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IN

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DOCTOR OF PHILOSOPHY

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By

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To

my teachers and friends who have

given me encouragement in many ways.

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AN INVESTIGATION FOR ALKALOIDS IN *CHARPENTIERA OBOVATA* GAUD.

By Tammanur R. Pattabhiraman

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 in chemistry, July 1964.

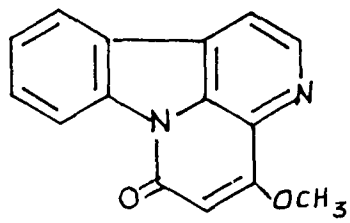
ABSTRACT

The genus *Charpentiera* belongs to the plant family Amaranthaceae and is endemic to the Hawaiian Islands. The Hawaiian name of the several members of the genus is *papala*. An investigation for alkaloids in the species *Charpentiera obovata* Gaud. was undertaken for several reasons. There is a reference to the general use of various species of this family against skin diseases. This is an example of the general phenomenon that physiological activity is frequently linked to the presence of alkaloids. Secondly, no structural work has been reported in the literature on alkaloids from the plant family Amaranthaceae. Thirdly, the plant family Amaranthaceae is one of several families referred to as the Centrospermae plants. A common characteristic of eight of these families is the unusual occurrence of nitrogenous pigments called betacyanins, structurally different from the other well-known class of pigments, anthocyanins. It was therefore felt that an investigation of the alkaloidal constituents of the species *Charpentiera obovata* might show whether a structural relationship exists between the alkaloids and the betacyanins.

Surprisingly, only one alkaloid could be isolated, and that in a very small yield of *ca.* 0.0005%, by solvent extraction and column

chromatography.

The alkaloid crystallized as pale yellow needles from an ethanol-acetone-hexane mixture, but could be sublimed to a white feathery substance, m.p. 220-221°. The molecular formula of $C_{15}H_{10}N_2O_2$ was supported by combustion analysis and by mass spectrum. The compound possesses one methoxyl group, one olefinic proton and six aromatic protons as shown by proton magnetic resonance spectrum. This information coupled with the ultraviolet spectrum in methanol sufficed to suggest that the alkaloid was probably 4-methoxycanthin-6-one, the structure of which is shown below.



Final confirmation was provided by a synthesis of 4-methoxycanthin-6-one along lines described previously and by comparison of ultraviolet and infrared spectra and of R_f-values (thin-layer chromatogram) and by mixed melting point determination which showed no depression.

This is the first instance that this alkaloid, 4-methoxycanthin-6-one has been isolated from natural sources. The significance of this finding to chemotaxonomic study is discussed.

Infrared spectra of some of the synthetic intermediates, e.g. harman, benzalharman, β-carboline-1-carboxylic acid, 4-hydroxycanthin-6-one have been determined.

CHAPTER I

INTRODUCTION

A. Botanical

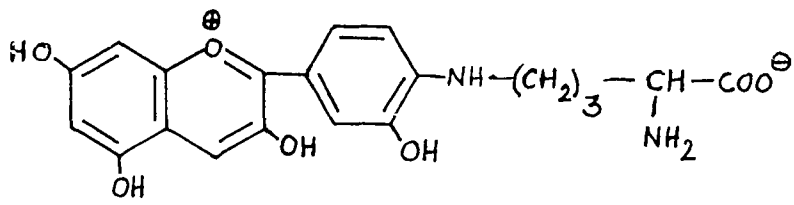
Charpentiera obovata Gaud. is a member of the plant family Amaranthaceae, which is a prominent contributor to the floras of the world, with the exception of the frigid zones.[1] The genus Charpentiera is peculiar to the Hawaiian Islands and consists essentially of two endemic species, C. elliptica (Hbd.) Heller and C. obovata Gaud. Though a third species, C. ovata Gaud.[2] is recognized by some people, it is considered to be conspecific with C. obovata by Skottsberg[3] and other botanists. A close examination of the specimens of both C. obovata and C. ovata preserved in the Herbarium of the Bishop Museum, Honolulu, revealed that they are almost identical except for some minor variations in the size and shape of the leaf and of the infloresence. It is therefore felt that both names apply to a single species under the name C. obovata Gaud. However, C. elliptica (Hbd.) Heller, present mostly on Kauai, is decidedly a different species according to Rock.[1] No specimen of this species is preserved in the Herbarium of Bishop Museum. The Hawaiian name for this genus is papala.[1]

Charpentiera obovata is usually found as a small tree of 15 to 35 feet in height and reaches its best development in drier habitats. It is a highly variable species, as was noticed by Gaudichaud, the variation depending mainly on the shape of the leaf. It occurs on all the islands of the group in wet land as well as dry forests, up to an elevation of 4,000 feet.[1] Its most outstanding recognizable features are seen when the plant is in full bloom. It is a rather attractive looking tree with

its long inconspicuous glabrous flowers in large paniculate spikes, hanging downwards, and its trunk usually divided in its portion into several column-like buttresses.[1] The wood is very soft and fibrous and when dry exceedingly light and will burn readily. It was because of this property and because of the ease with which the wood can be ignited that the tree was used by the Hawaiians for a most original and grand display of fireworks. The leaves may be as wide as twelve centimeters and as long as thirty centimeters.[1] Both leaves and bark when crushed emit a slightly pungent odor.

B. Chemical

The family Amaranthaceae is one of several families which comprise the order Centrospermae.[4,5,6] A common characteristic of eight families of this order is the unusual occurrence of nitrogenous pigments. The prominent genera in these eight families examined so far are shown in TABLE I.[7] These pigments were originally referred to as "nitrogenous anthocyanins" after Robinson and coworkers classified them as derivatives of anthocyanins containing nitrogen and proposed for consideration a structure comprising a pentahydroxyflavylium nucleus and an ornithine residue condensed by loss of one molecule of water thus leaving the more basic nitrogen of ornithine attached directly to one of the benzene rings,[8-13] in the following fashion:



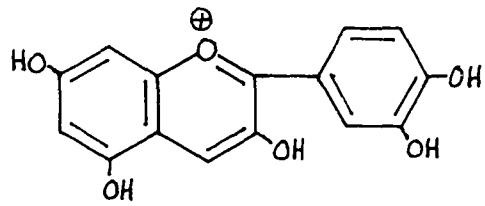
I

TABLE I. Centrospermae - Families and Genera in Which Betacyanins Have Been Found

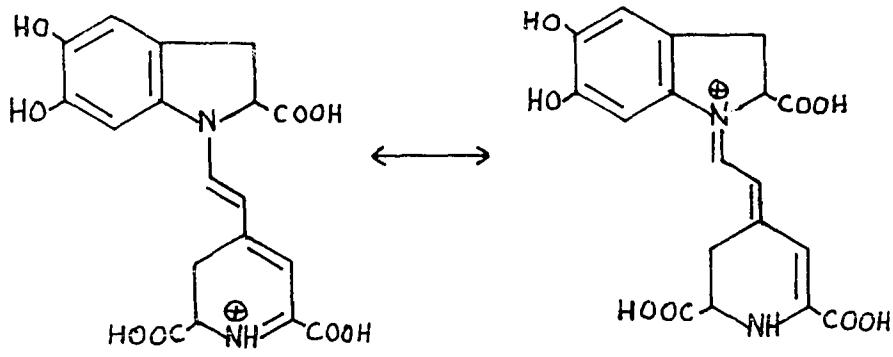
1. Chenopodiaceae - Beta (2 spp), Chenopodium (4), Atriplex (3), Corispermum, Kochia, Suaeda
2. Amaranthaceae - Amaranthus (5), Celosia (5), Aerva, Iresine (2), Gomphrena, Mogiphanes, Alternanthera (6)
3. Nyctaginaceae - Oxybaphus, Bougainvillea (2), Mirabilis (2), Boerhaavia (2), Cryptocarpus
4. Phytolaccaeae - Phytolacca (2), Rivina (2), Trichostigma
5. Aizoaceae - Sesuvium, Tetragonia, Malephora, Mesembryanthemum, Conophytum (17), Lampranthus (2), Pleiospilos (2), Lithops, Fenestraria, Gibbaeum (2), Dorotheanthus, Trichodiadema
6. Portulacaceae - Portulaca (3), Calandrinia, Anacampseros
7. Basellaceae - Basella (2)
8. Cactaceae - Pereskia, Mamillaria (7), Neoporteria, Melocactus, Aylostera, Hariota, Rebutia (4), Parodia (3), Lobivia (2), Cleistocactus, Notocactus (2), Gymnocalicium (3), Ariocarpus, Chamaecereus, Cereus (3), Selinocereus, Hylocereus, Opuntia (3), Zygocactus, Thelocactus, Monvillea, Nopalxochia

More recent work[14-21] has however shown that the nitrogenous pigments are not at all chemically related to the flavylium salt structures of the anthocyanins, although the two classes of compounds are visually indistinguishable, both having a visible absorption maximum around 540 mμ. These nitrogenous pigments are now called betacyanins after the red pigment betanin isolated from Beta and Bougainvillea plants.[11,12]

These betacyanins have been shown to contain the unusual chromophoric polymethylene cyanine group linking an indole and a pyridine residue. The distinction between the anthocyanins (II) and the betacyanins (III) will be clearly seen from the structures shown below:



II



III

The term betacyanin, derived from beet and cyanin, is used to express a certain undefined relationship to anthocyanins.

Structure II, the cyanidin cation, is characteristic of anthocyanin pigments, whereas the two resonance structures III incorporating the unusual polymethylene cyanine group, are characteristic of the betacyanins.[20] This structure of betacyanins is a new structural type among the naturally occurring colored substances, the chemical nature of which is very closely related to the alkaloids, and it is for this reason these pigments are also styled as "chromoalkaloids".[20]

Another interesting feature of the betacyanins is that their occurrence is exclusively limited to the group of families included in the Centrospermae and the two classes of pigments, anthocyanins and betacyanins, have never been found to occur in the same plant or even in the same family. Ordinarily, major taxonomic importance would not be expected of a single chemical character. However, the totally different structures of the two classes of pigments, which indicate different biogenetic pathways, their mutual exclusion and the limited distribution of the betacyanins make their presence of particular taxonomic significance.[22-24] This assumes greater importance in view of the fact that in recent years efforts have been made to determine whether the occurrence of certain chemical constituents in members of a genus or family can be related to the taxonomic classification of the plant. Research of this nature, which is usually termed, "Chemotaxonomy" has shown promising results, frequently confirming the classification already made on grounds of morphology, etc. Alkaloids, though they are secondary substances, furnish a great deal of information in this respect since they are present in about one-sixth of the vascular plants, and they can be very easily detected.[25-27]

A preliminary survey for alkaloids in Hawaiian plants has

indicated that C. obovata contains alkaloids,[28,29] and a search of the chemical literature[30] has shown that no work has been done so far to establish the molecular structure of any of these alkaloids. It seemed therefore interesting to see if, on the basis of the information concerning the presence of betacyanins among the eight families belonging to the Centrospermae order, a relationship exists between the alkaloids of this species and the betacyanine type of pigments. Normally, a given plant family will produce alkaloids of a certain structural type and since Amaranthaceae is a plant family belonging to the order Centrospermae, this family might be expected to produce a common and equally distinctive alkaloid structurally related to the betacyanins.

Furthermore, a member of this family, Achyranthus aspera Linn., has been found to be physiologically active against various diseases, such as inflammation of the internal organs, piles, dropsical afflictions, snake bites, hydrophobia and also in hysteria and nervous disorders.[31] Also, there is a reference to the general use of the various species of this family against skin diseases.[32] Since physiological activity has been claimed to be related to the presence of alkaloids, it may reasonably be expected that the alkaloids of C. obovata might also exhibit such activity.

C. Objectives

Little is known about the chemical constituents of C. obovata. Isolation and identification of the constituents may help to emphasize the uniqueness of the families in the order Centrospermae. This, in turn, may provide an opportunity to correlate chemical constituents and taxonomic classification. This research would also be of interest to the

pharmacologist since this species may produce physiologically active alkaloids.

The main object of this research was to isolate pure alkaloids and to characterize them by chemical analyses and physical methods such as the ultraviolet, infrared and nuclear magnetic resonance spectroscopy followed by comparison with, or conversion into known compounds. Finally, structural proof may be provided by total synthesis, if warranted. Once this is done, pharmacological tests may be carried out if the sample is obtained in sufficient amount. The chemotaxonomic significance may be assessed on the basis of the known chemical structure.

D. Acknowledgments

I deem it a very great pleasure in acknowledging the financial assistance by way of scholarship-grant provided by the Center for Cultural and Technical Interchange between East and West throughout the period of my study and research and wish to state that but for this generous financial help I would not have had the opportunity of working on this project in the Department of Chemistry.

The kind assistance given by Drs. R. E. Moore and Clifford W. J. Chang in taking the proton magnetic resonance spectrum and in providing useful discussions thereon is gratefully acknowledged.

It also gives me great pleasure to acknowledge the assistance given by Mr. Webster R. Hudgins unhesitatingly in helping to take the gas chromatogram.

CHAPTER II

EXPERIMENTAL

A. General Information about various standard apparatus and procedures is given below and will be referred to later.

The melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected.

Elemental analysis was performed by Dr. Koch, Microanalytical Laboratory, University of California, Berkeley.

Infrared absorption spectra were recorded with a Beckmann IR-5 double beam instrument either as potassium bromide pellets or in chloroform solution.

All ultraviolet (UV) absorption spectra were recorded with a Beckmann Dk-2 spectrophotometer using absolute methonol or dioxan as the solvent.

Nuclear magnetic resonance spectra (NMR) were recorded with a Varian A-60 (60 mc) analytical proton spectrometer, using tetramethylsilane (TMS) as the internal reference compound, and deuteriochloroform as the solvent.

Alumina (Woelm) both acid-washed and neutral, of Activity grade I and all other adsorbents were used as supplied by the manufacturers for chromatographic separation.

Aluminum oxide G and silica gel G for thin-layer chromatography were used as supplied by the manufacturers. The plates were prepared according to the method of Stahl[33] using a Desaga/Brinkmann standard applicator (Brinkmann Instruments, Inc.). Chromatograms were examined either under ultraviolet light or after development with modified

Dragendorff reagent.[34]

Alkaloid tests were considered positive if the test solution gave a precipitate with the usual alkaloidal reagents or at least with Mayer's and Dragendorff's reagents.[34,35]

B. Procurement and Processing of Plant Material

Plant material, chiefly bark and root bark for a preliminary investigation, was collected on the islands of Maui and Oahu. Material for the major work-up, however, was collected on Oahu alone, along the Kawaiiki Ditch Trail. Taxonomic identification was made by Drs. C. H. Lamoureux and G. W. Gillett of the Department of Botany, University of Hawaii.

The bark, both stem and root, was prepared for extraction by drying in a current of air at a temperature not exceeding 60° in a forced draft oven for ca. 48 hr, followed by grinding in a Wiley Mill to pass a 1 mm screen.

C. Isolation of Alkaloids

Conventional methods were employed to isolate the crude alkaloids from the plant material.[35-37] The plant materials from Maui and Oahu were worked up separately. Any shortcoming of a scheme was corrected in the subsequent work-up.

1. Root and Bark from Maui

For a preliminary investigation 500 g of dried and ground bark was used. All attempts to isolate alkaloidal material from the weakly positive fractions failed.

2. Root and Bark from Oahu

The entire extraction scheme is summarized in a flow sheet

shown in Fig. 1.

A total of 1,580 g of stem and root bark was extracted with refluxing methanol for ca. 72 hr in a soxhlet. The methanol was stripped off and the methanolic residue was digested with distilled water at 50° for a whole day. This was repeated twice and all aqueous extracts were collected and combined. The residue was rejected since it gave negative tests with the usual alkaloidal reagents. The aqueous filtrate was then extracted with chloroform in a continuous liquid-liquid extractor for 24 hr. The chloroform extract was dried over anhydrous sodium sulfate and removal of the solvent in a rotary evaporator under water pump vacuum yielded ca. 5 g of a brownish solid. This was labelled C(1). The aqueous phase was then extracted with n-butanol. The n-butanol extract was negative to the alkaloid tests and was therefore rejected. The remaining layer A_2 was faintly alkaloid-positive and was worked up separately.

The brownish solid C(1) was examined by thin-layer chromatography on aluminum oxide G and after trials with series of solvent mixtures of varying polarity, was chromatographed in a column containing 300 g of alumina (acidic, Woelm, Activity I) packed in chloroform and eluted with 100% chloroform. The first two fractions, 250 ml each, which were positive to alkaloid tests were collected. Subsequent fractions collected after eluting with solvents of increasing polarity were all negative to alkaloid tests and so were discarded. The two positive fractions were combined and again examined by thin-layer chromatography on aluminum oxide G to check the homogeneity of the alkaloid present. Based on the results of thin-layer chromatography, the combined positive fractions were again chromatographed in a column containing 150 g of

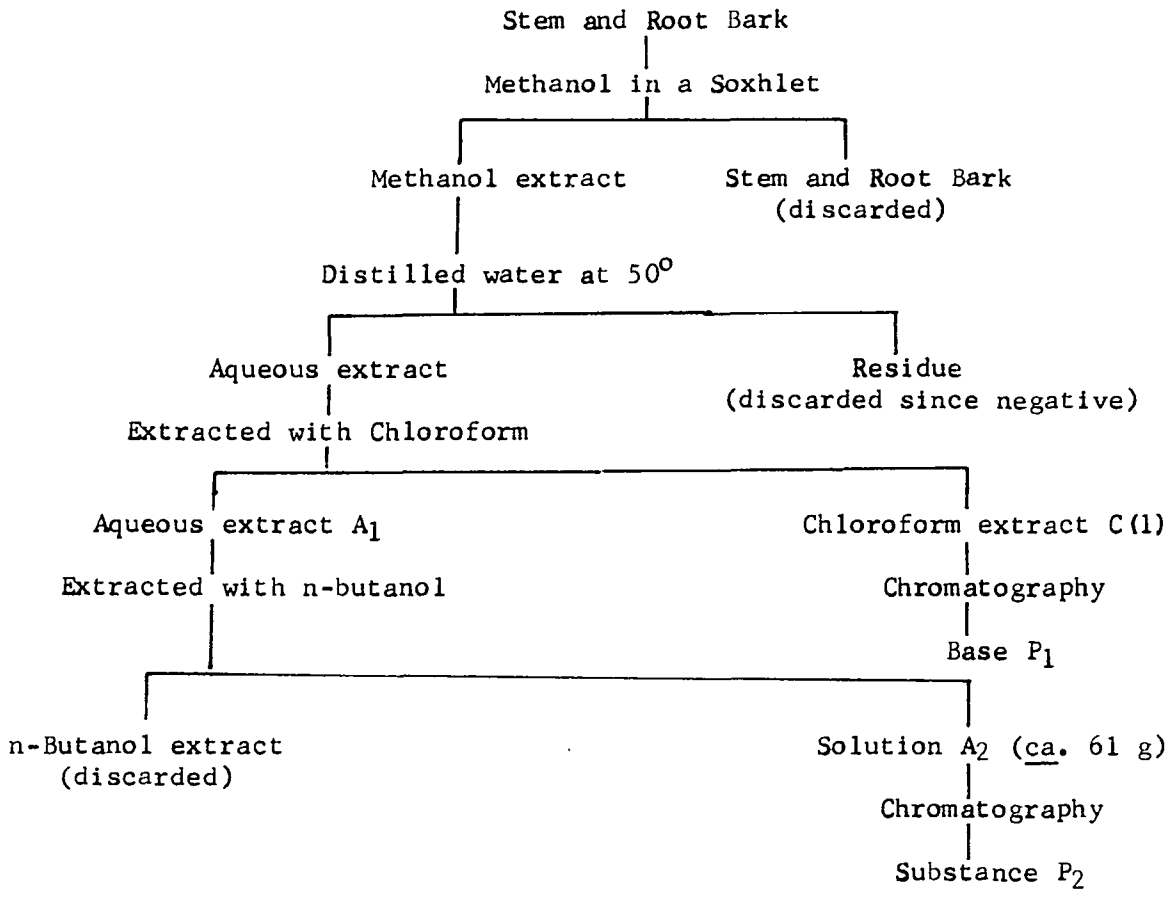


Fig. 1. Scheme 1 for Extraction of Stem and Root Bark (Oahu)

acidic alumina (Woelm, Activity I) in a mixture of 80% benzene and 20% chloroform. The column was eluted with the same mixture of solvents. The first 550 ml of eluant was negative to alkaloid tests. Subsequent fractions with a combined volume of 350 ml were positive to the alkaloid tests, after which the fractions were all negative. The combined positive fractions were again examined by thin-layer chromatography on alumina G and chromatographed again in a narrow column packed with 50 g of acid-washed alumina (Woelm, Activity I) in a mixture of benzene and chloroform, 80:20 respectively. The column was eluted with the same mixture and the positive fractions were collected after discarding the first three fractions with a combined total volume of 225 ml. The positive fractions were combined and examined by thin-layer chromatography on aluminum oxide G and deactivated silica gel. They were rechromatographed in a column packed with 15 g of silica gel (deactivated previously with 0.5N hydrochloric acid) in 100% chloroform and eluted also with the same solvent. The first 50 ml which was positive was collected and again analyzed by thin-layer chromatography on deactivated silica gel. Since the fraction was still found to be a mixture of two substances with very close R_f-values as indicated by thin-layer chromatogram examined by ultraviolet light and after development with modified Dragendorff's reagent, fractional crystallization was tried with a solvent mixture of acetone and ethanol, 90:10 by volume. This gave the pure base P_1 (ca. 2 mg), the homogeneity of which was confirmed by a single spot in thin-layer chromatography and by its sharp melting point, 218-220°.

The aqueous extract (A_2), after stripping off water weighed ca. 61 g and was dissolved in methanol and chromatographed over 500 g of

acid-washed alumina (Woelm - Activity I), eluting with chloroform-methanol (50:50 by volume). The very faintly positive fractions were obtained after discarding the first 6 fractions totalling 1700 ml. Fractions 7, 8 and 9 which were positive were combined. The solvent was removed and the residue was chromatographed over 450 g of acid-washed alumina (Woelm, Activity I) and eluted with acetone (100%). The first two fractions totalling 400 ml were faintly positive and were collected and the solvent was stripped off. A reddish brown liquid residue was obtained. This was again chromatographed over 30 g of neutral alumina with acetone as the eluant. The first two fractions were positive and were combined (70 ml) and the solvent was removed, resulting in a liquid residue P_2 with a pleasant odor. The liquid contained no nitrogen as determined by sodium fusion. It was then examined by gas chromatography and the gas chromatogram showed it to be a mixture of at least 10 components with very close retention times, making a separation unprofitable. The material was therefore discarded.

In another scheme of isolation, which is summarized in a flow sheet (Fig. 2), dried and ground stem and root bark from the island of Oahu, weighing about 4,250 g, was extracted with refluxing methanol in a soxhlet for 72 hr. The methanolic extract, after removal of the solvent was mixed with diatomaceous earth and extracted successively in a soxhlet with petroleum ether (b.p. 30-60°), chloroform and methanol. The petroleum ether extract was negative to the alkaloid tests and therefore was discarded.

The chloroform extract (C) after removal of the solvent weighed about 26 g and was chromatographed in a column containing 800 g of acid-washed alumina (Woelm, Activity I) and eluted with 100% chloro-

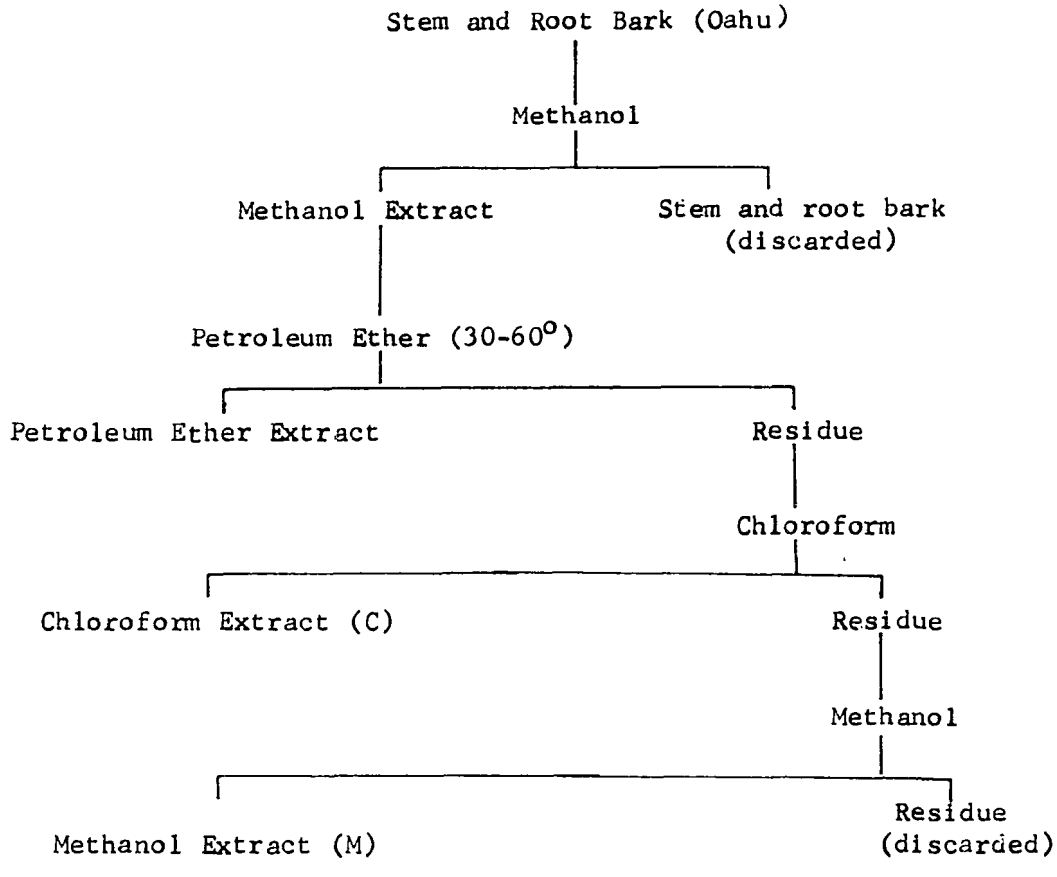


Fig. 2. Scheme 2 for Extraction of Stem and Root Bark (Oahu)

form. The first three fractions, which were positive to the usual alkaloid reagents, totalled 300 ml and the residue after stripping off the solvent weighed ca. 1 g. This was again chromatographed in a column containing 60 g of neutral silica gel (Woelm - Activity I) and eluted with 100% chloroform. The positive fractions were obtained after discarding the first 330 ml of the eluate. The next six fractions, 30 ml each, were all positive to the alkaloid reagents and were examined separately by ultraviolet spectra and by thin-layer chromatography on aluminum oxide G. Since these fractions turned out to be similar, they were combined. The thin-layer chromatogram on aluminum oxide G of this combined fraction showed however that it was not pure. This was therefore again chromatographed in a column containing 30 g of neutral alumina (Woelm - Activity I) and eluted with benzene-chloroform (60:40 by volume). The positive fractions were collected and combined. This combined fraction, when examined by thin-layer chromatography on aluminum oxide G and silica gel G, showed the same R_f-values as the base P_1 isolated in the first scheme and also the same ultraviolet absorption spectrum. The base crystallized from a mixture of ethanol, acetone and hexane (2:7:1) in pale yellow needles. The crystalline sample was filtered, washed with a little acetone and dried in a dessicator overnight. This melted sharply at 219-221°, as compared with 218-220° for base P_1. Total yield of the base was ca. 20.1 mg (0.0005% of dry plant material).

The methanol extract (M), after stripping off the solvent in a rotary evaporator under water pump vacuum, was worked up as shown in Fig. 3. The chloroform extract (A) obtained at pH 2 was negative to the alkaloid tests and so was discarded. But the chloroform extracts (B)

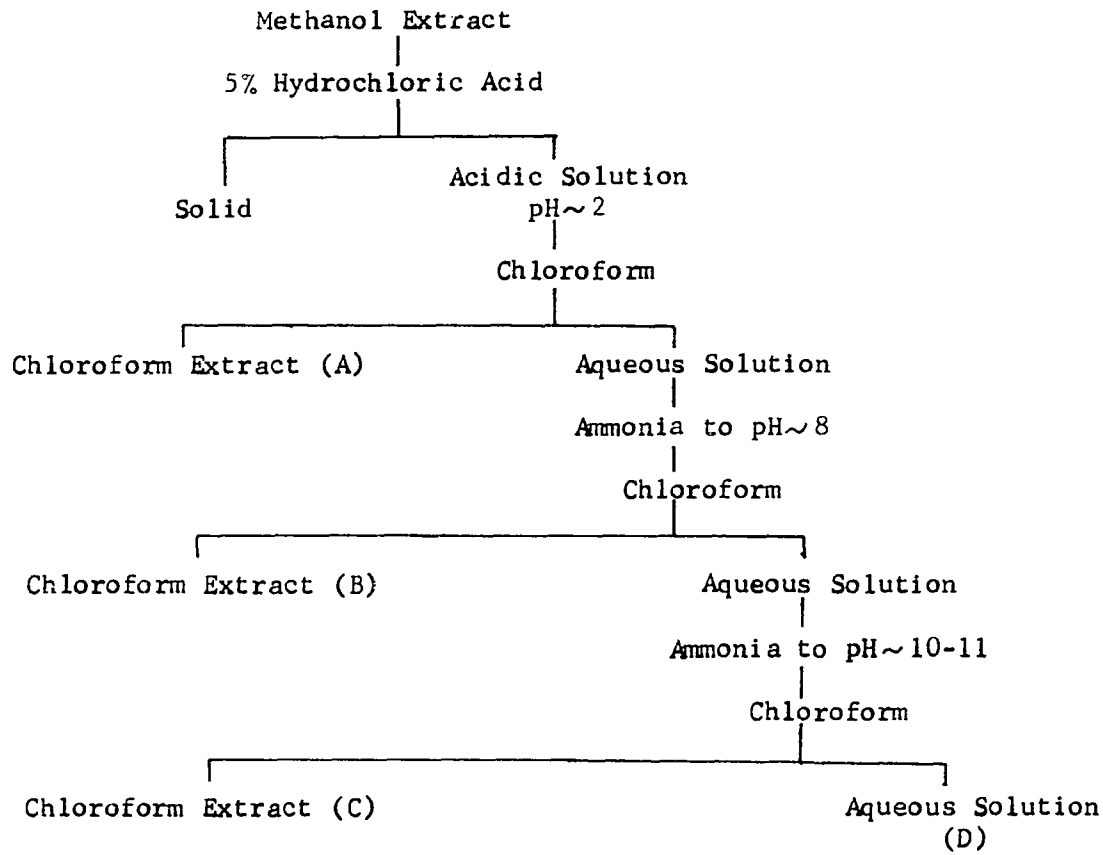


Fig. 3. Scheme for Work-up of Methanol Extract

and (c) obtained at pH 8 and pH 10-11 were both very weakly positive to the usual alkaloid reagents; but all attempts to isolate alkaloids failed. The aqueous fraction (D) left at pH 10-11 was negative to the alkaloid tests and was therefore discarded.

D. Characterization of the Alkaloid

Base P_1 sublimed readily at 130-140°/ $<$ 1 mm Hg pressure. The analytically pure base, white and feathery in appearance, melted sharply at 220-221°. It was very soluble in chloroform and alcohol, and somewhat soluble in benzene, but insoluble in water. The R_f-values in the thin-layer chromatograms were as indicated in TABLE II below.

TABLE II. R_f-Values of the Base in Thin-Layer Chromatograms

Adsorbent	Solvent System	R_f-Value
Aluminum oxide-G	Benzene and Chloroform in 60/40 by volume	0.61
Silica gel-G	Chloroform (100%)	0.32
Deactivated silica gel-G	Chloroform and Ethanol in 95/5 by volume	0.51

The sublimed sample was sent for ultramicro analysis and the following results were returned:

	C	H	N
Found:	72.32, 72.11	4.47, 4.53	10.74, 11.02
Calculated for $C_{15}H_{10}N_2O_2$:	71.99	4.03	11.20
Calculated for $C_{16}H_{10}N_2O_2$:	72.71	4.58	10.60

The analytical results were compatible with either of two formulae, $C_{15}H_{10}N_2O_2$ and $C_{16}H_{10}N_2O_2$, although the value for carbon showed better agreement with the C-15 formulation.

The proton magnetic resonance spectrum of the base in chloroform (Fig. 4) showed a three-proton peak at 4.08δ, a one-proton peak at 6.12δ, a three-proton peak at 7.58δ, three one-proton peaks at 7.87δ, 8.54δ, and 8.75δ, respectively, thus accounting for only 10 protons. This measurement agreed with a C-15 but not with a C-16 formula. A C-15 formula was also indicated by the molecular weight obtained from the mass spectrum (Fig. 5)[38] of the sample, which had its two highest peaks at 250 and 251 mass units.

A chloroform solution of the base had zero optical rotation.

The ultraviolet absorption spectrum of the base in methanol (Fig. 6) showed the following maxima and minima: λ_{max}^{MeOH} 365.9 m (log ϵ 2.32), 349.9 (2.28), $\sim$336.2 (2.04), 297.5 (2.19), 288.0 (2.19), 265.5 (2.58), 261.0 (2.53), $\sim$253.0 (2.29), 237.5 (2.61), $\sim$220.0 (5.07); λ_{min} 357.5 (2.20), 316.1 (1.55), 292.5 (2.17), 276.2 (1.99), 247.7 (2.21), 233.2 (2.59).

The ultraviolet spectrum of the base in methanolic potassium hydroxide (Fig. 6) was identical with the spectrum in neutral medium. In acid (Fig. 6) a bathochromic shift occurred which was reversed on rebasification.

The major peaks in the infrared absorption spectrum (Fig. 7) in KBr occurred at the following wave lengths, with the most prominent peak at 6μ. 2.9 w, 3.3-3.4 b, 6.0 s, 6.29, 6.41, 6.79 s, 6.95, 7.15, 7.51 s, 7.7, 7.95 s, 8.25 s, 8.35 sh, 8.65, 8.94, 9.13, 9.8-10.15 b, 11.5, 11.8, 12.58, 13.09 s, 14.05, 14.41.

(s = strong; b = broad; sh = shoulder; w = weak)

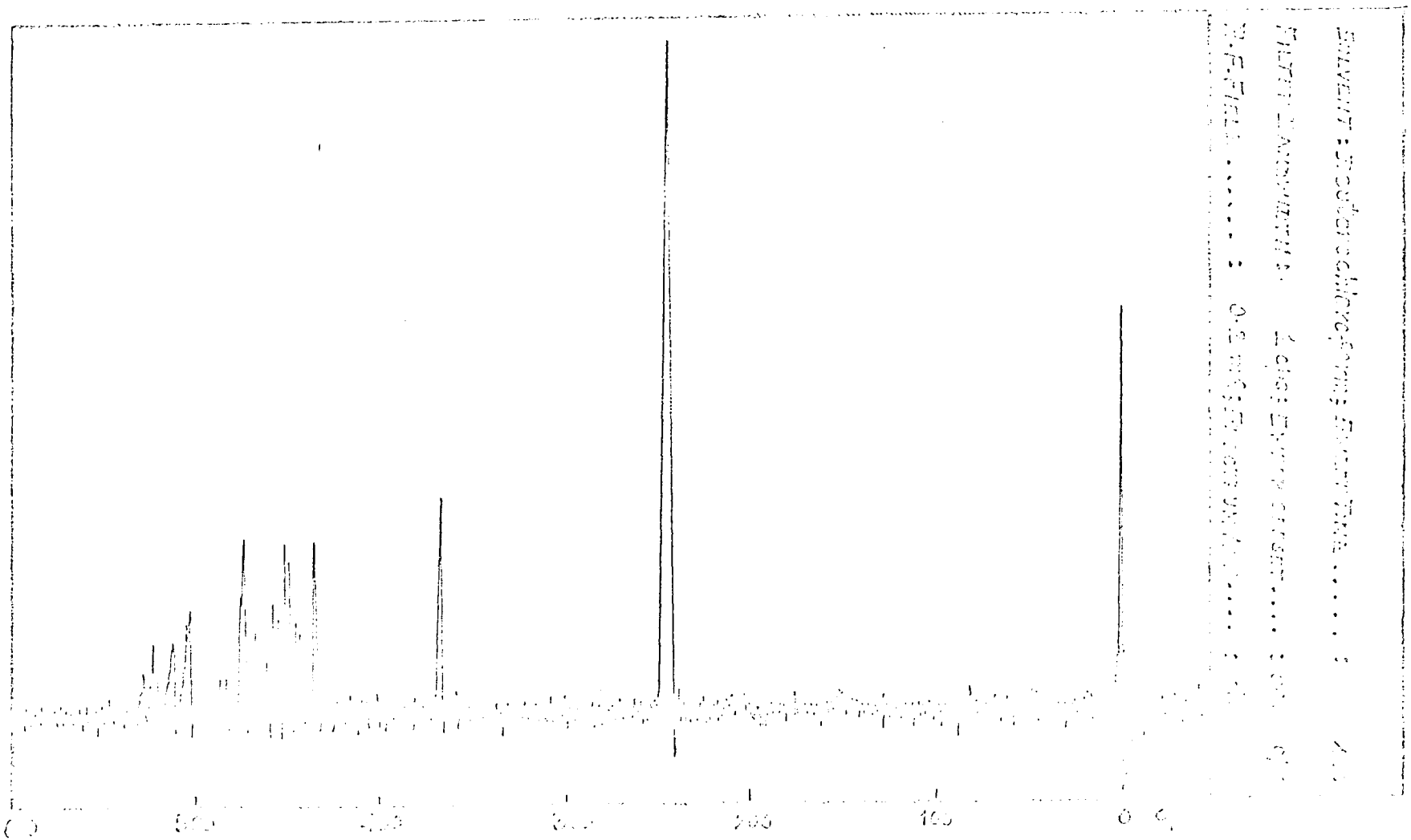


Fig. 4. Nuclear Magnetic Resonance Spectrum of the Base

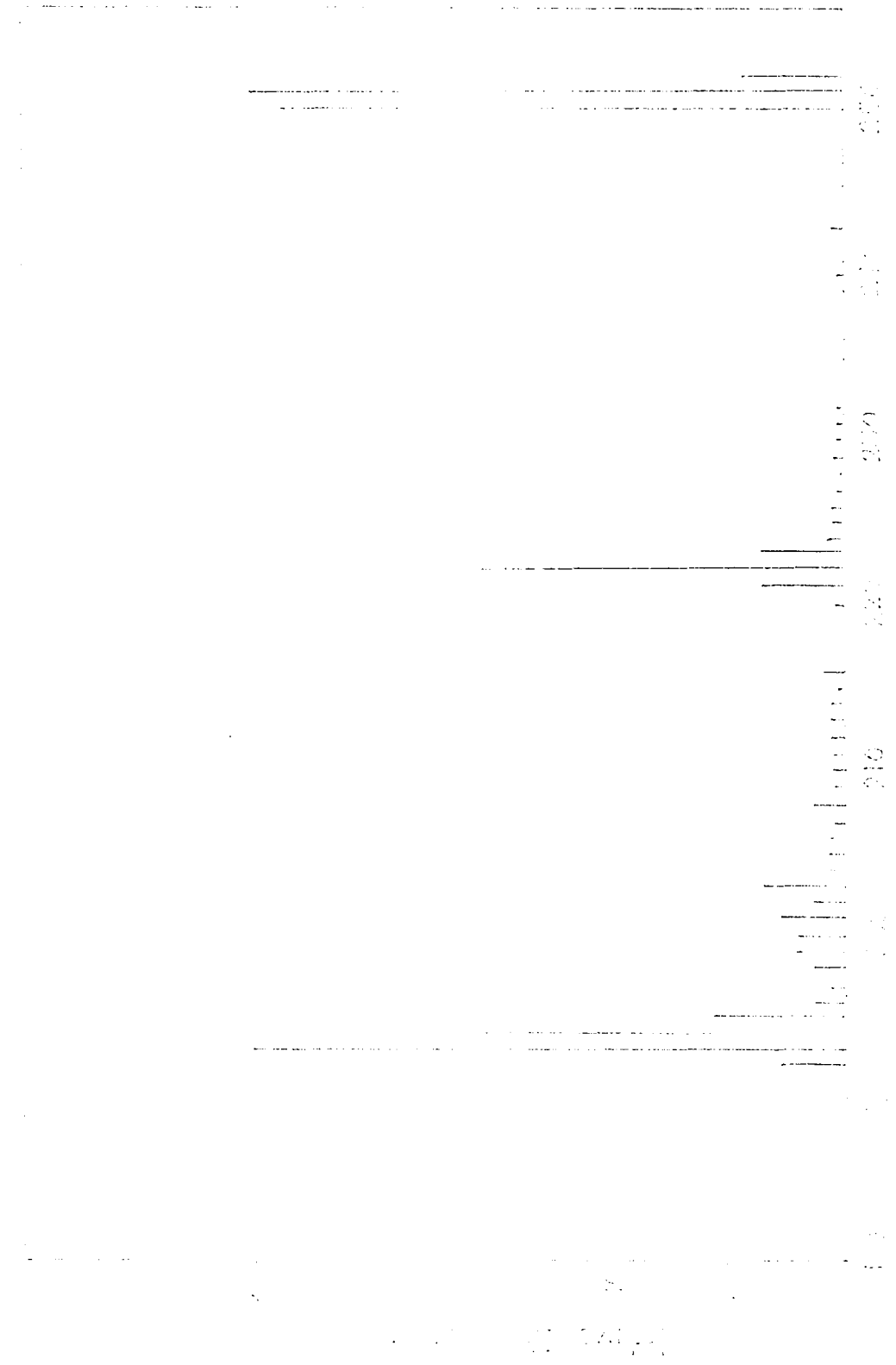


Fig. 5. Mass Spectrum of the Base

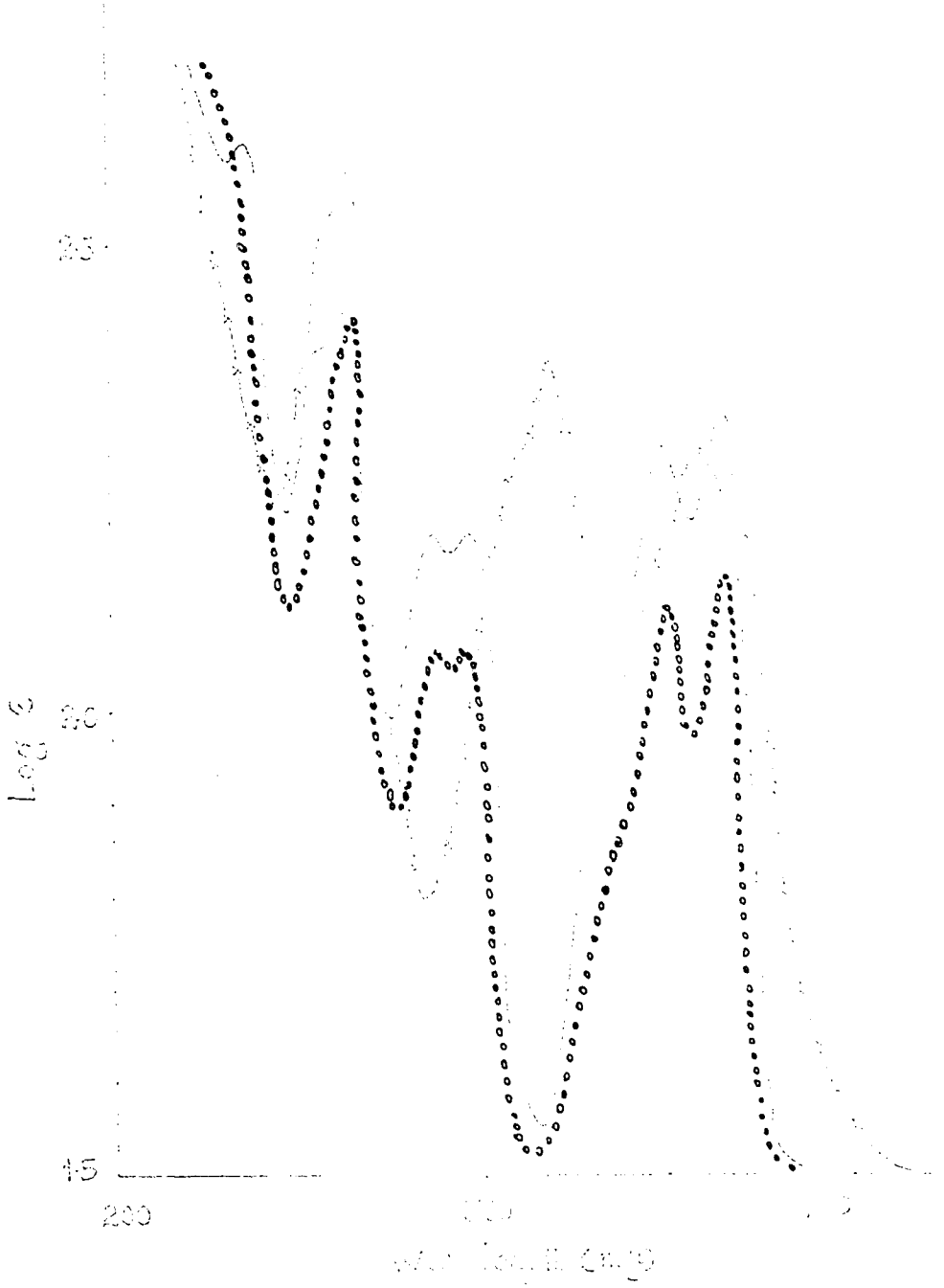


Fig. 6. Ultraviolet Spectra of the Base in MeOH (—), Methanolic HCl (x-x-) and Methanolic KOH (∘∘∘∘)

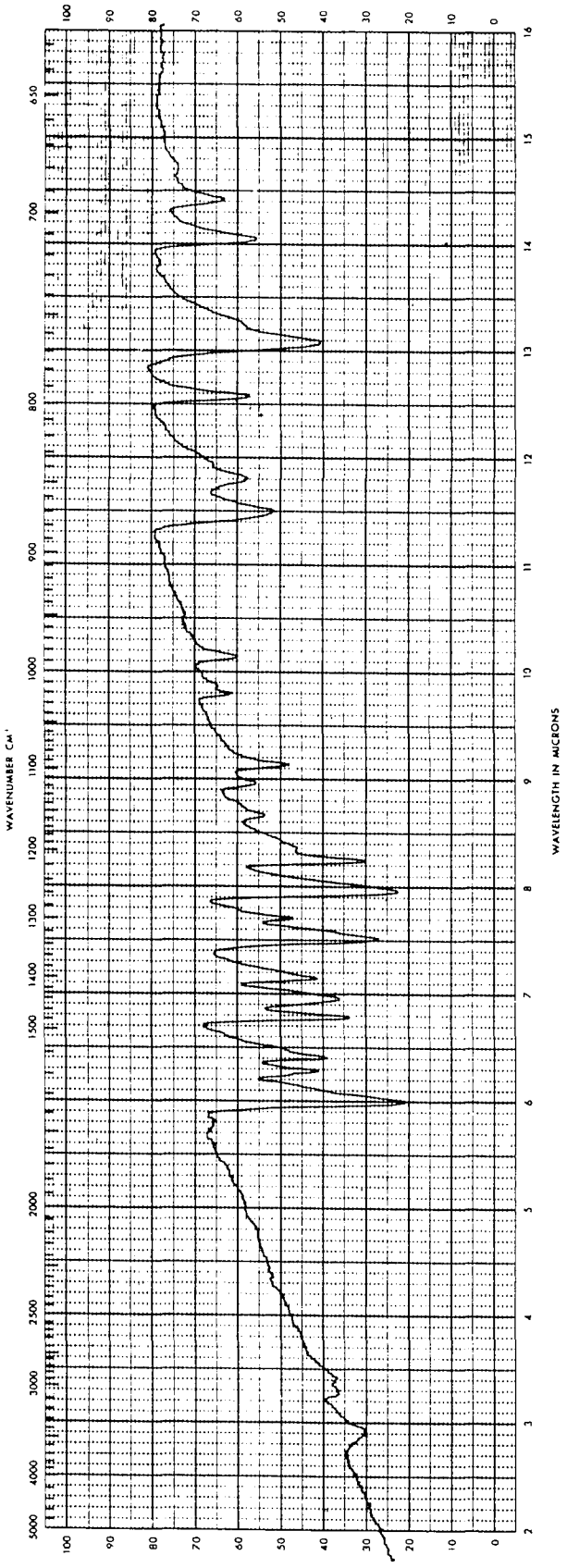


Fig. 7. Infrared Spectrum of the Base in KBr

E. Synthesis

1. Harman. Harman was prepared as described by Kermack, Perkin and Robinson.[39] Tryptophan (15 g, 0.073 mole) was heated with acetic anhydride (100 ml, 1.051 mole) (Merck) in a three-necked round-bottomed two-liter flask provided with a reflux condenser and a glycerine-sealed stirrer with Teflon blade until it dissolved completely. Anhydrous granular zinc chloride (10 g, 0.073 mole) (Allied Chemicals) was then added and the mixture boiled for 1 min. The excess of acetic anhydride was decomposed by careful addition of 750 ml of hot water. This led to the separation of a brown oil, which was converted into a yellow, sparingly soluble chromium complex by addition of 300 ml of a 10% solution of potassium dichromate (Merck), to which 15 ml of conc. sulfuric acid had been added previously. The resulting solid was broken up and the mixture boiled until nearly the whole of the chromium complex had passed into solution, which took about 20 min. The contents of the flask were cooled in ice and excess of a saturated solution of sodium hydroxide was added to liberate the base. The base was then extracted thrice with ethyl acetate. The extracts were combined and washed with a little water and then further extracted twice with dilute hydrochloric acid. The acidic extracts were combined, cooled in an ice bath, and neutralized carefully with conc. ammonia. The resulting free base was then repeatedly extracted with ether. The ethereal extracts were combined, dried over anhydrous magnesium sulfate overnight and the ether stripped. Yield of crude harman was ca. 4 g (28%). Part of the crude product was chromatographed over neutral alumina (Woelm - Activity I) with 100% chloroform as the eluant. The Dragendorff-positive fractions were collected, combined and stripped. The solid obtained was then

sublimed at 150-160°/ < 1 mm Hg pressure. Almost colorless needles, melting sharply at 235-237° (Lit. m.p. 237-238°)[39] were obtained. The infrared spectrum of the sublimed sample in KBr was recorded and is reproduced in Fig. 8.

2. Benzalharman.[39] Harman (3.8 g, 0.021 mole) was placed in a 100 ml round-bottomed flask and 12 ml (0.119 mole) of freshly distilled benzaldehyde was added. The mixture was refluxed gently for 4 hr, with a condenser short enough to permit the slow distillation of water formed during the course of the reaction. After cooling, the brown mixture was diluted with 50 ml of ether. Dilute hydrochloric was then added when the yellow hydrochloride of benzalharman was precipitated. This was separated by filtration under suction and the precipitate washed first with large amount of ether and then with a little water. The yellow hydrochloride thus obtained was then suspended in 10-15 ml of 95% ethanol and neutralized with dilute aqueous ammonia (ammonia/water 1:5 by volume). The free base thus liberated was repeatedly extracted with ether. All extracts were collected and dried over anhydrous magnesium sulfate overnight. The ethereal extracts exhibited a brilliant bluish-green fluorescence. The ether was removed in a rotary evaporator under water pump vacuum and the crude benzalharman (ca. 6 g) was collected. This was purified by chromatography over 200 g of neutral alumina with 100% chloroform as the eluant. The Dragendorff-positive fractions were collected, combined and stripped (yield 5.1 g, 90%). A portion of the sample was sublimed at 170-175°/ < 1 mm. This sublimed sample melted sharply at 195-197° (Lit. m.p. 197-199°).[39] The infrared spectrum of the sublimed sample was measured in KBr and is shown in Fig. 9.

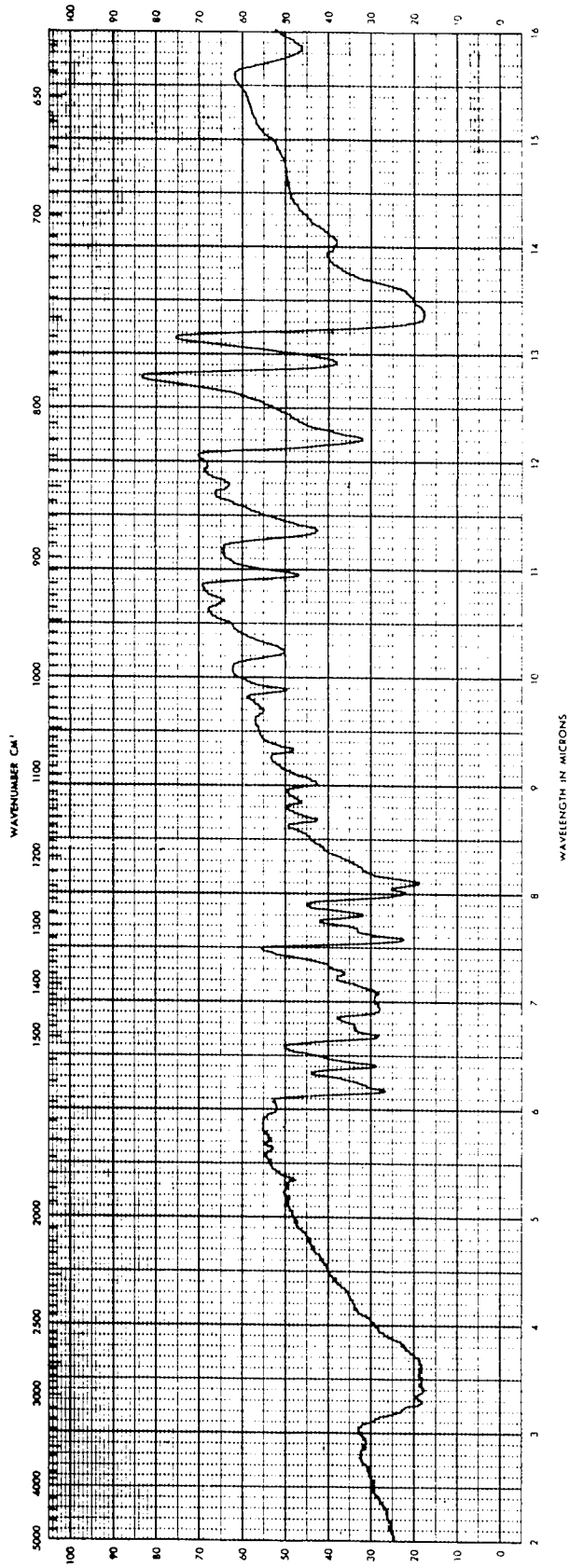


Fig. 8. Infrared Spectrum of Harman in KBr

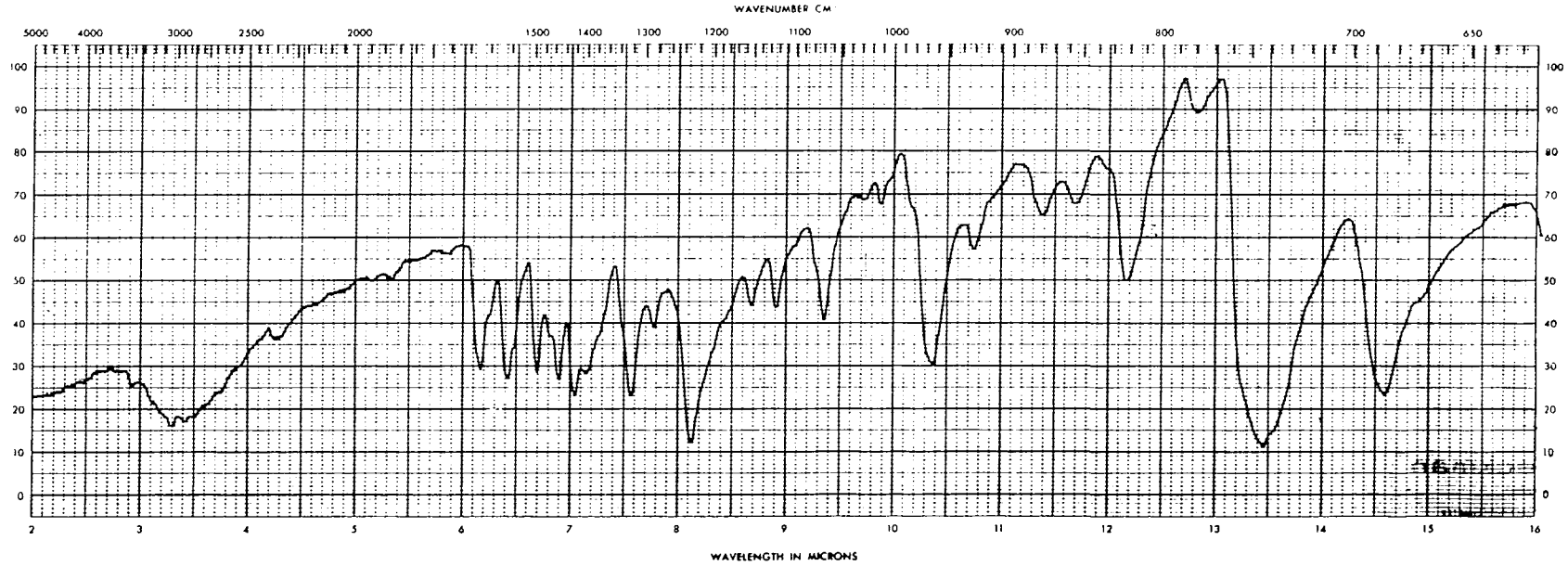


Fig. 9. Infrared Spectrum of Benzalharman in KBr

3. β-Carboline-1-carboxylic acid.[40] Crude benzalharman (5 g, 0.019 mole) was placed in a 250 ml Erlemeyer flask and dissolved in 25 ml of pyridine. The solution was cooled in ice. A saturated aqueous solution of potassium permanganate (about 150 ml) was added dropwise while keeping the solution stirred with a magnetic stirrer, until the permanganate color persisted for at least an hour. The excess potassium permanganate was then destroyed by heating the mixture with 5 ml of 95% ethanol on a steam bath. The dark suspension was filtered while hot and the filtrate concentrated to about 50 ml. This was then acidified with dilute hydrochloric acid when a beautiful canary-yellow hydrochloride precipitated, which was collected. The dark residue was suspended in 50 ml of water and acidified with dilute hydrochloric acid. The mixture was treated with sodium bisulfite to reduce the manganese dioxide, and the additional hydrochloride precipitated was collected. The combined hydrochlorides were dissolved in 750 ml of hot aqueous ammonia, the solution was filtered, cooled and the free acid precipitated by neutralization with dilute acetic acid. The yellow precipitate was collected, washed with water and dried. The acidic mother liquor yielded more of the acid on standing. The acid, a bright yellow crystalline powder, could not be purified further since it was insoluble in all common solvents. Total yield was 2.04 g (52%). Part of the sample was sublimed at 190°/ < 1 mm and the sublimed sample melted in the range 250-255° (Lit. m.p. 235°).[40] The infrared spectrum of the sample was measured in KBr and is shown in Fig. 10. For further identification of the acid, its methyl ester was prepared through its acid chloride and reaction with absolute methanol. The methyl ester melted at 160-162° (Lit. m.p. 164-5°).[41]

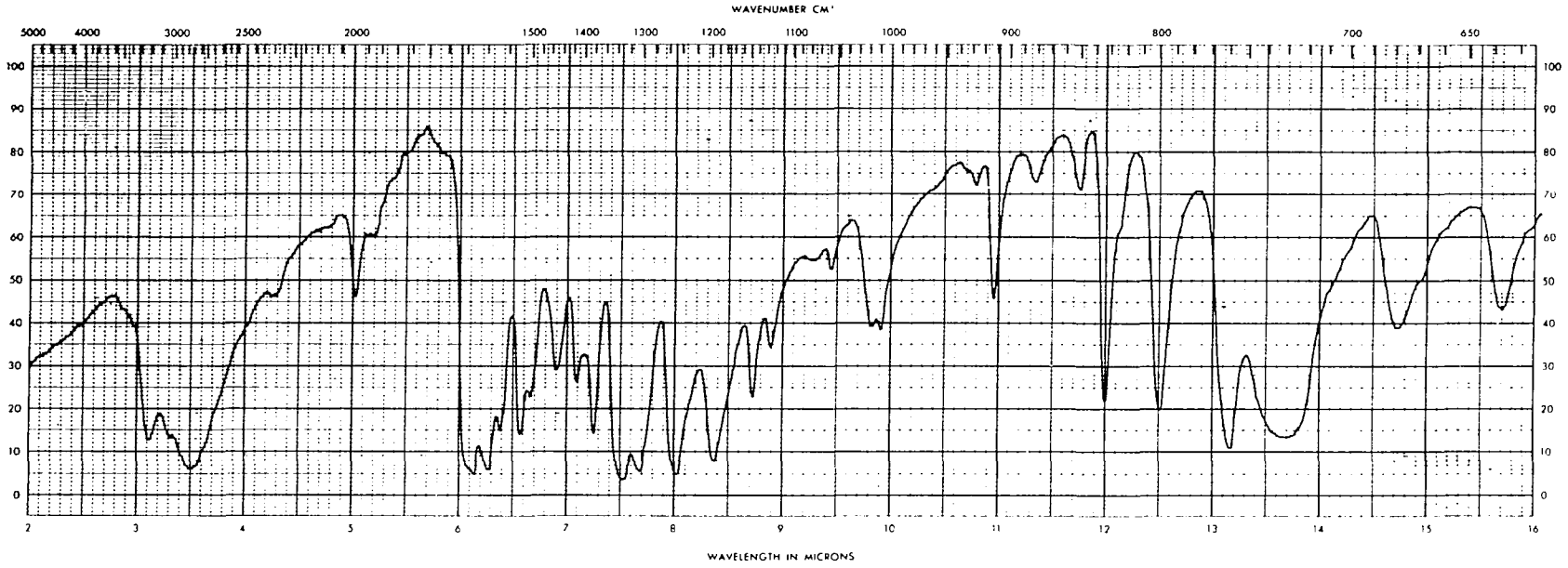


Fig. 10. Infrared Spectrum of β-carboline-1-carboxylic Acid in KBr

4. 4-Hydroxycanthin-6-one. The compound was prepared as described by Nelson and Price.[42]

a. β-Carboline-1-carboxylic acid chloride.[43] β-Carboline-1-carboxylic acid (finely powdered, 1.2 g) was refluxed with 30 ml of thionyl chloride for 10-15 min in a 100-ml round-bottomed flask provided with a calcium chloride guard tube, on a water bath at 75-80°. After refluxing, the excess thionyl chloride was distilled off under vacuum on a water bath at 40-50°. The product, which is the hydrochloride of the acid chloride, was then dried in vacuum over potassium hydroxide.

b. Magnesium ethoxymalonic ester. This compound was prepared using the procedure of Walker and Hauser.[44] In a 100-ml three-necked flask equipped with a glycerine-sealed stirrer, dropping funnel and a reflux condenser protected by a calcium chloride drying tube, was placed 0.5 g (0.021 mole) of magnesium turnings. The flask was heated gently over a low flame applying suction at the same time through the condenser in order to remove moisture. After cooling, a few drops of carbon tetrachloride were added, followed by 0.5 ml of absolute ethanol. The mixture was stirred gently and reaction was induced by scratching the magnesium turnings against the flask. Once the reaction had started, it was allowed to proceed for a few minutes after which the flask was cooled. Absolute ether (7.5 ml) was then added cautiously to the flask. The resulting mixture was placed on a steam bath and a solution of 3.5 ml (0.023 mole) of diethylmalonate, 2 ml of absolute ethanol and 2.5 ml of absolute ether was added at such a rate that rapid refluxing was maintained, heating the reaction mixture, when necessary. The mixture was refluxed for 3 hr, or until no magnesium remained undissolved.

c. Condensation of the Acid Chloride and the Magnesium Ethoxy Derivative. Crude β-carboline-1-carboxylic acid chloride (1.124 g) was powdered well and added gradually in small portions with shaking to the magnesium ethoxy derivative of malonic ester contained in the flask and diluted with a further 30 ml of absolute ether. The mixture was refluxed for 15 min and the ethereal solution was decanted from the unreacted greenish solid. The ethereal extract was then acidified with dil acetic acid when a pale green precipitate formed. This was collected and washed with a little ether and refluxed for 4.5 hr in a 100-ml round-bottomed flask provided with a condenser with a mixture of conc. hydrochloric acid (7.5 ml) and absolute ethanol (7.5 ml). On cooling the reaction mixture, a yellow solid separated, which was dissolved in dilute sodium hydroxide and then acidified with dilute acetic acid, when the free base 4-hydroxycanthin-6-one, separated. This was filtered under suction, washed with a little water and dried (yield 85 mg, 6.3%). A part of this was sublimed, m.p. 285-290° (Lit. m.p. 288-290°).[42] The infrared spectrum of this compound was measured in KBr and is reproduced in Fig. 11.

5. 4-Acetoxycanthin-6-one.[42] The hydroxy compound (80 mg) was placed in a 100-ml round-bottomed flask and refluxed with 5 ml of acetic anhydride and 3-4 drops of pyridine for about 15 min. The reaction mixture was then poured into ice-cold water, when the 4-acetoxy compound appeared as a pale brown precipitate. This was filtered, washed with water and dried (47.5 mg, 50.4%) m.p. 200-202° (Lit. m.p. 206-206.5°).[42] The ultraviolet spectrum of the compound in dioxan (Fig. 12) was identical with the one reported by Price and Nelson,[42] confirming the identity of the compound.

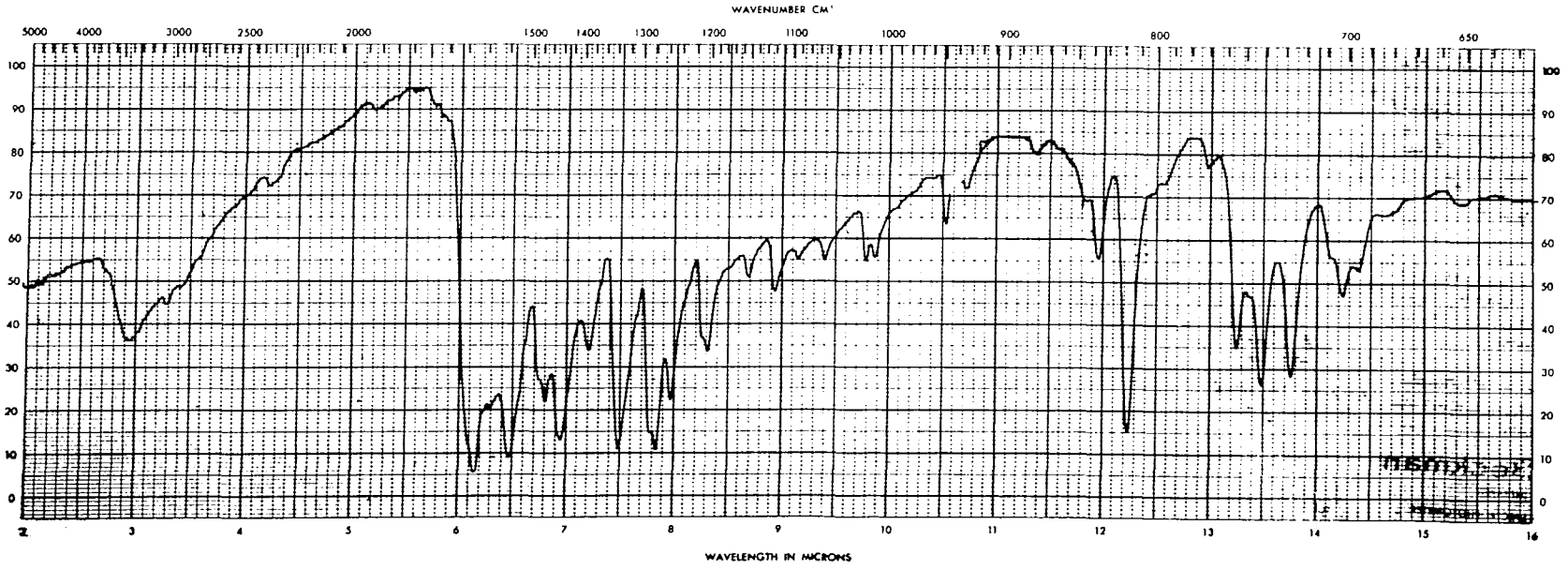


Fig. 11. Infrared Spectrum of 4-Hydroxycanthin-6-one in KBr

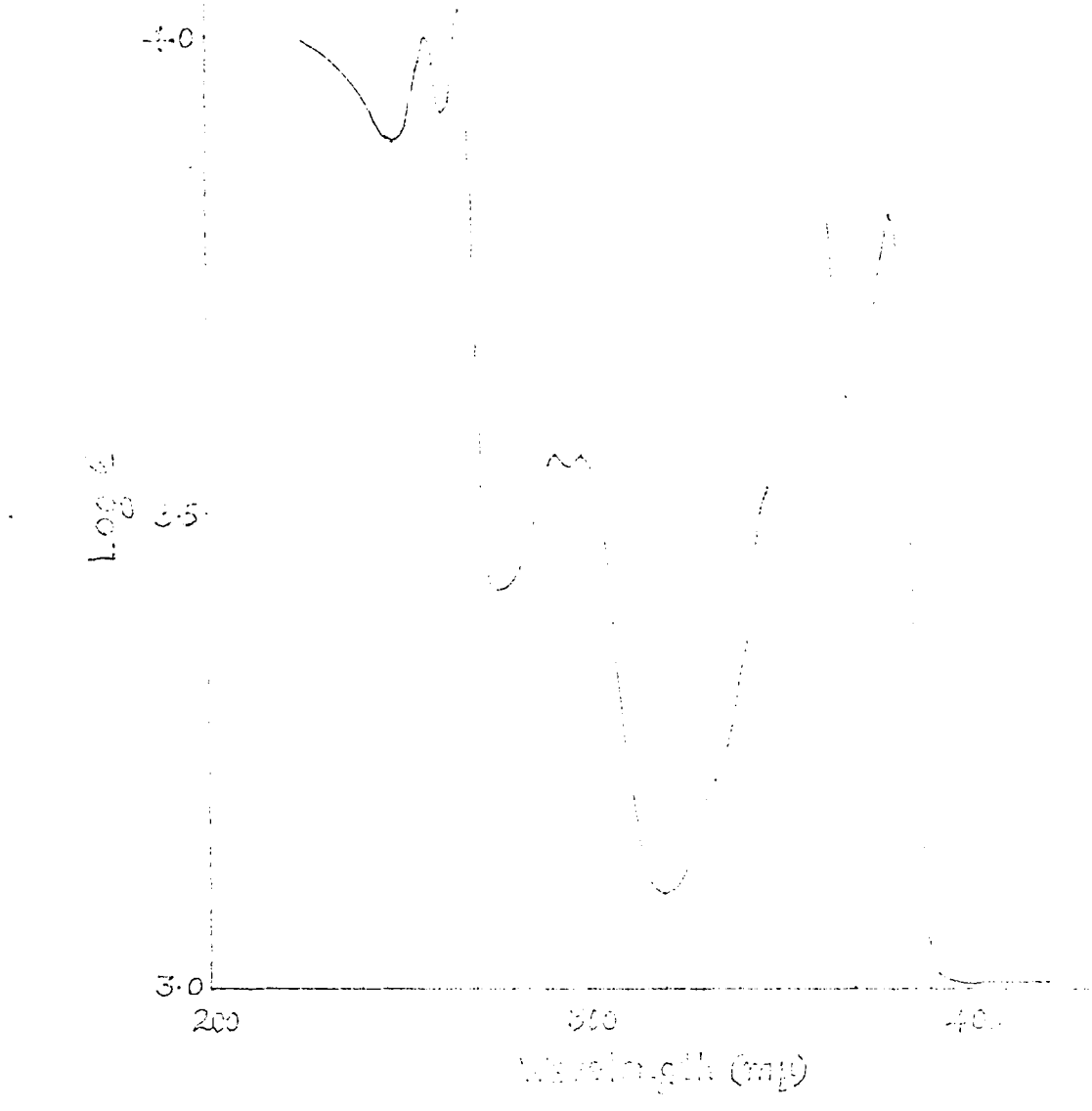


Fig. 12. Ultraviolet Spectrum of 4-Acetoxycanthin-6-one in Dioxan

6. 4-Methoxycanthin-6-one.[42] - Diazomethane. An ethereal solution of diazomethane was prepared from Aldrich's Diazald (N-methyl-N-nitroso-p-toluenesulfonamide) as follows:[45] Ethanol (95% 25 ml) was added to a solution of potassium hydroxide (5 g) in water (8 ml) in a 100-ml distilling flask fitted with dropping funnel and an efficient condenser set downward for distillation. The condenser was connected to a receiving flask, containing 20-30 ml of absolute ether and immersed partially in a dry ice-carbon tetrachloride bath. The flask containing the alkaline solution was heated on a water bath to 65°, and a solution of 21.5 g (0.1 mole) of Diazald in about 130 ml of absolute ether was added through the dropping funnel in about 25 min with the rate of distillation being almost equal to the rate of addition. When the dropping funnel was empty, another 20 ml of absolute ether was added slowly and the distillation continued until the distilling ether was colorless. The ethereal distillate contained about 3 g of diazomethane in ca. 125 ml of ether. Etherification. To the acetoxy compound (47 mg) dissolved in 3 ml of dioxan, 5 ml of the diazomethane solution prepared above in moist ether was added. The reaction flask was kept tightly closed for 3 days after which a red crystalline solid which had formed was filtered off. The filtrate was evaporated almost to dryness, and extracted with 2% sodium hydroxide solution. This was then extracted twice with chloroform. The combined chloroform extracts were stripped and chromatographed over neutral alumina with a benzene-chloroform mixture (60:40 by volume) as the eluant. The fractions, positive to the usual alkaloid reagents, were collected, combined and stripped. The crude 4-methoxycanthin-6-one (ca. 15 mg) was then sublimed at 130-160°/ < 1 mm. Yield 7 mg. m.p. 218-220° (Lit. m.p.

220-220.5°).[42] The infrared spectrum of 4-methoxycanthin-6-one in KBr was taken and is shown in Fig. 13.

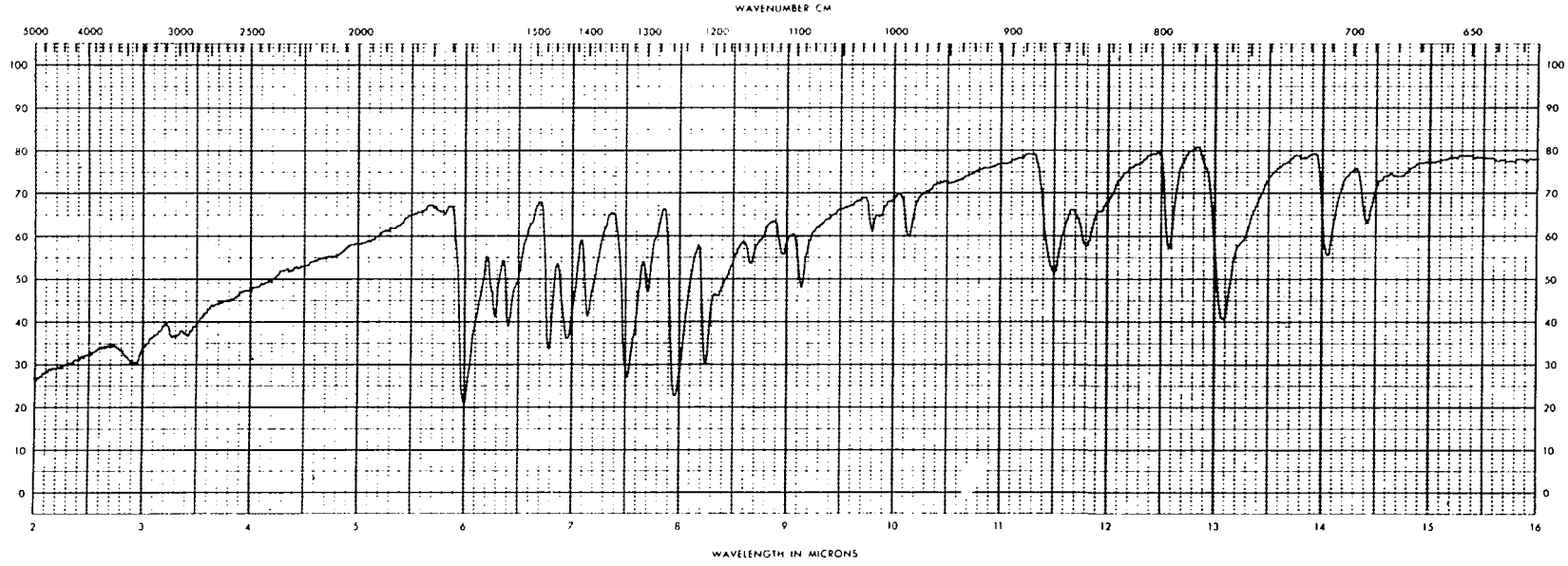


Fig. 13. Infrared Spectrum of 4-Methoxycanthin-6-one in KBr

CHAPTER III

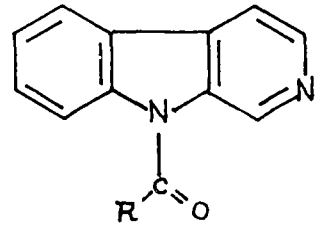
RESULTS AND DISCUSSION

The base P_1 was isolated from the root and stem bark collections on the island of Oahu but not from those on Maui. A total of 4.25 kg of plant material was processed yielding 20.1 mg of chromatographically pure base (ca. 0.0005% of dry plant material).

The homogeneity of the base was evident from its sharp melting point and from the single spot in the thin-layer chromatograms obtained in each of three systems (TABLE II). This thin-layer chromatographic technique, besides providing a tool to check the homogeneity of the substance, is further valuable since it helps to define the conditions for successful separation of a mixture on a preparative scale. This, however, holds true only if the conditions viz. adsorbents, solvent systems, temperature and humidity are exactly reproducible in the preparative experiment. Another point to be emphasized is the variability of the R_f-values of a substance if the conditions viz. thickness of the layer, temperature, etc., are not exactly reproducible when the chromatograms are run at different times for purpose of comparison. In the present research attempts have been made to conform to reproducible conditions as far as possible[46] in all the chromatographic separations.

The ultraviolet spectrum of the base in neutral medium, the bathochromic shift in acid medium, which was reversed on rebasification, and the absence of any shift in basic medium suggested the possibility of protonation and deprotonation of a basic nitrogen atom. Furthermore, a search of the literature[47-51] for a plausible structure of the base, having the characteristic absorption peaks in the ultraviolet region

indicated that it belonged to the class of alkaloids incorporating a β-carboline moiety with possibly an acyl group attached to the indole nitrogen as shown in Structure IV.

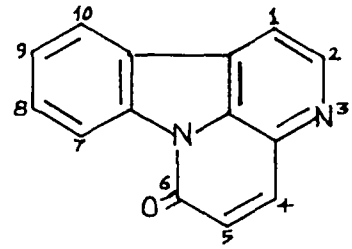


IV

The infrared spectrum with a prominent peak at 6 μ indicated a carbonyl function.[52]

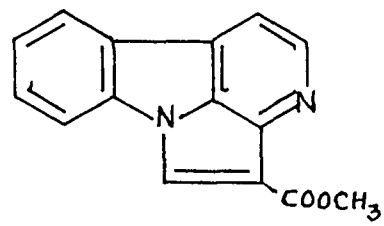
The elemental analysis suggested a molecular formula of $C_{15}H_{10}N_2O_2$. The proton magnetic resonance spectrum confirmed the number of protons arrived at by the elemental analysis and the mass spectrum confirmed the molecular weight based on the above formula.

A search of the literature for a compound with the molecular formula $C_{15}H_{10}N_2O_2$ and having similar absorption characteristics led to three papers by Nelson, Price and Haynes,[42,53,54] which describe the isolation and characterization of the canthinones. They are a group of alkaloids which derive their name from the product obtained in the reaction between phthalic anhydride and calycanthine,[53] and which have been isolated from the Australian plant, Pentaceras australis Hook F., Family Rutaceae. The canthin-6-ones have the basic structure with a carbonyl function at position 6,[55] as shown in Structure V. Their ultraviolet absorption characteristics are similar to that of the base isolated from Charpentiera. Surprisingly, it was noticed that the melting point of the base, 220-221°, was almost the same as that of 4-methoxycanthin-6-one as were the maxima and the minima in the ultra-



V

violet spectrum of the base in dioxan (Figs. 14 and 15). This suggested the possibility that the base was identical with 4-methoxycanthin-6-one. The proton magnetic resonance spectrum (Fig. 4) of the base also strongly suggested this structure for the base. But the possibility of an alternative structure VI could not, however, be ruled out since structure VI also agrees with the proton magnetic resonance and ultraviolet spectral data quite satisfactorily. A final confirmation of the structure was therefore in order.



VI

It was felt that 4-methoxycanthin-6-one should be synthesized and a direct comparison of melting point, ultraviolet and infrared spectra and R_f-values in the thin-layer chromatogram should be made between the natural base and the synthetic substance: this would lead to unequivocal identification of the natural base.

A perusal of the three papers by Price and coworkers,[42,53,54] however, showed that 4-methoxycanthin-6-one, though it was not isolated from a natural source, had been synthesized in order to compare the

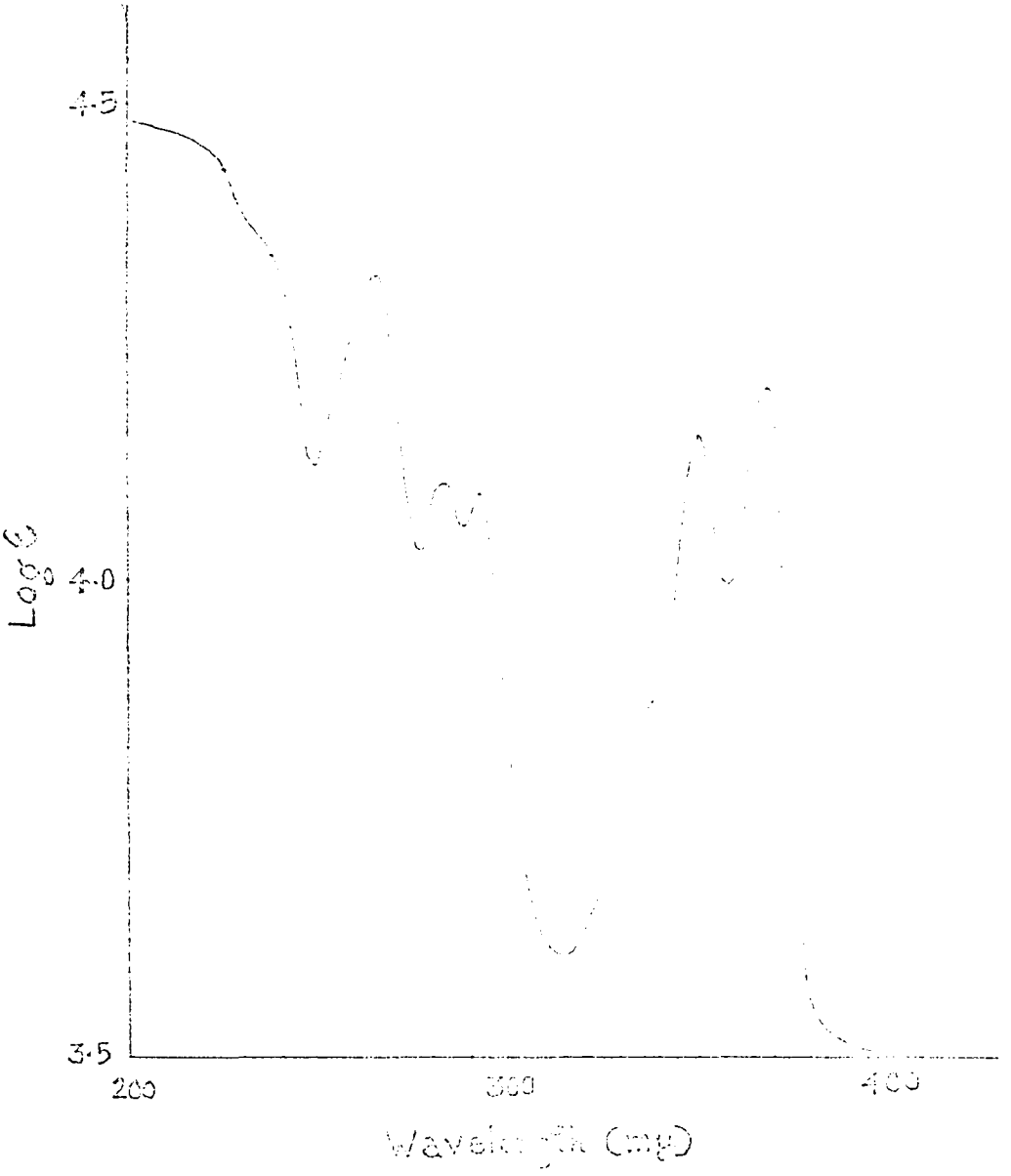


Fig. 14. Ultraviolet Spectrum of 4-Methoxycanthin-6-one in Dioxan

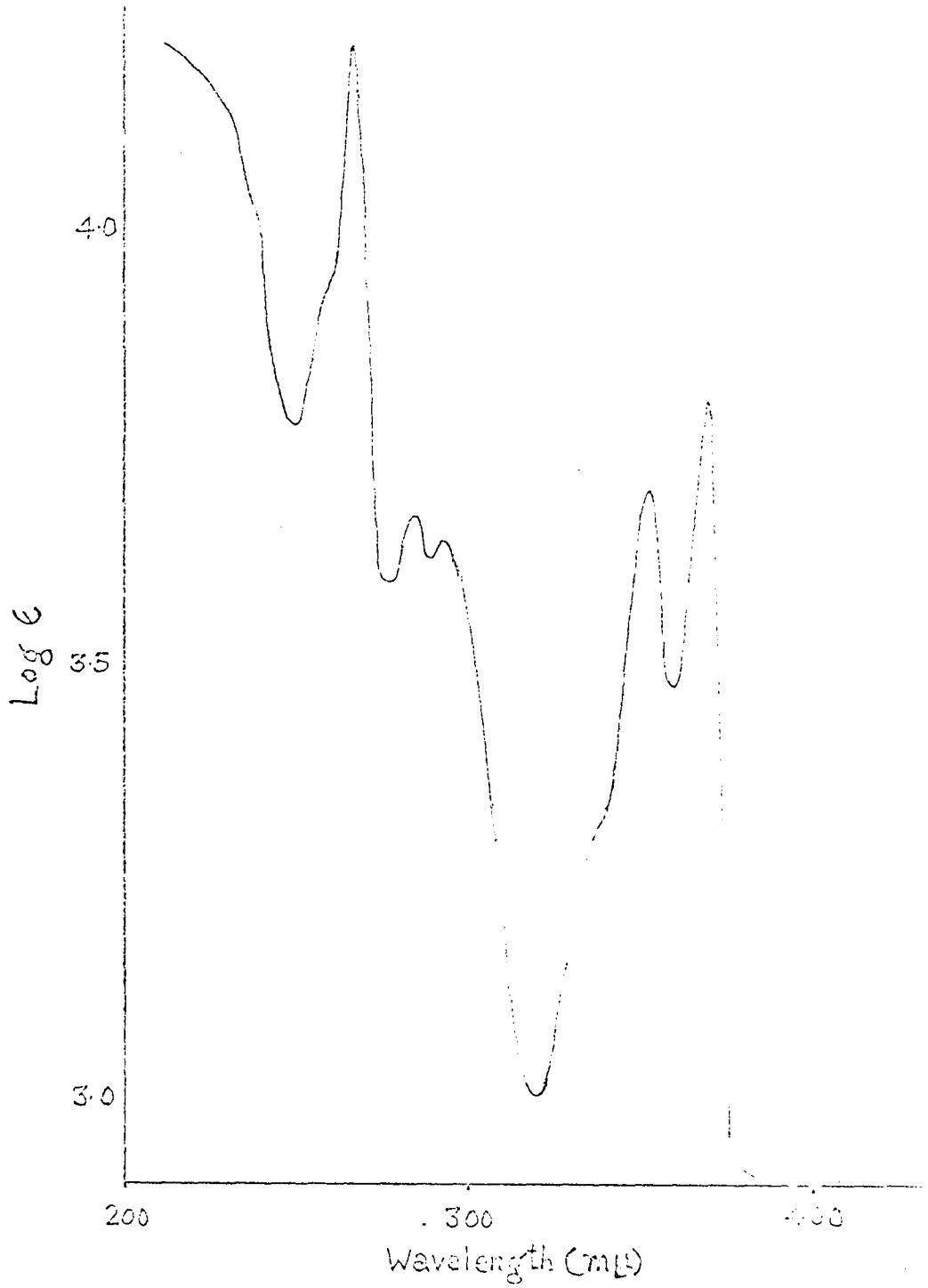
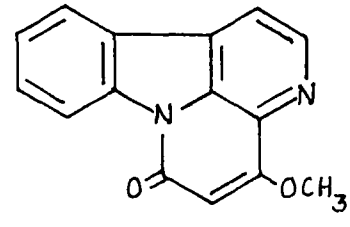


Fig. 15. Ultraviolet Spectrum of the Base in Dioxan

physical and chemical properties of the different canthinones isolated, viz. canthin-6-one, 5-methoxycanthin-6-one and 4-methylthiocanthin-6-one. The synthesis of 4-methoxycanthin-6-one was therefore attempted along the lines adopted by Nelson and Price.[42] The entire synthetic scheme is summarized in Fig. 16.

A comparison of melting point, ultraviolet and infrared spectra and R_f-values of thin-layer chromatograms between the natural base and the synthetic 4-methoxycanthin-6-one indicated beyond doubt that the natural base was 4-methoxycanthin-6-one, VII.



VII

TABLE III. Comparison of R_f-Values of the Natural Base and 4-Methoxycanthin-6-one

Compound	Adsorbent-Solvent System: Alumina-G Benzene-Chloroform (60:40 by vol)	Silica Gel-G Chloroform (100%)	Deactivated Silica Gel + Chloroform - Ethanol (95:5 by vol)
Natural base	0.61	0.32	0.51
4-methoxycanthin-6-one	0.60	0.32	0.51

The low yield of synthetic 4-methoxycanthinone (Fig. 16) may be attributed to any or all of the following factors. It may be well to note that the various steps involved in the synthesis have not been worked out with a view to obtain optimum conditions for maximum yield.

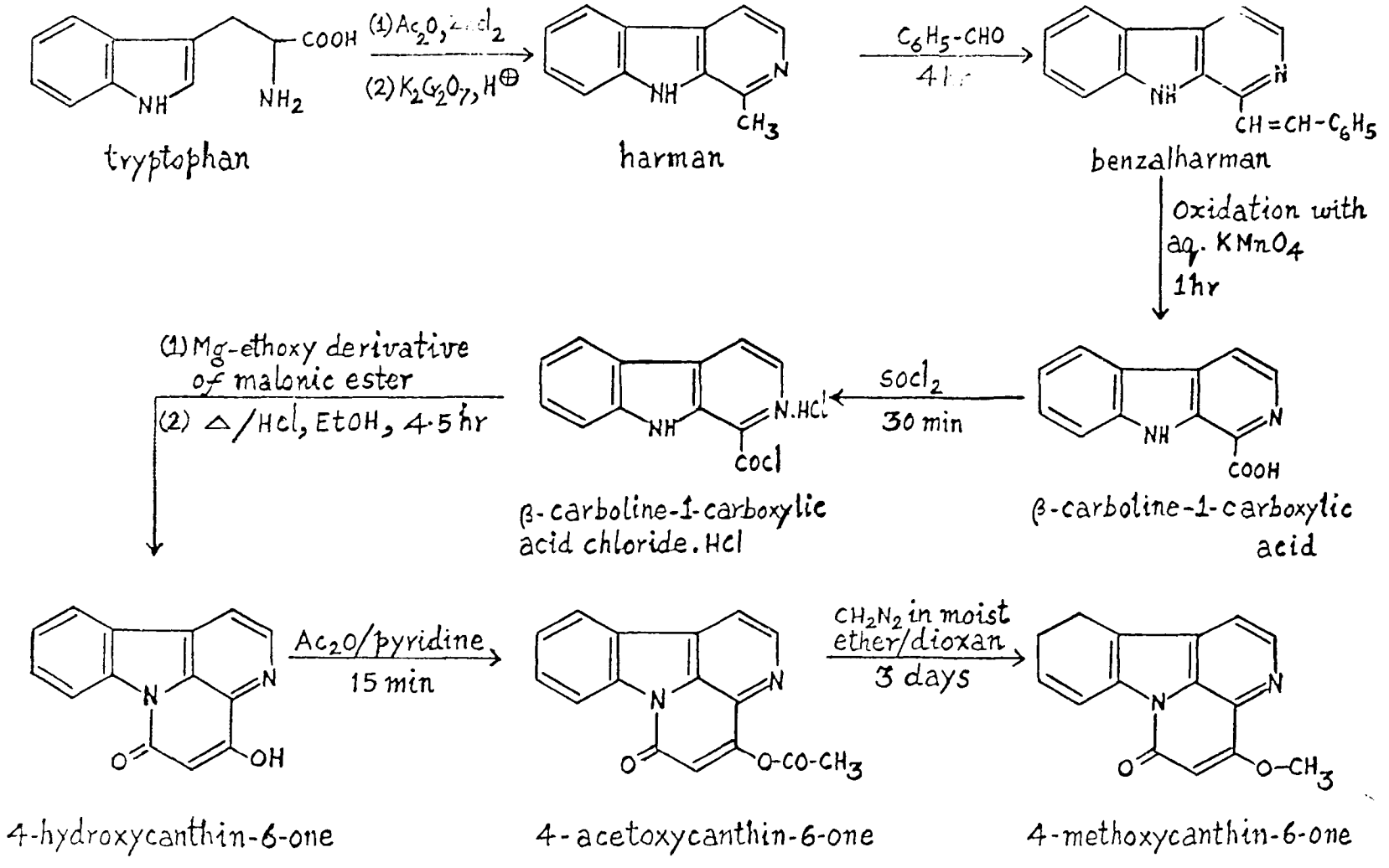


Fig. 16. Scheme for Synthesis of 4-Methoxycanthin-6-one

Even in the first step leading to synthesis of harman, though several methods have been worked out, in none of them is a yield of more than 25% reported. The yield of harman, viz., 28% obtained in our synthetic work is actually slightly above the reported figures. The second step, leading to synthesis of benzalharman, was almost quantitative, with yields as high as 90%. The next step, involving oxidation of benzalharman to β-carboline-1-carboxylic acid, resulted in only 52% yield though this is slightly better than the yield reported in the literature (43.3%) by Kermack, Perkin and Robinson.[39] The subsequent step, leading to the formation of the acid chloride resulted only in a very low yield. It was noted that optimum conditions for refluxing of thionyl chloride with the acid were very difficult to achieve. When the refluxing was carried out even slightly above the boiling point of thionyl chloride, rapid decomposition of the acid chloride took place; when, on the other hand, refluxing was done at a lower temperature, the acid was only partially converted into the acid chloride. Again, in the next step involving the condensation of the magnesium ethoxy derivative with the acid chloride much of the acid chloride seemed to remain unreacted. Also, after decarboxylation of the condensed product with a mixture of hydrochloric acid and ethanol, there was only an incomplete separation of the 4-hydroxycanthin-6-one hydrochloride on cooling. This may also account for the low yield of the final product. And again in the final step, involving the methylation of the acetoxy compound with diazomethane, much of the reacting mixture seemed to precipitate out as a red solid, thereby accounting for a low yield.

CHAPTER IV

SUMMARY AND CONCLUSION

Only one alkaloid could be isolated from the plant Charpentiera obovata Gaud. collected on Oahu and the total yield of the alkaloid amounted to ca. 0.0005% by weight of the dry plant material. This alkaloid, m.p. 220-221°, was shown to have a molecular formula $C_{15}H_{10}N_2O_2$ and was identified to be 4-methoxycanthin-6-one by a study of its ultraviolet, infrared and proton magnetic resonance spectra. This was further confirmed by a comparison of these spectral data and of the R_f-values of thin-layer chromatograms with an authentic sample of 4-methoxycanthin-6-one which was synthesized along the lines followed by Nelson and Price.[42] Final confirmation was provided by a mixed melting point determination of the natural base with 4-methoxycanthin-6-one, which showed no depression.

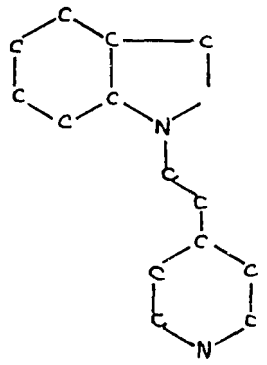
The detection of 4-methoxycanthin-6-one in C. obovata Gaud. brings to five the number of canthin-6-one and its derivatives isolated from natural sources (TABLE IV). Incidentally, this is also the first instance of the isolation of 4-methoxycanthin-6-one from a natural source, although it had been synthesized earlier for comparative studies.[42]

As it was already referred to, one of the reasons for undertaking the present research was to investigate the chemotaxonomic relationship among the families of the order Centrospermae based on the isolation of betacyanin-type pigments from several members of this order (TABLE I). It was considered plausible that alkaloids elaborated by a member of the Centrospermae might be constructed along the same skeleton as the pigments of the Centrospermae, whose structures have recently been

TABLE IV. Canthin-6-one and Its Derivatives Detected in Plants

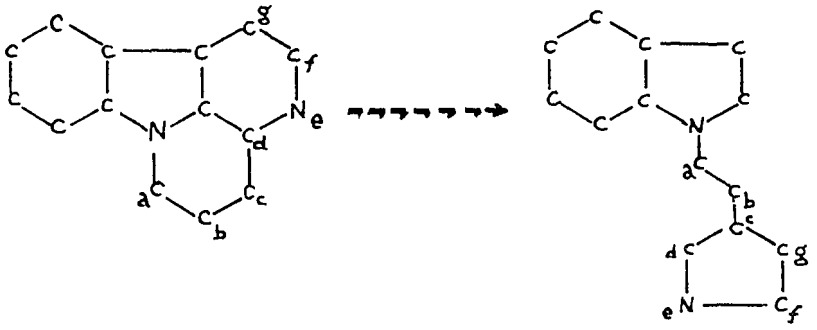
	Alkaloid	m.p.	Binomial	Family
1.	Canthin-6-one	163°	a) *Pentaceras australis* Hook. f[53]	Rutaceae
			b) *Zanthoxylum suberosum*[56]	Rutaceae
2.	5-Methoxycanthin-6-one	242°	*Pentaceras australis* Hook. f[54]	Rutaceae
3.	4-Methylthiocanthin-6-one	253°	*Pentaceras australis* Hook. f[42]	Rutaceae
4.	4, 5-Dimethoxycanthin-6-one	147.3°	*Picrasma ailanthoides* Planchon[41]	Simarubaceae
5.	4-Methoxycanthin-6-one	220-221°	*Charpentiera obovata* Gaud.	Amaranthaceae

established and found to involve an indole and a pyridine ring system with a 2-carbon chain connecting them. This is illustrated schematically as follows:



VIII

These pigments are generally referred to as betacyanins. However, the alkaloid which was isolated was not constructed along this pattern. In fact, a betacyanin pigment contains fifteen skeletal carbon atoms and two nitrogens while a canthinone contains but fourteen carbons and two nitrogens. If one proceeds to "unfold" a canthinone skeleton in a formal manner to obtain a betacyanine-type compound, one can construct a pyrrole conjugated with an indole but not a pyridine. This is shown schematically as follows:



IX X

There may of course be no biogenetic connection between pigments and alkaloids in a given plant. Yet a number of interesting problems present themselves for investigation. First of all, it becomes necessary to isolate the pigments elaborated by Charpentiera in order to be certain that they are indeed of the betacyanine type. Far more interesting, however, would be a biosynthetic study with labelled precursors in any member of the Centrospermae which elaborates alkaloids and betacyanine in order to discover the relationship--if any--of the biogenetic pathways. Such a study, incidentally, might shed some light on the long-puzzling question of the role of alkaloids as plant constituents.

Another point of chemotaxonomic interest deserves comment. A generally accepted, though at present not understood, relationship seems to exist between the molecular structure of alkaloids and the taxonomy of the plants from which they are isolated. Among well-known examples may, for example, be cited the occurrence of indole alkaloids in Apocynaceae, furoquinolines in Rutaceae, morphines in Papaveraceae, tropanes in Solanaceae. Of the four heretofore isolated canthinones (TABLE IV), three were found in Rutaceae and the fourth in the closely allied family Simarubaceae. The present finding, the isolation of a canthinone from a member of Amaranthaceae which is phylogenetically distant from Rutaceae and Simarubaceae,[4,5,6] is a sharp break with a long-accepted pattern. It is, however, only a single example and, unless canthinones will be found in other families phylogenetically distant from the Rutaceae, it will remain an isolated instance of nonconformity.

In this connection, it may be well to quote from an article by

Price,[57] "....The difference between presence and absence of a substance might be the reflection of profound biochemical and genetical differences or of nothing more than a single gene difference occasioning inability to synthesize or accumulate detectable amounts of the substance," sought for.

Pharmacological studies could not be carried out because of the small amount of the pure base available.

CHAPTER V

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