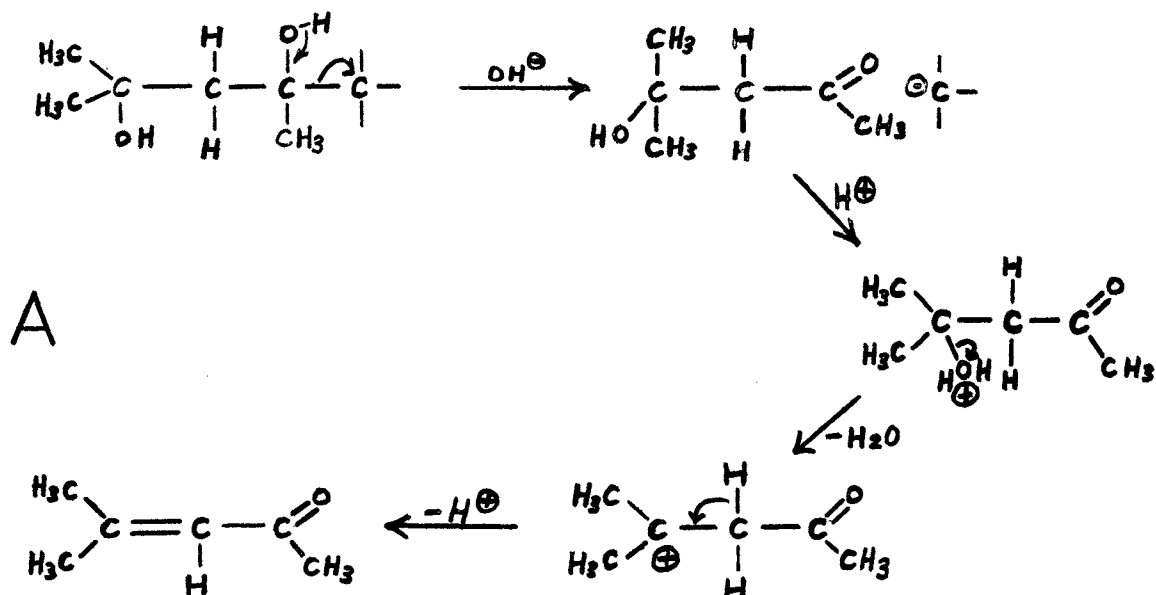
Mesityl oxide was rigorously established as the major component of the volatiles and acetone was indicated by comparison of retention times (GLPC, see Fig. 7). In order to examine the structural implication of these two fragments, it must be shown that neither mesityl oxide nor acetone were artefacts (i.e. produced by secondary reaction of a precursor), but were products of degradation of taccalin itself.

When authentic mesityl oxide was heated with aqueous base under the same conditions as during the hydrolysis of taccalin, no acetone was produced as evidenced by a GLPC. This indicates that the acetone was not produced by secondary attack on mesityl oxide, but that acetone originated from taccalin.

Since mesityl oxide can be prepared by refluxing acetone with a basic catalyst, followed by dehydration of the resulting diacetone alcohol with acid (56), it is possible that acetone was the sole product of hydrolysis of taccalin, and mesityl oxide resulted from self-condensation of acetone, followed by dehydration. If this in fact was

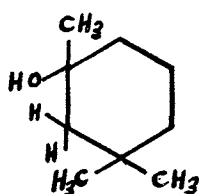
the case, then some diacetone alcohol should be detected in the volatiles, even under the most favorable equilibrium conditions. Diacetone alcohol, b. p. 166°C ., was chromatographed (GLPC) and compared with the volatiles from hydrolysis. The volatiles contained no component corresponding to diacetone alcohol. In support of this contention, no hydroxyl band was observed in the IR spectrum of the crude volatiles. It appears that both mesityl oxide and acetone were produced from taccalin and did not arise from secondary reactions.

In the discussion concerning the carbon skeleton it was shown that fewer than 14 carbon atoms were involved in the three carbon rings of taccalin. The exact number would depend on the length of side chains. The acyclic part structure shown below could account for the isolation of mesityl oxide and acetone in the following ways:

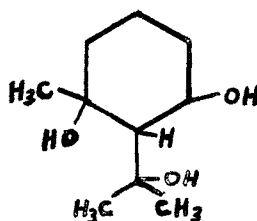


In scheme A normal dehydration is involved while in scheme B a carbon-carbon cleavage takes place. Although both schemes are plausible, they are unlikely, since six carbons would be involved in the side chain leaving at most only eight carbon atoms for the three carbon rings.

The fragments must have arisen by ring fission, from structures such as C and D below. The arguments to follow would apply equally as well to 5-membered rings.

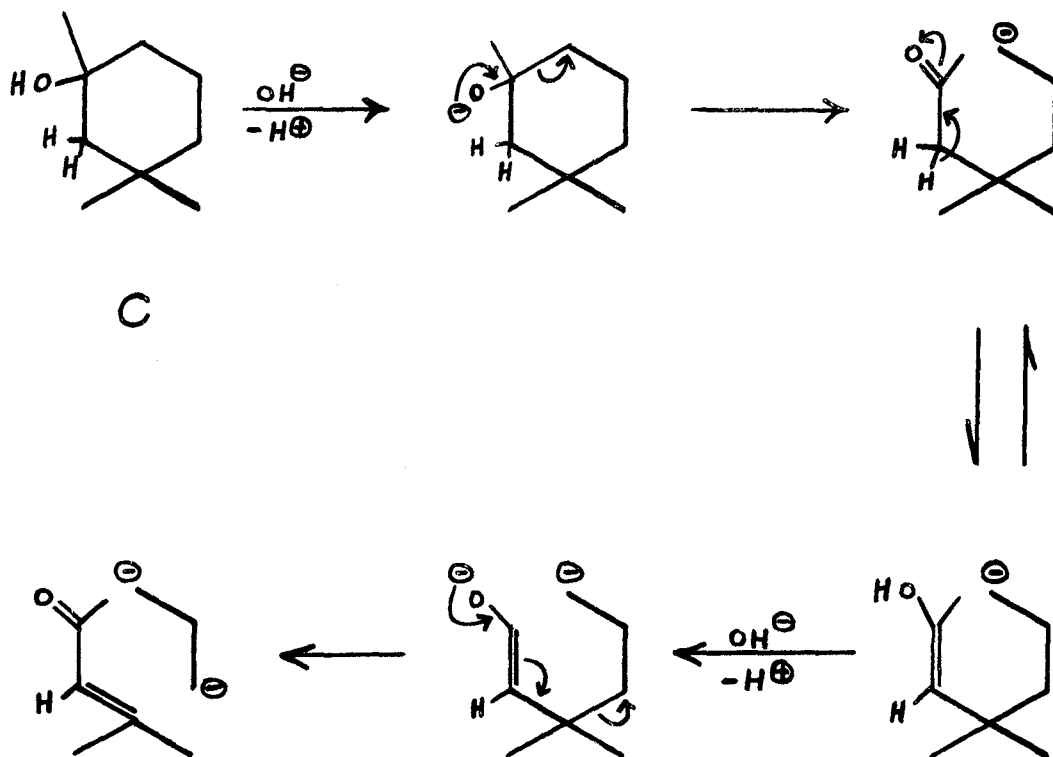


C

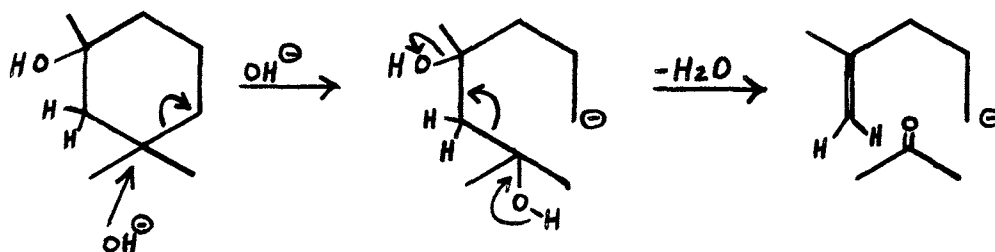


D

A mechanism can be envisioned to produce mesityl oxide from C as follows:

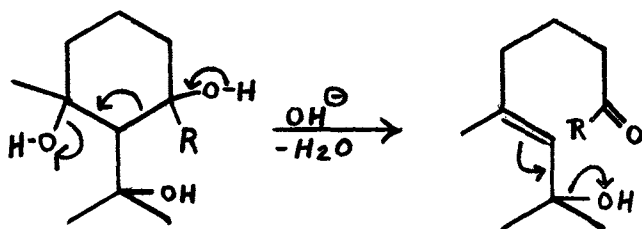


and acetone could arise by the following transformation:

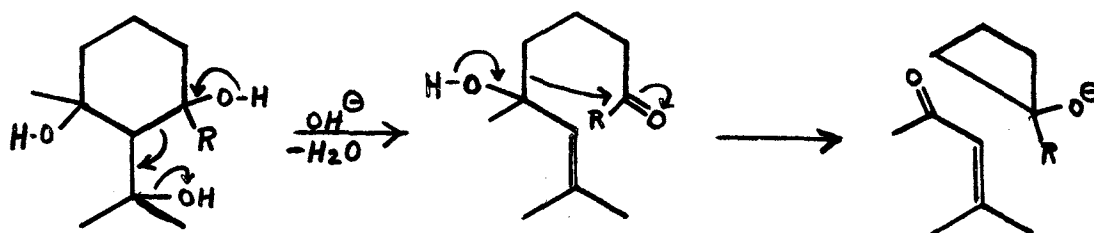
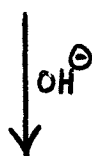


C

Structure D could produce mesityl oxide by two possible paths involving beta-elimination as follows:

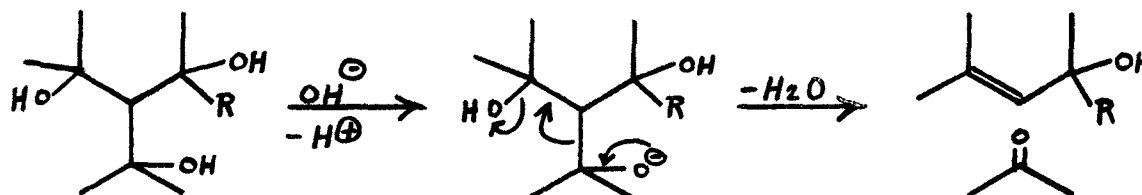


D



D

Acetone can arise simply by dehydration of D.



D

In either C or D, dehydration to produce acetone competes with the carbon-carbon cleavage to produce mesityl oxide. Whether structure C (gem-dimethyl group) or structure D (isopropyl residue) is involved, the conformations of the participating groups must be important factors in determining the type and course of the eliminations.

Unfortunately, only a few milligrams of acid (III) were isolated so that its formula could not be established, but it is likely that all the carbon atoms of taccalin were retained in the acid plus mesityl oxide i.e. acid (III) contains twelve carbon atoms. If structure C represents a part structure of taccalin, then acid (III) must contain six oxygen atoms, but if structure D is the correct formulation, acid (III) would contain only five oxygen atoms. That acid (III) contains at least one hydroxyl is evident from the IR spectrum (see Fig. 6).

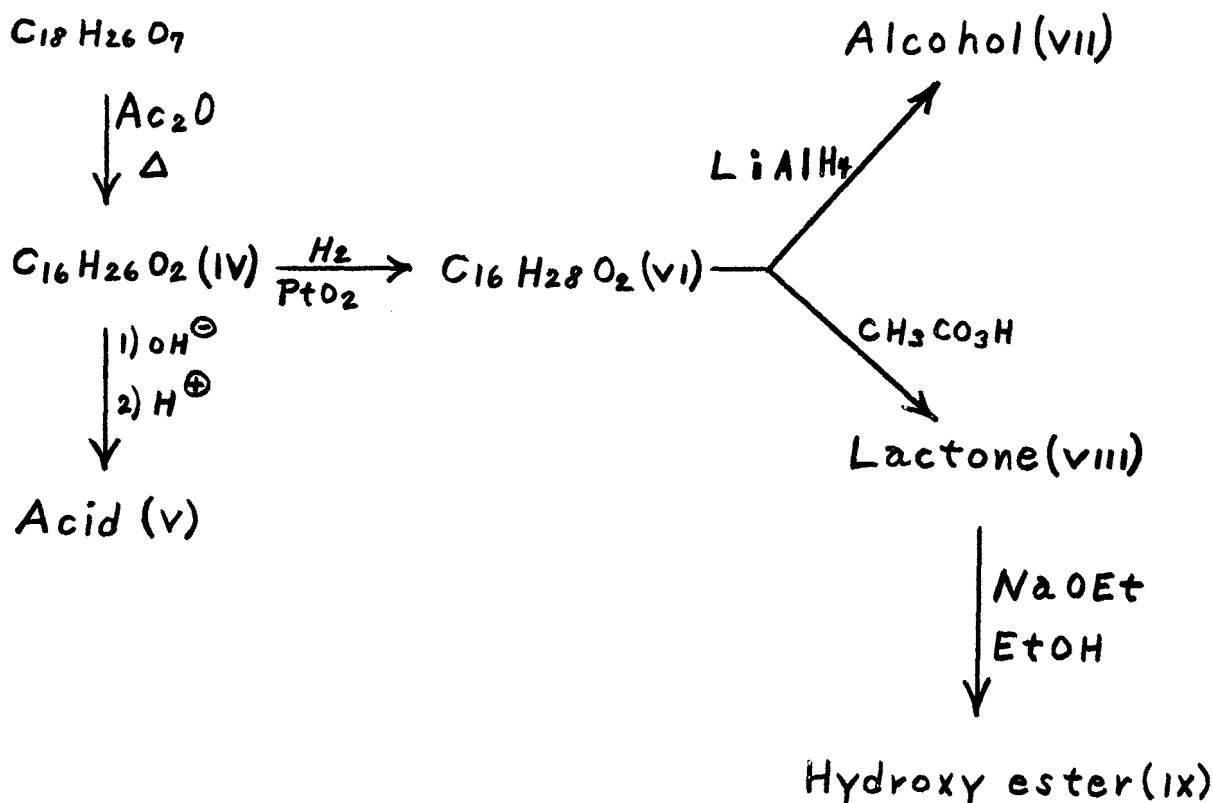
2. Degradation of Taccalin.

Taccalin was degraded to a substance, $C_{16}H_{26}O_2$ (IV), by hot acetic anhydride. Compound (IV) could be hydrolyzed to an acid (V) and could be hydrogenated to a substance, $C_{16}H_{28}O_2$ (VI), which in turn could be reduced to an alcohol (VII) by lithium aluminum hydride.

Compound (VI) could be oxidized under Baeyer-Villiger conditions to a neutral substance (VIII), which, when treated with sodium ethoxide in ethanol, produced a hydroxy ester (IX). These transformations are illustrated schematically below.

TACCALIN

$C_{18}H_{26}O_7$



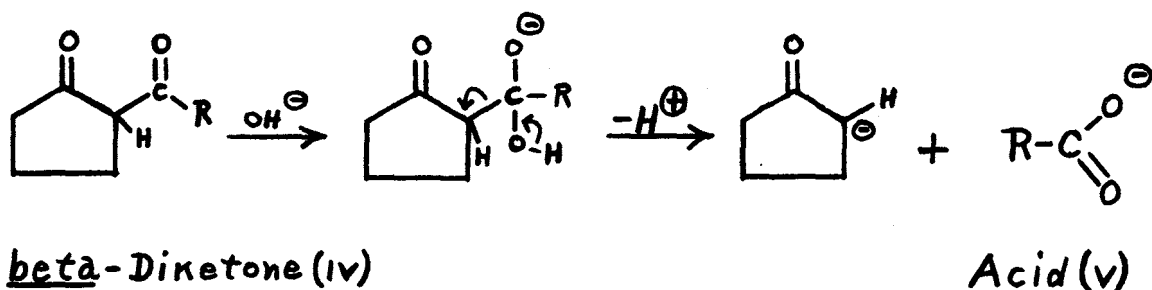
The $C_{16}H_{26}O_2$ (IV) compound has been formulated as a beta-diketone on the basis of the following evidence:

- (1) It forms iron (III) and copper (II)* chelates.
- (2) IR spectrum (see Fig. 10) shows absorptions at 3300 (hydroxyl), 1735 (free carbonyl), 1659 (conjugated carbonyl), 1630 cm^{-1} (enol chelate), in good agreement with literature spectra (57-63).

* No explanation is apparent for the exceedingly low value for copper in the analysis (see Experimental Section). An impure sample would not account for this large discrepancy.

- (3) The UV spectrum (see Fig. 9) is in accord with published spectra of beta-diketones (64, 65). The 33 $m\mu$ bathochromic shift observed in base is typical of an enolate anion (66).
- (4) The IR spectrum of the copper (II) chelate (see Fig. 11) supports the beta-diketone formulation (59).
- (5) The visible spectrum of the iron (III) chelate (see Fig. 12) is consistent with spectra of similar chelates (67).

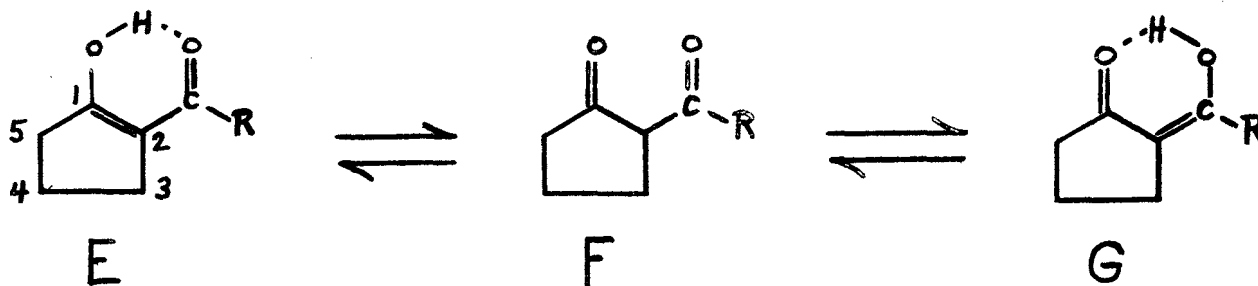
The 1735 cm^{-1} band of beta-diketone (IV) is characteristic of a cyclopentanone (68) and the second carbonyl must be located in an acyclic position as basic hydrolysis produced an acid containing no carbonyl group (see acid (V)).



An alpha-substituted, beta-diketone as shown above is further supported by the low wavelength minimum of the iron (III) chelate, which is characteristic of alpha-monoalkyl-beta-diketones (69). In addition, the exalted UV absorptions, without concurrent wavelength shifts, obtained by increasing the concentration, are characteristic of alpha-substituted beta-diketones (see Fig. 9).

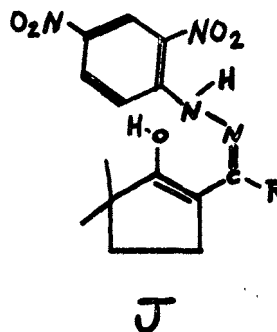
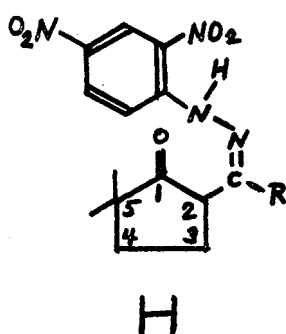
Although the cyclopentanone carbonyl would be expected to undergo nucleophilic attack preferentially, the isolation of acid (V), above,

requires attack to take place at the acyclic carbonyl. In connection with this nucleophilic preference, the failure of beta-diketone (IV) to react with 2,4-dinitrophenylhydrazine is informative. The normal course of reaction involves condensation with one carbonyl group to form the substituted hydrazone followed by intramolecular cyclization of the enol to the substituted pyrazole (70). The acyclic carbonyl must be the enolic contributor, since the cyclopentenol tautomer E would be less



stable than G due to the endocyclic double bond in the 5-membered ring (71). The preference for nucleophilic attack at the acyclic carbonyl rather than at the ring carbonyl can be accounted for by the presence of hindering groups in the 5-position of the ring. This contention is supported by the failure to isolate a benzylidene derivative.

Attack then by 2,4-dinitrophenylhydrazine must take place at the acyclic carbonyl H. The resulting hydrazone cannot cyclize since this would involve introduction of a double bond into the 5-membered ring, J. The inability of the 5-membered ring to accomodate the double bond and



the steric hindrance offered by the two substituents in the 5-position, effectively prevent the approach of the nitrogen atom and thus cyclization to the pyrazole.

beta-Diketone (IV) must involve the following functions:

- (1) One hindered carbonyl in a 5-membered ring,
- (2) A second carbonyl, beta to the first, responsible for the enolic properties and preferential nucleophilicity,
- (3) A second ring and one C-CH₃ group in the molecule.

Degradation of beta-diketone (IV), although not successful in producing a compound of known structure, substantiated the proposed partial structure.

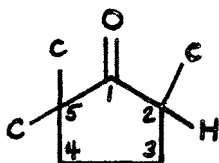
Ketone (VI): Hydrogenation of beta-diketone (IV) produced a substance, C₁₆H₂₈O₂, thought to be the expected beta-ketol, but which showed no absorption in the hydroxyl region of the IR. A possibility that the beta-ketol was dehydrated to the alpha, beta-unsaturated ketone was considered. However, an alpha, beta-unsaturated ketone would be expected to absorb much lower than the observed 1740 cm.⁻¹ frequency. In addition, a maximum at 296 μ (Log E = 1.98; see Fig. 14) was observed, which is consistent with a cyclopentanone (72) and not an alpha, beta-unsaturated ketone, which would be expected to absorb strongly near 240 μ . Confirmation of the cyclopentanone formulation was obtained by lithium aluminum hydride reduction to a secondary alcohol (VII), containing the expected bands at 3268 and 1090 cm.⁻¹. No absorption was present in the C-O-C stretching region characteristic of ethers (see Fig. 16), which indicates that ketone (VI) contains only one oxygen. The possibility of a hydrate was considered due to the observed unusual melting behavior (see Experimental). Although additional evidence was lacking for a hydrated ketone, no other

oxygen function could be discovered. A formula of $C_{16}H_{26}O \cdot H_2O$ requires a tricyclic system, the nature of which is not obvious. A tentative formulation as a tricyclic cyclopentanone involving steric hindrance of the carbonyl, as evidenced by its failure to form an oxime or a 2,4-dinitrophenylhydrazone, is proffered for ketone (VI).

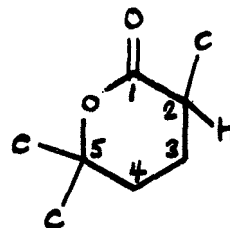
Further evidence as to the correctness of this formulation was afforded by the peracetic acid oxidation of this ketone to a neutral substance, IR absorption at 1740 cm^{-1} (delta-lactone), which was opened to the hydroxy ester (IX) with sodium ethoxide in ethanol. IR absorption bands at 3448 (hydroxyl), 1731 (acetate), 1179 (tertiary hydroxyl) cm^{-1} (see Fig. 17). The presence of the tertiary hydroxyl supports the contention that both beta-diketone (IV) and ketone (VI) are disubstituted in the alpha position to the ring carbonyl.

3. Partial Structures of the Degradation Products.

In the peracetic acid oxidation of ketone (VI), the tertiary carbon-5 should migrate in preference to the secondary carbon-2 (73) and lactone



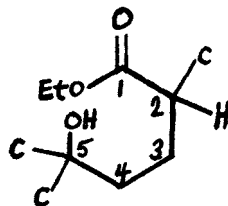
Ketone (VI)



Lactone (VIII)

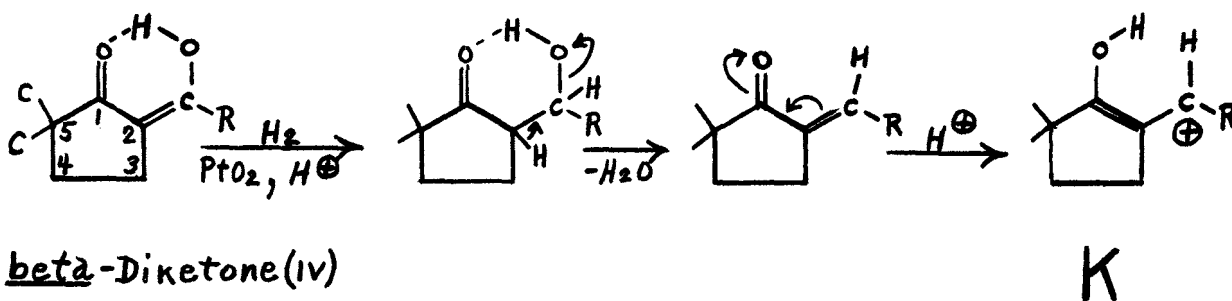
(VIII) should be formulated as shown if ketone (VI) is formulated correctly. In support of this hypothesis, hydroxy ester (IX) contained a tertiary hydroxyl as inferred from the IR spectrum. This confirms the disubstitution at the 5-position in ketone (VI) as well as in beta-

diketone (IV).

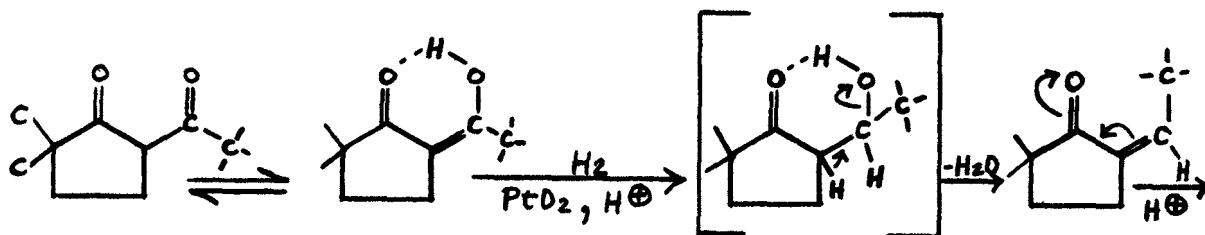


Hydroxy ester (IX)

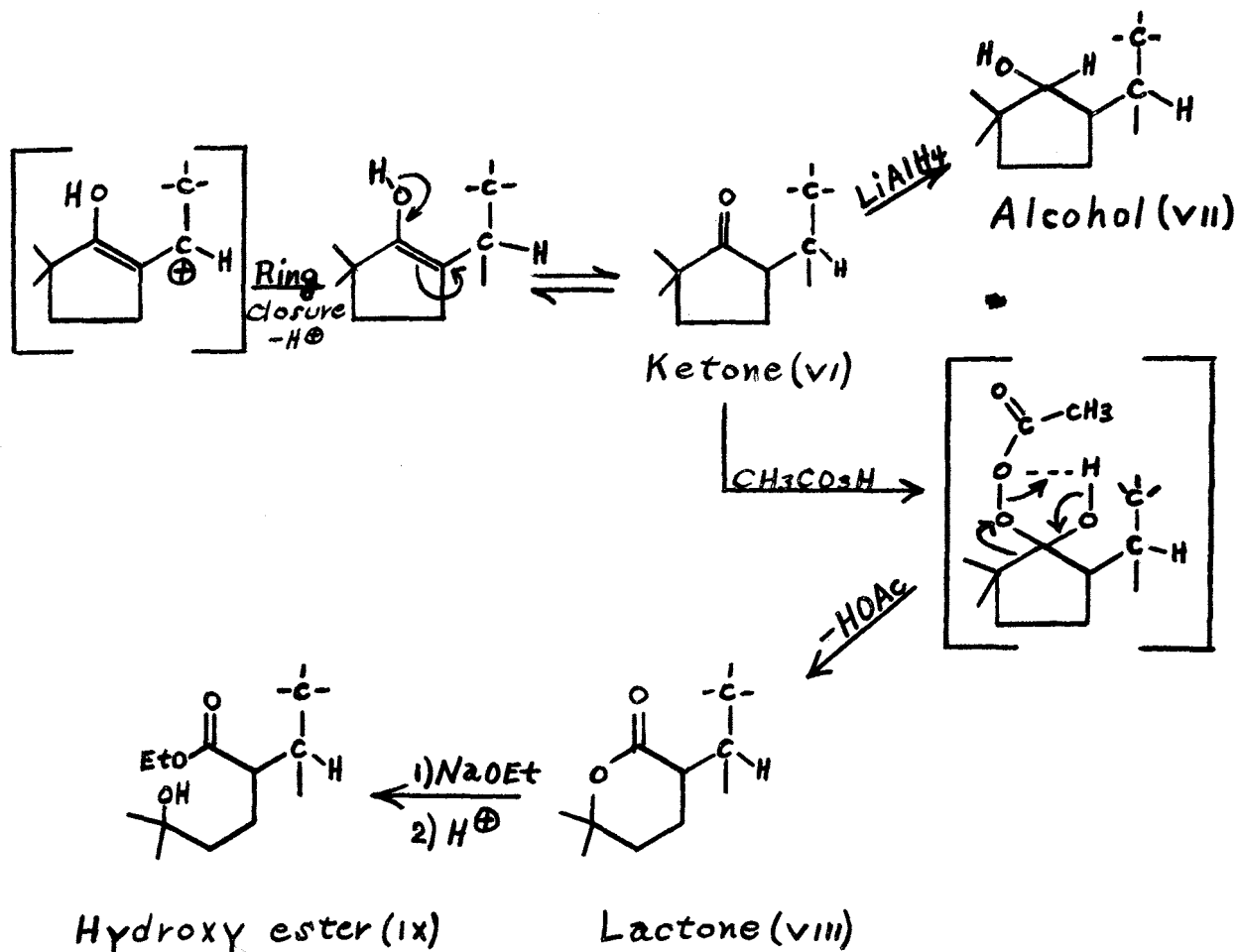
If we accept the fact that ketone (VI) contains only one oxygen atom, then the molecule must be tricyclic. This means that the bicyclic beta-diketone (IV), $C_{16}H_{26}O_2$, formally lost one oxygen atom upon hydrogenation to give the tricyclic ketone (VI), $C_{16}H_{26}O$. The nature of this transformation is not clear, but if the reaction proceeds to K as shown, then the carbonium ion K may cyclize with a carbon having anionic character by proton elimination, with or without prior rearrangement.



The degradation scheme may now be formulated with the use of part structures.



beta-Diketone(IV)

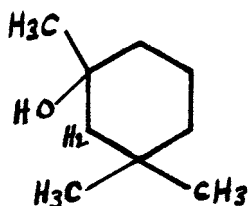


The nature of the conversion of taccalin, $\text{C}_{18}\text{H}_{26}\text{O}_7$, to beta-diketone (IV), $\text{C}_{16}\text{H}_{26}\text{O}_2$, must be a complex transformation involving the formal loss of C_2O_5 . If both carbonyls of the anhydride are lost, which is likely, then two more oxygens must be lost from hydroxyls with no overall loss of hydrogen. In addition, two carbonyl groups are generated beta to each other - probably from the two remaining hydroxyls - with concurrent opening of one carbon ring!

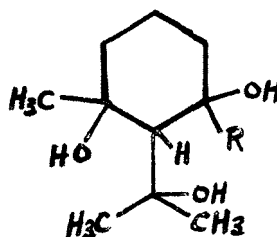
E. Conclusions

Evidence was presented which demonstrated that taccalin, $\text{C}_{18}\text{H}_{26}\text{O}_7$, is tricyclic and contains four tertiary hydroxyl groups and a 6-membered cyclic anhydride grouping. The molecule contains three or four $\text{C}-\text{CH}_3$ groups.

Basic hydrolysis of taccalin afforded mesityl oxide and acetone, which requires that a grouping such as A or B be incorporated into the taccalin molecule.

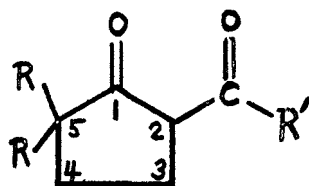


A



B

Taccalin was degraded by hot acetic anhydride to a new substance, $C_{16}H_{26}O_2$, which was shown by spectral data and by a series of degradations to be a bicyclic, 2-acylcyclopentanone derivative C. The ring carbonyl was shown to be sterically hindered by two substituents in the



C

5-position ($R \neq H$). The nature of the transformation of taccalin to this beta-diketone remains obscure.

IV. SUMMARY

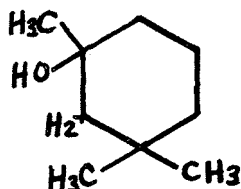
The extraction of dry pia tubers with acetone, following a prior petroleum ether extraction, produced a red-brown solid from which a white, amorphous bitter principle could be isolated in $3-10 \times 10^{-3}\%$ yield (dry weight). Purification of the bitter principle was accomplished by fractional precipitation from acetone: water and ethyl ether: petroleum ether. Although this bitter principle (named taccalin) failed to crystallize, evidence which demonstrated its homogeneity was obtained by paper chromatography and UV absorption characteristics.

Elemental analyses, combined with molecular weight and Zerewitinoff determinations, indicated a formula of $C_{18}H_{26}O_7$ for taccalin. The active hydrogen determination indicated the presence of four hydroxyl groups, all of which appeared to be tertiary as evidenced by repeated failures to be esterified. The remaining three oxygen atoms were found to be involved in a 6-membered cyclic anhydride. This assignment is based on spectral evidence.

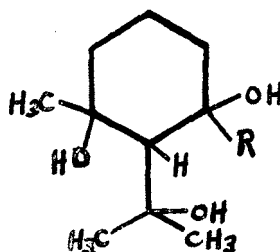
Microhydrogenation indicated the absence of unsaturation, thus requiring taccalin to be tetracyclic - a tricarbocyclic skeleton in addition to the cyclic anhydride. The presence of three or four $C-CH_3$ groups restricted the number of carbon atoms in the three rings to thirteen or fewer.

Basic hydrolysis afforded a small amount of an hydroxy acid, which was not characterized, and a volatile fragment, shown to be predominantly mesityl oxide with a lesser amount of acetone. Plausible mechanisms were presented which suggest that a grouping such as A or B must be incorporated

in the taccalin molecule in order to account for these hydrolysis products. A decision between the gem-dimethyl A and isopropyl B types could not be made on the basis of the information available.



A

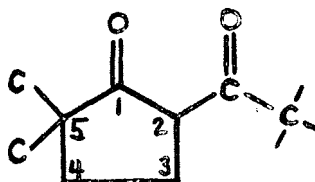


B

Acetylation of taccalin produced a crystalline solid which was shown not to be an acetate, but a lactonic acid, which probably retained the eighteen carbon atoms of taccalin. Insufficient material prevented further investigation of this lactonic acid.

Hot acetic anhydride converted taccalin to a new substance, $C_{16}H_{26}O_2$. This compound was formulated as a 2-acylcyclopentanone derivative largely on the basis of its spectral properties and its hydrolysis by base to a non-ketonic acid. Support for this formulation was furnished by chemical and spectral properties of its iron (III) and copper (II) chelates. Additional support was obtained by hydrogenation of this substance to a tricyclic cyclopentanone derivative, $C_{16}H_{26}O$, which could be reduced with lithium aluminum hydride to a secondary alcohol. The tricyclic ketone was further degraded by peracetic acid oxidation to a delta-lactone, which was opened with sodium ethoxide in ethanol to the hydroxy ester. The hydroxyl group of this compound was shown to be tertiary indicating that the 2-acylcyclopentanone and the tricyclic ketone were disubstituted in the five-

position in accord with their failure to react with carbonyl reagents and to form benzylidene derivatives. The part structure of the 2-acylcyclopentanone, which is consistent with all the data is shown below.



C

The nature of the transformation of taccalin to the beta-diketone with hot acetic anhydride remains unexplained. Indeed, the hydrogenation of the bicyclic beta-diketone to a tricyclic ketone is not clear. These questions must await further experimental work before answers will be forthcoming.

Only three substances have been found in natural products which contain an acid anhydride grouping, none of which were found in flowering plants. They are: the well-known vesicant cantharidin from Cantharides beetles (74), stipitatic acid from Penicillium stipitatum (75), and puberulonic acid from Penicillium puberulum (76), the last two being mold metabolites containing a tropolone ring. Taccalin is evidently a new compound, hitherto unknown and deserves the interest of future workers.

APPENDIX: COMPANION SUBSTANCES OF TACCALIN

I. Introduction

A yellow substance accompanied taccalin tenaciously in the isolation stage of this study and it seemed desirable to obtain some knowledge of its properties in order to devise a purification scheme for taccalin. This yellow oil, which eventually crystallized to yield ester (1), was shown to be a rather large molecule of some complexity.

During the initial extraction of the pia tubers with petroleum ether, a small amount of taccalin was invariably carried over into the solvent with the residual water in the tubers. Although the amounts lost in this way proved later to be negligible, recovery of the taccalin from the "fatty" extract led to the observation that several crystalline components of the "fat" were easily isolated. Two of these components were investigated on a preliminary basis and the results are given below.

II. Experiments and Results*

A. Ester (1)

The first chloroform eluate from the Florisil column (see Thesis Experimental Section) contained a yellow, viscous oil free of taccalin. Additional quantities were obtained from the first eluates before the taccalin break-through in subsequent chromatograms.

This yellow oil, which had a characteristic odor of fatty acids, gave negative permanganate and ceric ammonium nitrate tests. A

* See Experiments and Results section in the thesis proper for details of apparatus and analyses.

2,4-dinitrophenylhydrazine test was also negative. A sample of this oil was dried at room temperature/25 mm. for 12 hours for analysis.

Analysis. Found: C, 74.8 and 74.7% H, 10.6 and 10.7% C-CH₃, 5.4%

$$[\alpha]_D^{27} = 0 \pm 45^\circ \text{ (c = 40 g./l. in acetone).}$$

The IR absorption spectrum showed prominent hydroxyl and carbonyl bands, but the characteristic anhydride bands of taccalin were absent.

Preliminary hydrolyses with hot aqueous base afforded a waxy solid and a solid acid upon acidification indicating the presence of an ester group. A quantitative saponification was undertaken in order to obtain the neutralization equivalent and to recover the hydrolysis fragments.

Saponification: Exactly 2 ml. of 0.1993 N NaOH was added to 97.8 mg. of ester (1) and the mixture refluxed one hour. The solution was cooled and back-titrated potentiometrically with 0.0945 N hydrochloric acid. A volume of 1.701 ml. of acid was required to neutralize the excess base. The calculated neutralization equivalent was 411.

Molecular weight by isothermal distillation: Ca. 18 mg. of ester (1) was equilibrated with acetone against azobenzene in the usual way (vide supra). Equilibrium was established in 480 hours at room temperature; MW = 446. Ester (1) began to crystallize in the apparatus after standing for several more days (vide infra).

Alcohol (XII): The white, waxy solid which precipitated from the basic solution during the saponification of ester (1), was filtered, washed with water and recrystallized from methanol to give 21 mg. of colorless crystals. One recrystallization from aqueous acetone furnished colorless crystals

of alcohol (XII), which melted poorly near room temperature.

IR max. = 3367, 3300, 3145 (strong triplet), 1730 (S), 1661 (S),
1623 (S) cm.^{-1} (see Fig. 19).

Acid (XIII): The basic filtrate from the preparation of alcohol (XII) was acidified with conc. hydrochloric acid to pH 4 and a waxy solid formed. The solution was decanted and the aqueous phase extracted with 5 X 5 ml. of ethyl ether, the ether washed once with water and the ether dried and stripped in vacuo. The solids were combined, dissolved in aqueous sodium hydroxide, filtered and precipitated by acidification. The white solid was crystallized once from chloroform to give 16 mg. of acid (XIII), colorless crystals, m.p. 126-141°C.

IR max. = 1718 (S) cm.^{-1} (see Fig. 20).

Crystallization of ester (1): Ester (1) was separated from the yellow oil by crystallization from acetone. One more recrystallization from acetone and once from methanol gave light yellow plates, m.p. 62-63.5°C. which remained unchanged after further crystallization from methanol. Dried at room temperature / 25 mm. for 6 hours for analysis.

Analysis. Calc'd. for $\text{C}_{27}\text{H}_{48}\text{O}_5$:	C, 71.6%	H, 10.7%	M. Wt., 453
Found	: C, 71.9	H, 10.5	M. Wt., 1271 (Rast, Camphor)

A small residue was noted upon combustion. The C and H values are calculated from an adjusted sample weight.

$\lambda_{\text{inflection}} = 240 \text{ m}\mu$ (log E = 4.0; see Fig. 2).

IR max. = 3378 (S), 3322 (sh), 1730 (S), 1695 (S), 1653 (S) cm.^{-1}
(see Fig. 18).

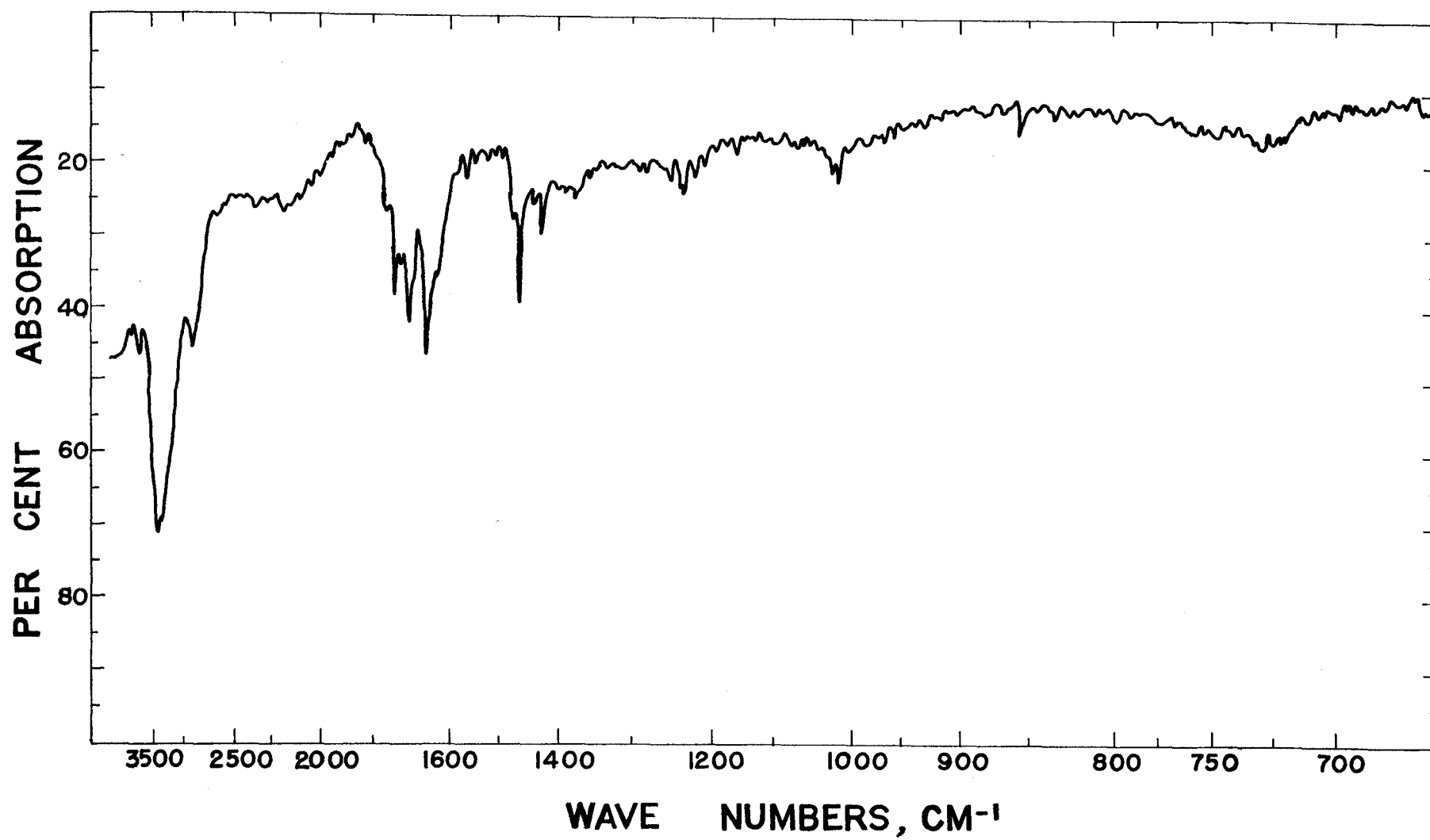


FIG.18 IR SPECTRUM OF ESTER (I); SOLID FILM

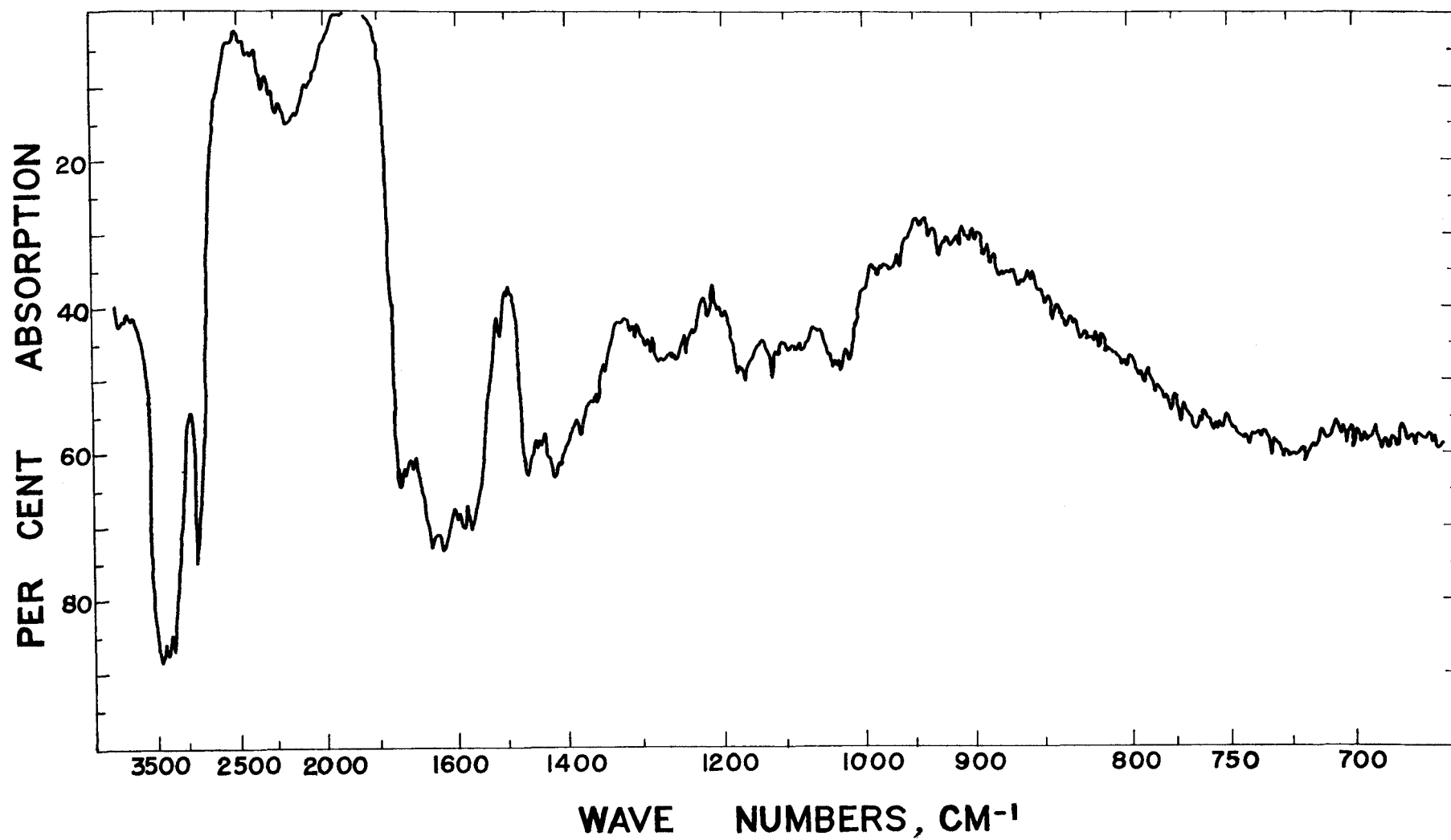


FIG.19 IR SPECTRUM OF ALCOHOL (XII); SOLID FILM

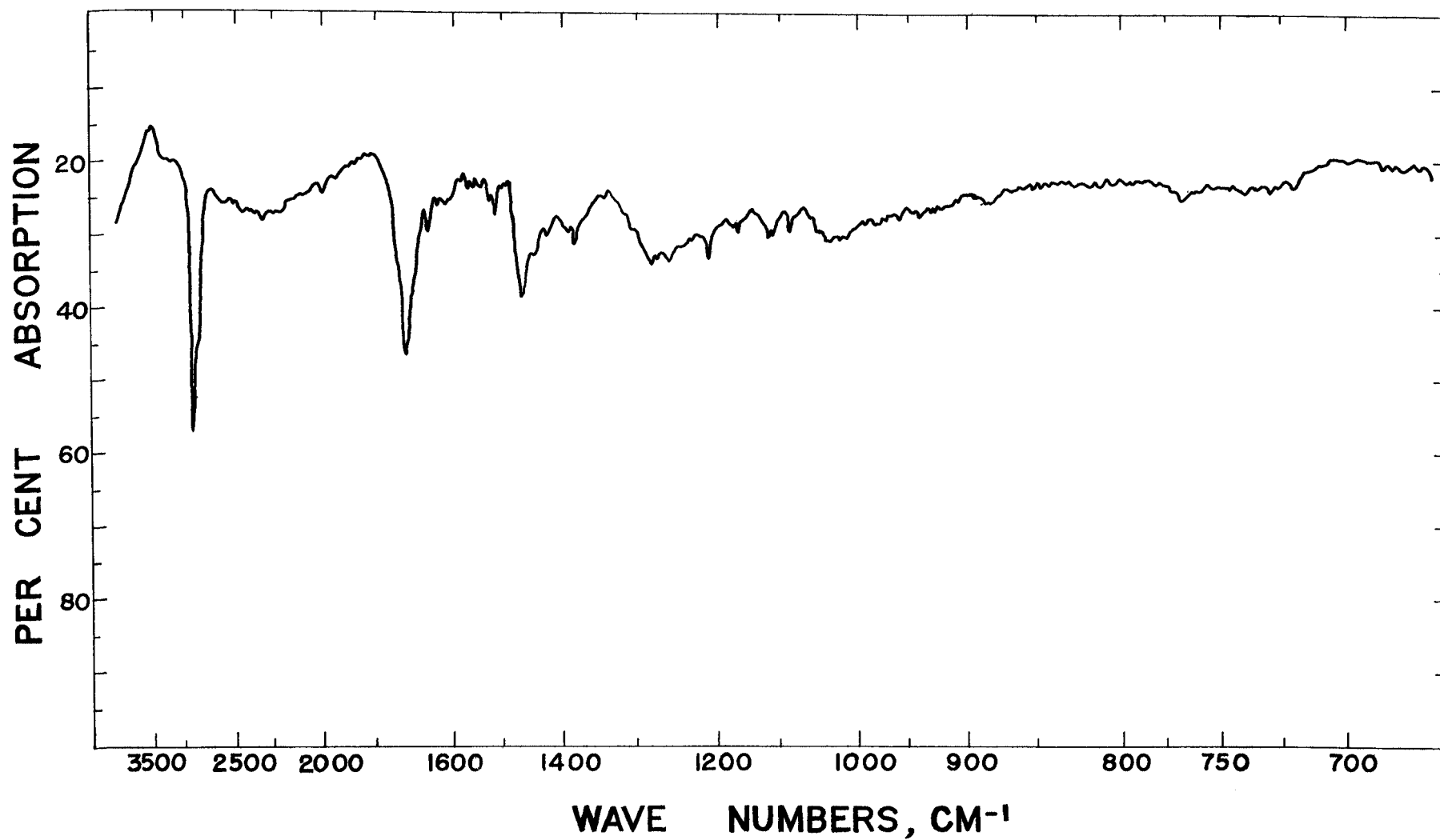


FIG. 20 IR SPECTRUM OF ACID (XIII); SOLID FILM

B. Alcohols (X) and (XI)

A small portion of the petroleum ether extract was evaporated to dryness in vacuo to yield ca. 2 g. of "fatty material". This waxy solid was extracted with cold acetone and the residue (300 mg.) was dissolved in a minimum amount of hot n-heptane and allowed to stand in the refrigerator. A white solid was deposited and a 2-fold concentration of the mother liquor afforded additional solid; total yield, 190 mg. of light, cream-colored solid. The solid was dried and dissolved in hot acetone, water was added slowly and the solution was allowed to evaporate slowly. A yellow oil appeared on the surface of the solution, the oil was separated and the acetone stripped from the water. The aqueous solution was extracted continuously with ethyl ether to remove additional yellow oil which was not investigated. The aqueous solution was stripped to dryness in vacuo and the white solid (56 mg.) was crystallized from acetone-ether. The solid could be separated by fractional crystallization from acetone-ether into two solids, the more soluble appearing as colorless needles and the less soluble as colorless plates. The more soluble solid was recrystallized from water containing a small amount of acetone and finally from methanol-acetone to give 18 mg. of colorless cubes, m.p. 168-70°C. alcohol (X).

Analysis. Found: C, 57.7% H, 7.9% A gray residue was found after ignition, invalidating the analysis. The substance was not checked for hetero atoms.

IR max. = 3333 (M), 1618 (W), 1580 (W), 1042 (Br and S) cm.^{-1} (see Fig. 21).

Alcohol (XI): The less soluble solid was recrystallized twice from

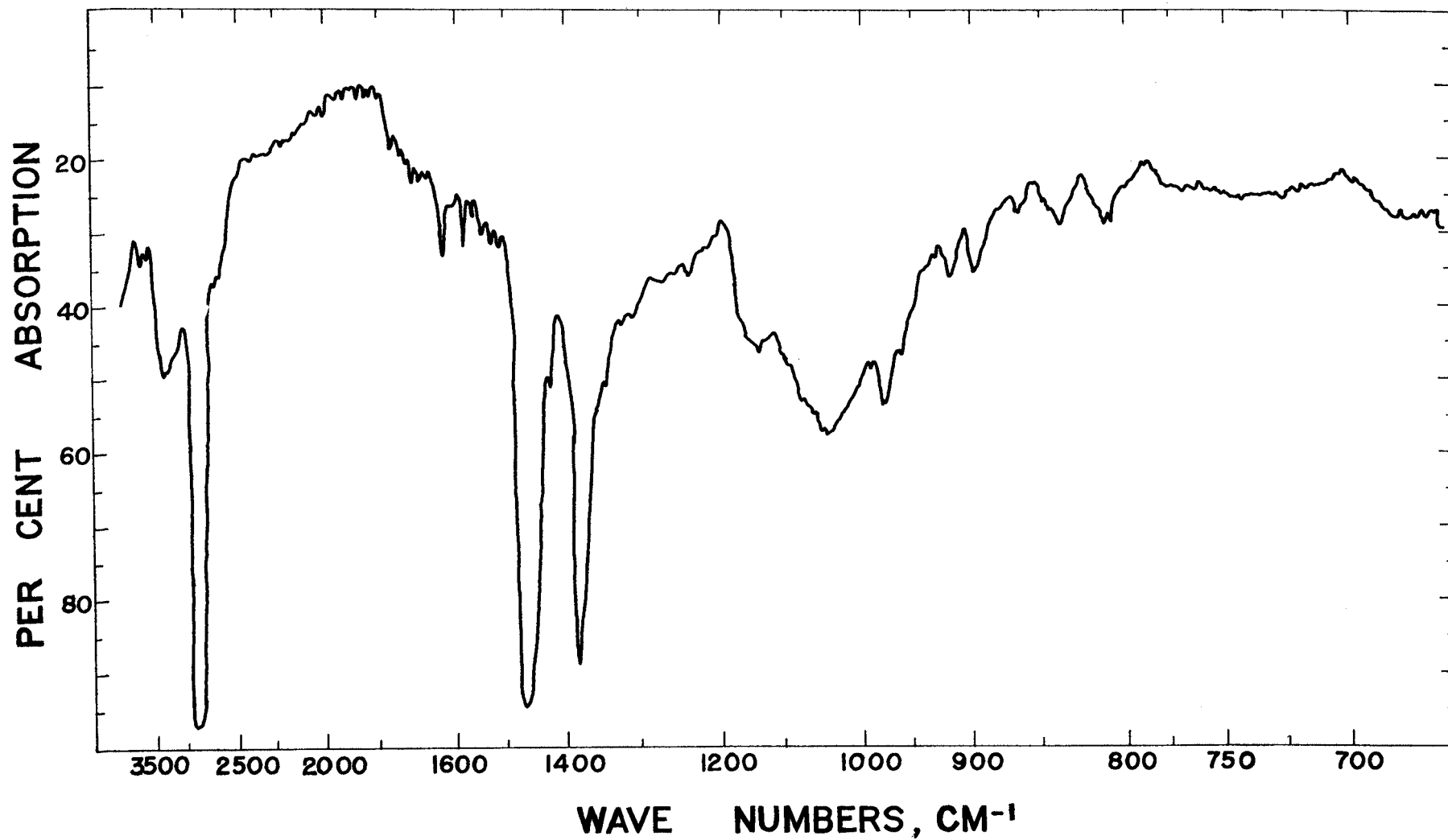


FIG. 21 IR SPECTRUM OF ALCOHOL (X); MINERAL OIL MULL

ethanol to yield 14 mg. of glistening needles, m.p. 278-80°C.

IR max. = 3356-3311 (Br and M), 1658 (M), 1044 (S) cm^{-1} (see Fig. 22).

III. Discussion of Results

A. Ester (I)

The ester, $\text{C}_{27}\text{H}_{48}\text{O}_5$, appears to contain an ester grouping which can be hydrolyzed by base to a solid alcohol and a solid acid. The 725 cm^{-1} band due to the methylene rocking was absent in the ester and in both hydrolytic fragments. This indicates a polycyclic structure, as a methylene chain of four or more units will absorb strongly near 725 cm^{-1} (77).

The carbonyl region of the ester is complex, showing absorptions at 1730 (ester), 1695 (conjugated carbonyl) and 1653 (conjugated carbonyl or exalted double bond) cm^{-1} . In addition, a hydroxyl band at 3378 cm^{-1} is prominent. The acid moiety shows only the carboxylic acid absorption (1718 cm^{-1}). The alcohol, on the other hand, retains all of the IR-active chromophores. The strong triplet in the 3300 cm^{-1} region is indicative of a polyhydroxy molecule, although the 1730 cm^{-1} band, assigned to the ester previously, is still present. Another carbonyl band may have been superimposed on the ester band and survived the hydrolysis.

Since the molecular weights obtained by saponification and isothermal distillation were in agreement, the Rast molecular weight must have been in error.

The UV inflection at 240 $\text{m}\mu$ ($\log E = 4.0$) is consistent with an alpha, beta-unsaturated ketone with alpha, beta or beta, beta-substituents,

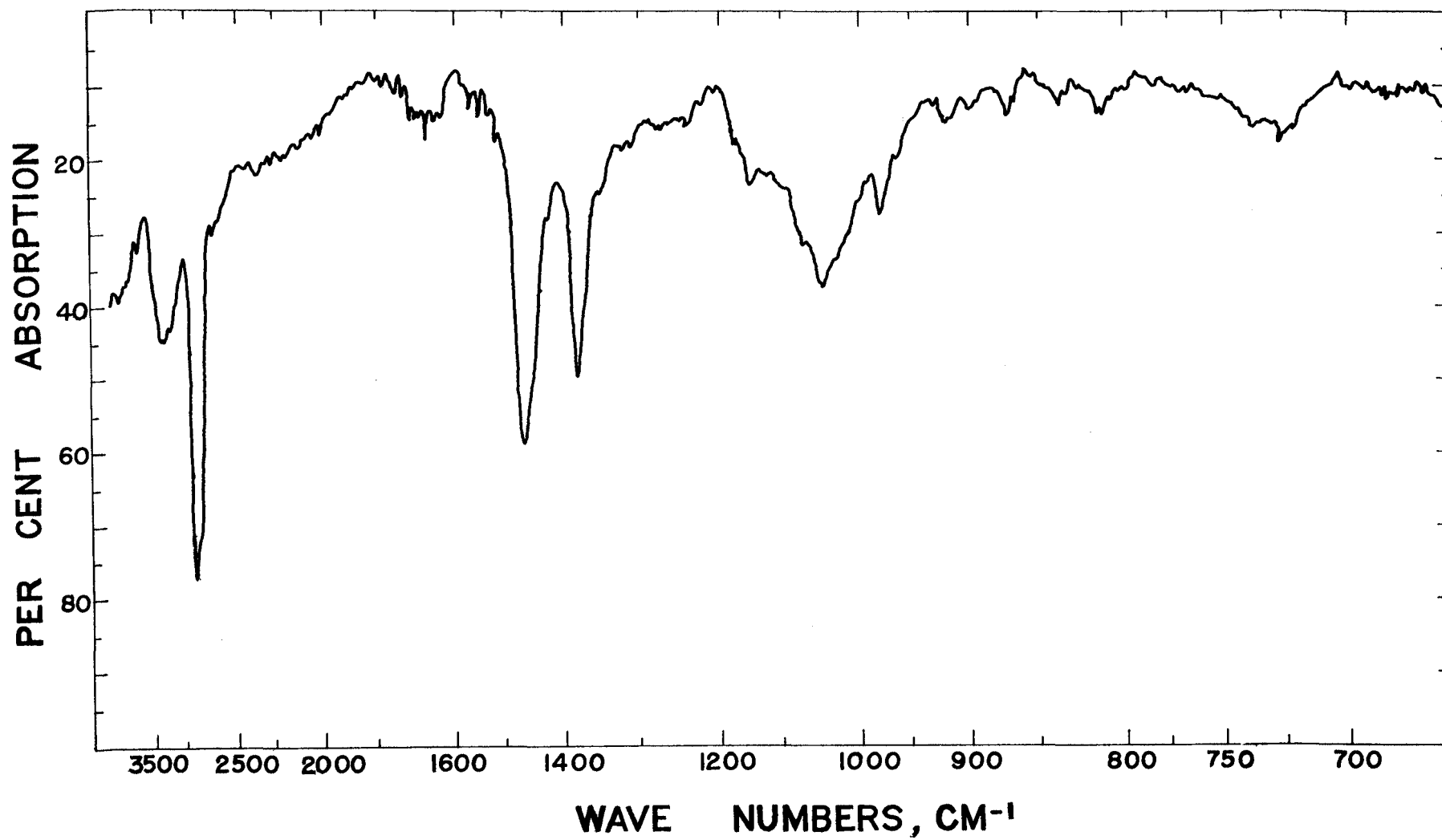


FIG. 22 IR SPECTRUM OF ALCOHOL (XI); MINERAL OIL MULL

in which the double bond is exocyclic. The IR maximum at 1653 cm^{-1} in the ester, which is retained in the alcohol moiety (1661 cm^{-1}), is consistent with this formulation.

This ester probably contains, a conjugated ketone, a second carbonyl and one hydroxyl. The alcohol moiety contains all of the functional groups which shows that the ester is composed of a simple acid esterified with a polycyclic alcohol of some complexity.

B. Alcohols (X) and (XI)

Little can be said concerning the chemical nature of these substances as very little experimental work was done. Alcohol (X) is fairly soluble in water and is probably a polyhydroxy compound containing no carbonyl functions. The analysis, although useless in predicting a molecular formula, indicates a very high oxygen to carbon ratio similar to sugars. No methylene rocking vibration near 725 cm^{-1} is observed indicating a cyclic structure rather than acyclic.

Alcohol (XI), obtained in small yield, appears to contain more than one hydroxyl group and either an exalted double bond or a conjugated carbonyl which is reduced in intensity. A significant absorption near 725 cm^{-1} indicates a methylene chain in the molecule of at least four units.

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