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Pachycereus Marginatus Alkaloids

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PACHYCEREUS MARGINATUS ALKALOIDS

By
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INTRODUCTION

Serturner's paper, 1817, "Morphis, a New Salt-forming Substance, and Meconic Acid, as the Chief Constituents of Opium", opened a new era of discovery in organic plant chemistry. His isolation of the first alkaloid was soon followed by the isolation of narcotine by Robiquet and strychnine by Pelletier and Caventou. These basic compounds were at first called vegetable alkalies, but were later renamed alkaloids, meaning alkali-like[11].

Alkaloids have been among the most extensively studied of the naturally occurring organic compounds. The empirical formula for over 2000, found in 3670 plant species, is listed by the U. S. Department of Agriculture[20]. Nevertheless, authoritative phytochemists estimate that not more than two percent of the known flowering plants have been investigated for possible alkaloidal content[11].

Notwithstanding, the many extremely valuable synthetic medicinal and antibiotic agents that have been added to the list of weapons against disease, the alkaloids still constitute an indispensable and most potent group of substances for the treatment and mitigation of functional disturbances and relief from suffering. In addition, proof of molecular structure of the natural physiological active alkaloids has helped locate the therapeutically active portion of the molecule so that active and better synthetic agents can be prepared as replacements, for example, the highly addicting cocaine has now been replaced with non-addicting procaine.

Alkaloids are, as a rule, white crystalline solids containing carbon, hydrogen, nitrogen, and oxygen. Several alkaloids which do not contain oxygen are liquids[9]. Generally, the alkaloidal bases are practically insoluble in water but soluble in water-immiscible solvents.

All alkaloids are basic owing to the presence of nitrogen in the primary, secondary, or tertiary form.

"The Dangerous Magic of LSD," an article in the Saturday Evening Post[10], vividly portrays the "bizarre mental transformations" of the users of d-lysergic acid, a constituent of all ergot alkaloids, and mescaline, an alkaloid obtained from cactus. Mescaline, a primary amine alkaloid, causes beautiful colored hallucinations and has been used for generations in an impure form by the Indians of U. S. and Mexico for this purpose. The presences of this alkaloidal hallucinogen in cactus furnished early impetus for investigation of other cacti for alkaloids.

One of the most common of the giant cacti (Cereanae) is Pachycereus marginatus. It was reported[14] as early as 1931 that this cactus species contained alkaloids. The isolation of one alkaloid, pilocereine[8], and the presence of others[17] was described by Djerassi, Smith, and co-workers.

This thesis describes the isolation and investigation of the structure of the other alkaloids found in conjunction with pilocereine. Research was carried out on the same cactus extract as was used by Djerassi, Smith, et al.[8] and carried out in the following manner:

1. Number of alkaloids was determined.
2. Separation of the alkaloids was accomplished and purification attempted.
3. Some chemical and physical properties were ascertained.

HISTORICAL REVIEW

Serturner, when he isolated the first alkaloid in 1817, probably used a "shaking out" extraction procedure. This procedure is briefly described in the National Formulary XI[12] as:

The "shaking out" process is carried out by treating the drug, or a concentrated liquid extract of it, with a solvent immiscible with water, in the presence of an excess of alkali which liberates the alkaloid. The free alkaloid is dissolved by the immiscible solvent from which it is removed by means of an excess of dilute acid. The acid solutions are then extracted with an immiscible solvent in the presence of an excess of alkali, and the immiscible solvent evaporated to obtain the alkaloid.

The "shaking out" method of isolation and separation of alkaloids is still used today, after over a hundred and forty years. The literature, however, proves this method has faults and more sophisticated procedures are needed. "Ion-exchange Resins and Electrodialysis in the Extraction and Purification of Alkaloids,"[3] "Determination of Some Alkaloids by Means of a Photocolorimetric Method Using Reinecki Salt,"[19] "Polarography in Analytical Chemistry of Alkaloids,"[15] and "Microdetermination of Alkaloids Employing Radiometric Titration,"[16] are examples of titles of recent papers.

Isolation of pilocereine from the first cactus species (Lophocereus schottii) in 1953 was carried out using extraction and column chromatography procedures[6]. In 1955 the same alkaloid was isolated from P. marginatus by extraction, column chromatography, and countercurrent distribution techniques[17].

The U. S. Department of Agriculture[20] has catalogued fifty-five species of cactus that contain alkaloids. Twenty-seven of these species contain seventeen different alkaloids of known molecular formula (see Table 1, page 4).

Table 1

CACTUS ALKALOIDS

Alkaloid	Molecular Formula	Number of Cactus Species Containing
Anhalamine	$C_{11}H_{15}NO_3$	2
Anhalidine	$C_{12}H_{17}NO_3$	2
Anhaline (Hordenine)	$C_{10}H_{15}NO$	2
Anhalinine	$C_{12}H_{17}NO_3$	2
Anhalonidine	$C_{12}H_{17}NO_3$	3
Anhalonine	$C_{12}H_{15}NO_3$	6
Cactine	---------	1
Caffeine	$C_8H_{10}N_4O_2$	5
Candicine	---------	3
Carnegine	$C_{13}H_{19}NO_2$	2
Coryneine	$C_{11}H_{19}NO_3$	1
Lophocerine	---------	1
Lophophorine	$C_{13}H_{17}NO_3$	2
Mescaline	$C_{11}H_{17}NO_3$	5
N-methylmescaline	$C_{12}H_{19}NO_3$	2
O-methylanhalonidine	$C_{13}H_{19}NO_3$	1
Oxycandicine	---------	1
Pellotine	$C_{13}H_{19}NO_3$	3
Piloceredine	$C_{30}H_{44}N_2O_4$	1
Pilocereine	$C_{30}H_{42}N_2O_4$	5
Tricocereine	$C_{13}H_{21}NO_3$	1

The U. S. D. A.[20] also lists Mammillaria lewinii as the cactus species which contains the largest number (twelve) of known alkaloids. This species, the first that was known to contain mescaline, was most extensively studied because of the unusual physiological effects of this alkaloid. "Mescal buttons," the dried plant, is the form used by the Indians to produce the effects, colored hallucinations, and according to legend, increased physical endurance. Indians were able to run for hours while under their influence.

The cactus alkaloids, see Table 1, page 4, are with two exceptions, C_{13} or below. It was Djerassi and co-workers[7] that found piloceredine and pilocereine to be C_{30} alkaloids. C. R. Smith[17] isolated pilocereine from P. marginatus and indicated the presence of other alkaloidal material.

The subject of this thesis is the several other alkaloids found in conjunction with pilocereine in P. marginatus.

EXPERIMENTAL*

General Data

The crude base was in dry, marble-size, amber colored lumps. It had been stored at room temperature for seven years in a tightly sealed, clear, glass bottle. The base was ground to a powder using a mortar and pestle. This powdered base was used throughout the investigation. The crude base dissolves in ether, ethanol, methanol, chloroform, and ethyl acetate giving a clear straw colored liquid which darkens upon aging.

Paper Chromatography

To hasten the running of paper chromatograms, the simple apparatus illustrated in Figure 1, page 6, was used.

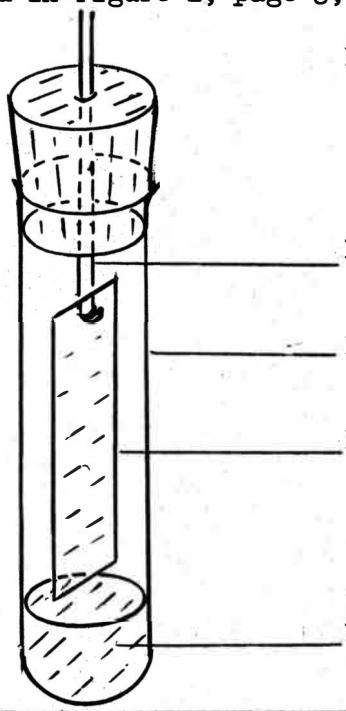
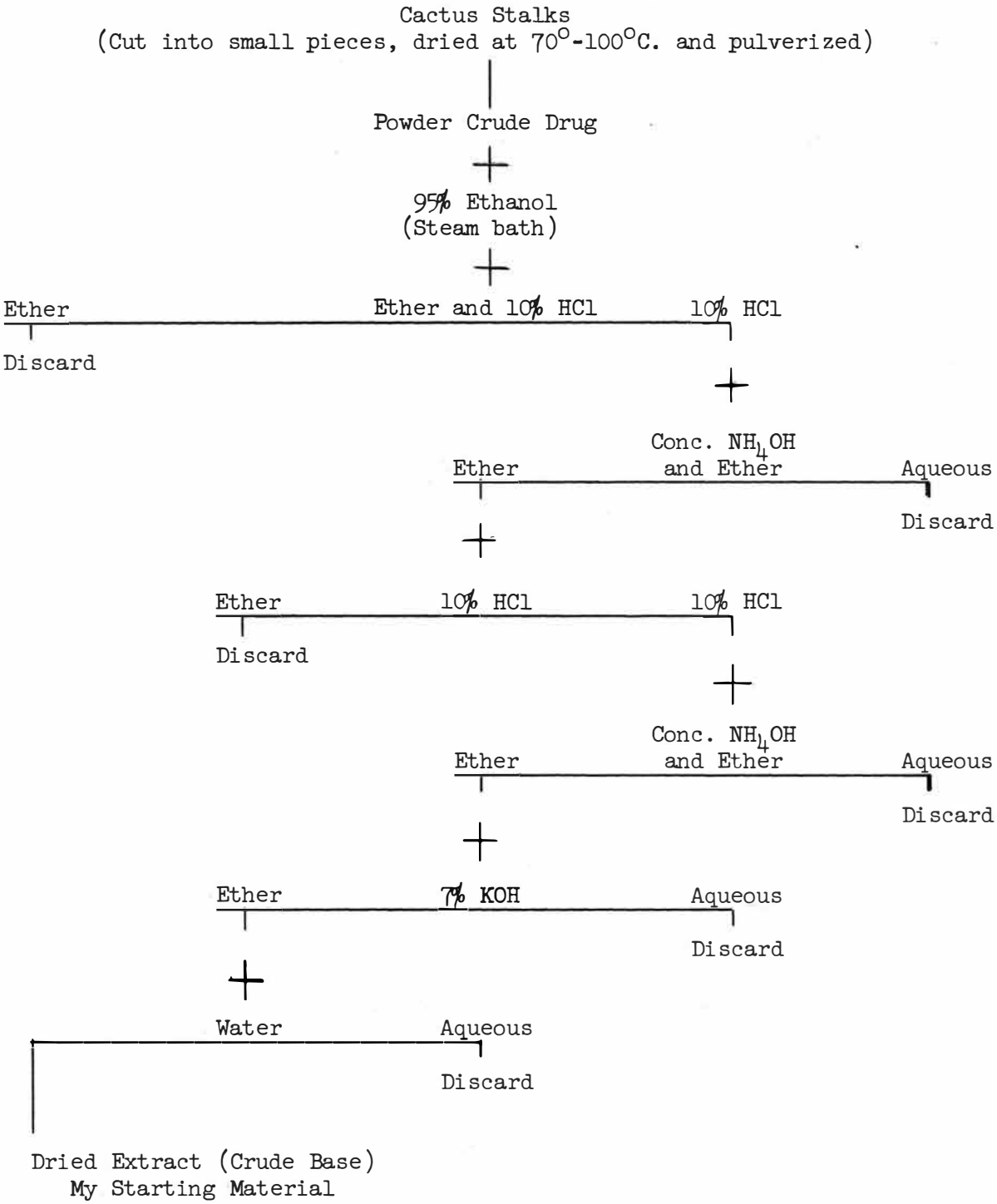


Figure 1

*For this research twenty to twenty-five grams of crude base, prepared by Carl Djerassi, C. R. Smith, et al[8] was available. The method used for isolation of the crude base is diagrammed in Figure 2, page 7.

Figure 2

METHOD OF ISOLATION OF CRUDE BASE



After spotting, the seventeen centimeter paper was suspended in a saturated atmosphere of the development solvent. The ascending solvent front was allowed to proceed to within three to five centimeters of the top of the paper.

Whenever one of the small test papergrams showed separation, the chromatograph was repeated using fifty centimeter paper for either descending or ascending chromatograms. These employed conventional equipment and technique[2].

Developing Solvents Used

A number of developing solvents was used. Their composition and effectiveness are summarized in Figure 3, page 9.

Chromatographic Papers Tested

Whatman's papers No. 1, 4, 11, and 54 were used; No. 1 and 4 were best. No. 54 was much too fast. The small papers were seventeen centimeters long and one to one and one-half centimeters wide. These were usually spotted three to four centimeters from the bottom.

Concentration of Spotting Material

Five or ten microliters of either ten milligrams of crude base per milliliter of ethanol or ninety-one milligrams per milliliter was used as spotting material for the seventeen centimeter papers. The larger papers required larger amounts—up to one-tenth milliliter.

Paper Buffering and Additive Systems

The following additive solutions were used:

No. A - 2 ml. dimethyl formamide, 6 ml. acetone, and 5 ml. of the developing solvent.

No. B - 1 g. benzoic acid and 25 ml. formamide.

No. C - 3 ml. acetone and 1 ml. formamide.

Figure 3

COMPOSITION OF DEVELOPING SYSTEMS USED FOR PAPER CHROMATOGRAPHY

Liquids were combined on a v/v ratio.

Developing System No.	Glacial Acetic Acid	3% NH_4OH Solution	Benzene	n-butanol	Isobutanol	t-butanol	Chloroform	Citric Acid	Cyclohexane	Ethanol	Ethyl Acetate	Ethyl Ether	Methanol	Petroleum Ether	Toluene	Deionized Water	Resolution
1	1			25												sat.	Poor
2	1			10												sat.	Poor
3				*50				lg.								50	Buffered pap two spots
4				2								5					Buffered pap two spots
5		1		2													Poor
6				1					1								Two spots
7						*25		lg.	25							50	Two spots
8							all										Buffered pap two spots
9							1			1							All to solve front
10							5			1							All to solve front
11										all							All to solve front
12																all	No movement
13							1		1	1							Considerable spreading
14											all						Three spots on some
16		all															No movement
17															all		Two spots
18					all												Two spots one spread
19													all				Some spreadi
21							1				1						Nothing
22							1		1		1						Two spots
25							all									acid sat.	Three spots
34			4											1			Two spots one spread

*butanol phase

No. D - 1 g. benzoic acid, 5 ml. developing solvent, and 25 ml. formamide.

The chromatographic papers were dipped and allowed to dry before use. Better separation was obtained when No. C paper additive solution was used.

Staining Solutions Tried

1. Dragendorff's Reagent
2. Dichlorofluorescein
3. Fluorescein
4. Alkaline bromphenol blue
5. Picric acid
6. Potassium ferricyanide
7. Tannic acid
8. Phosphotungstic acid
9. Phenolphthalein
10. Methyl red

After thoroughly air drying, the papers were dipped rather than sprayed with the stain. The P. marginatus alkaloids are soluble in some of the staining solutions and can easily be washed off the paper.

Dragendorff's Reagent gave a positive test (orange spot on a yellow background) for the following known alkaloids: atropine, ergot alkaloids, quinine, reserpine (faint) and caffeine and was most frequently used. Dragendorff's Reagent should be freshly prepared from equal amounts of the following two solutions, glacial acetic acid and water. Mix 5 ml. solution A, 5 ml. solution B, 20 ml. glacial acetic acid, and add water to make 100 ml. Prepare solution A by dissolving 0.85 g. bismuth subnitrate in 40 ml. water and 10 ml. glacial acetic acid and prepare

solution B by dissolving 8 g. potassium iodide in 20 ml. of water.

Alkaline bromphenol blue, dichlorofluorescein, and fluorescein also gave acceptable results.

When an acetic acid-butanol-water developing solvent and Dragendorff's Reagent for staining are used, the P. marginatus alkaloids have R_F values of 0.9 to 0.95.

Countercurrent Distribution Experiments

Five countercurrent distribution experiments were completed on the thirty-plate, ten milliliter per phase instrument manufactured by H. O. Post Scientific Instrument Co., Inc., Middle Village, N. Y. and one on the two hundred-plate, robot-driven, fifty milliliter per phase instrument also manufactured by H. O. Post, (courtesy of Unit 140 of The Upjohn Company). Alkaloids from these distributions required additional separation. Thin layer and column chromatography were used.

Solvent Systems

Two types of two-phase solvent systems were employed.

1. All volatile liquids (no solids added).
2. Aqueous phase buffered to a definite pH with nonvolatile solids.

The first solvent system has the advantage of recovery of the dissolved alkaloidal material by evaporation. The buffer solids must be removed from the second system before the alkaloids can be recovered.

Technique

Technique employed for the thirty-plate instrument was standard except for an addition to the instrument on later runs. This addition consisted of a rest bar which was fixed to the instrument in such a

manner that the drain position was always at exactly the same angle.

Figures 4 and 5, pages 13 and 14, show the distribution of solids obtained from countercurrent distribution experiments numbers 1 and 3 which employ solvent systems type 1.

The two hundred tube distribution experiment is described in more detail because the alkaloidal material from the individual tubes was used as a spotting material for many thin layer chromatograms.

Previous work done by C. R. Smith and the success of Svoboda[18] in his work on veratrine alkaloids determined the choice of solvents. The stationary phase was chloroform U.S.P. and the movable phase was an aqueous phosphate-citrate buffer prepared as follows: Solution A: 360 g. sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$) dissolved in five liters deionized water. Solution B: 169 g. of citric acid ($C_6H_8O_7 \cdot H_2O$) dissolved in seven and one-half liters of deionized water. Solution A was added to solution B until the pH reached between 4 and 4.5; seven and one-tenth liters were required. Two hundred fifty milliliters of lower phase (chloroform) was funneled into each tube. The machine was started and run thirty to forty minutes to allow equal distribution of lower phase before the upper phase was added. The upper phase is fed in automatically after each transfer into tube number 0 from a reservoir. The sample (8.3 g.) was dissolved in 250 ml. of lower phase. Fifty milliliters of the sample containing lower phase was put into tubes 0 through 4. The robot control of the instrument was set for a two minute shake period, three minute rest, and a one minute transfer; the cycle to be repeated 190 times. The instrument was started. One hundred and ninety transfers were completed in nineteen hours.

Figure 4

TOTAL SOLIDS FOR COUNTERCURRENT DISTRIBUTION EXPERIMENT #1

Solvent (parts by volume):

Deionized Water	5 parts
Ethyl Acetate	4 parts
Ab. Ethanol	1 part

Sample Weight	0.65 g.
Estimated Recovery	0.615 g.
29 Transfers	

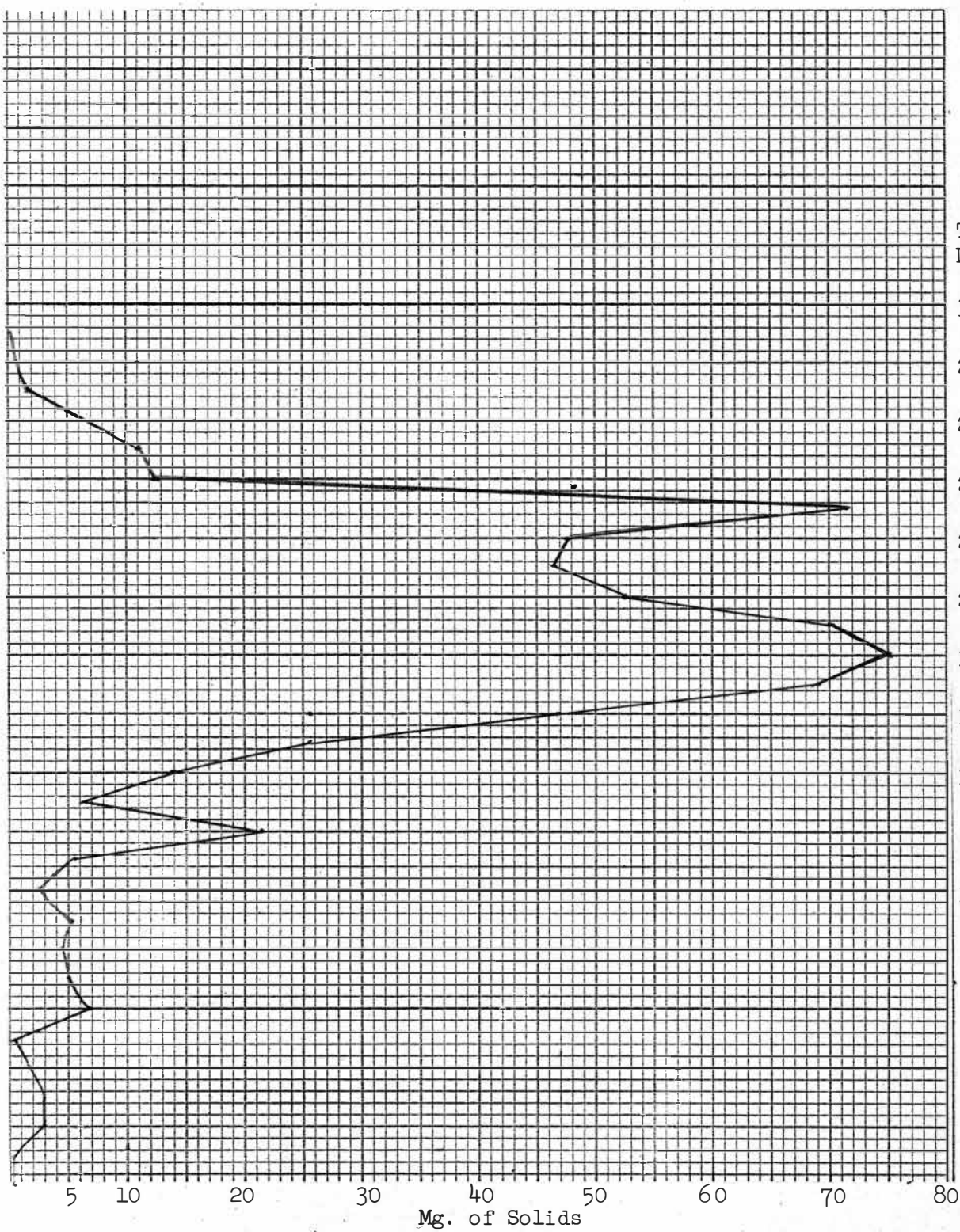


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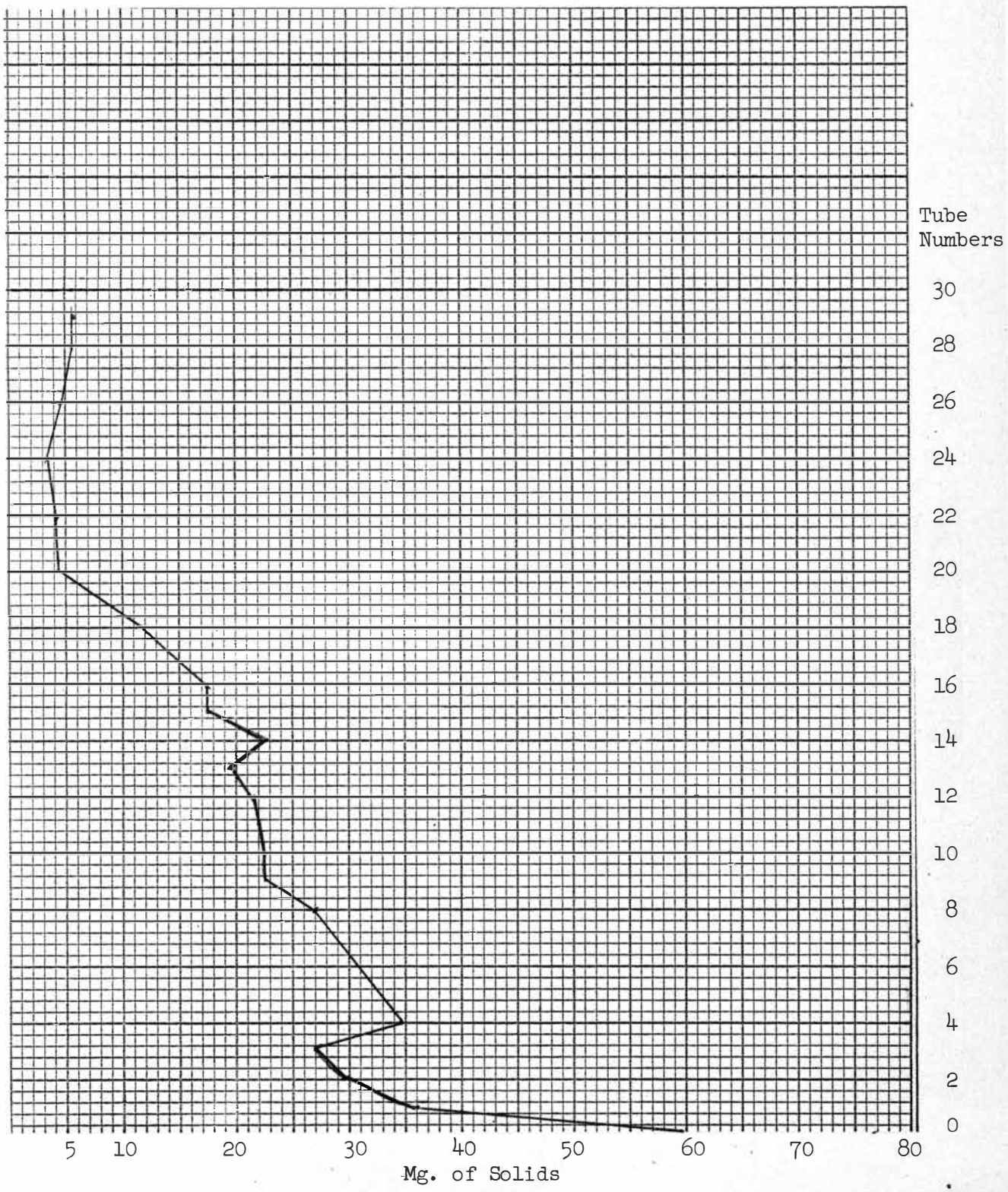
Figure 5

TOTAL SOLIDS FOR COUNTERCURRENT DISTRIBUTION EXPERIMENT #3

Solvent (parts by volume):

Ethyl Acetate	4 parts
Deionized Water	3 parts
Cyclohexane	1 part
Methanol	2 parts

Sample Weight 0.2 g.
Recovered 0.23 g.
29 Transfers



Work Up of Countercurrent Distribution

Upon completion of distribution, upper and lower phase of each tube was withdrawn into a four fluidounce bottle. These 190 bottles were stored in a dark, cool place, temperature 15° to 25°C., until they could be processed.

The first extraction was made in the bottle used to collect the liquid remaining in the tube after the countercurrent distribution had been run. Extraction was carried out in the following manner: three milliliters of 4 M sodium hydroxide were added to the one hundred milliliter two-phase chloroform-aqueous buffer system, thus the water-soluble alkaloidal salts were converted to the chloroform-soluble alkaloidal bases. The first extraction was accomplished by shaking the two-phase system already in the bottle. The chloroform layer was separated from the aqueous layer by using a separatory funnel and filtered through dry cotton to remove moisture. The aqueous layer was further extracted by using two twenty-five milliliter portions of chloroform. These chloroform extracts were combined with the original chloroform layer and the resulting one hundred milliliters used for the following experiment.

To determine amount of solids present in each tube, exactly two milliliters of the above chloroform solution were removed, placed in a small tared tin foil dish and evaporated to dryness on a steam bath. To remove the last traces of chloroform, the dishes were placed into a steam-heated vacuum column and dried for ten minutes. The tin foil dish containing the solid residue was weighed on a Mettler Balance manufactured by Mettler Instrument Corporation, Highstown, New Jersey. This balance is accurate to ± 0.025 milligram. This procedure gave the distribution of the material in the experiment. Total solids, for every

fifth bottle, are plotted in Figure 6, page 17. The color and physical state of the material appear in Table 2, page 18.

The remaining ninety-eight milliliters of chloroform solution were concentrated in a current of air, without heat, to about five milliliters. Considerable darkening of certain fractions occurred during the evaporation process. Some of the darkening could be attributed to an increase in concentration of the alkaloidal material. Five to ten microliters of the remaining five milliliters of chloroform solution were used to spot thin layer chromatography (TLC) plates and the balance pooled if TLC plates indicated the presence of the same alkaloids.

Because countercurrent distribution experiments numbers 2, 4, and 5 were duplications of experiments numbers 1, 3, and 6, data for 2, 4, and 5 is not included.

Figure 6

TOTAL SOLIDS FOR COUNTERCURRENT DISTRIBUTION EXPERIMENT #6

Solvent:
Chloroform 1 part
Aqueous Citrate Phosphate Buffer 1 part

Sample Weight 8.3 g.
Estimated Recovery 5.8 g.
190 Transfers

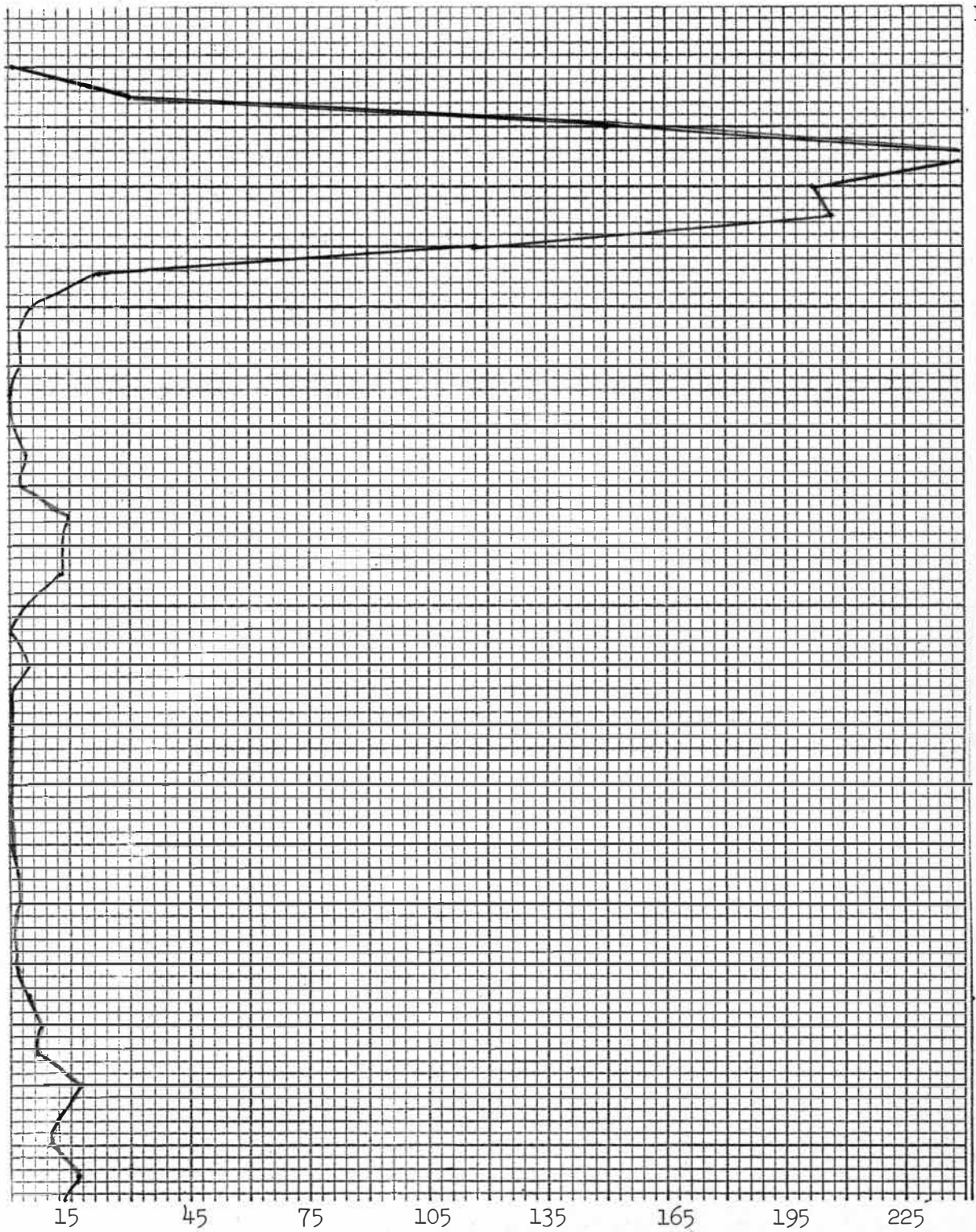


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Table 2

PHYSICAL APPEARANCE OF SOLIDS FROM

COUNTERCURRENT DISTRIBUTION EXPERIMENT #6

Tube Number	Color	Form
0 - 6	Brownish-yellow	Amorphous
7 - 11	Yellow	Amorphous crystalline edges
20	White	Fairly crystalline
90	Pale yellow	Fairly crystalline
110	Pale yellow	Fairly crystalline
140	Yellow	Waxy
160	Yellow	Waxy
168	Yellow-orange	Waxy
172	Pale yellow	Waxy
185	Pale yellow	Fairly crystalline

THIN LAYER CHROMATOGRAPHY FOR SEPARATIONS

Preparation of Plates

All thin layer plates, except the first few, used silica gel G which contains calcium sulfate ($CaSO_4 \cdot 1/2H_2O$) 13%, has a pH of 6.7, and is distributed by Brinkmann Instrument, Inc. Dry silica gel G was mixed with water in the ratio of one to two and spread 0.25 mm. thick with a Desaga Heidelberg Spreader also distributed by Brinkmann. Two plate sizes were used: twenty by twenty centimeter and five by twenty centimeter. The plates were spread and either air dried over night or air dried for several hours and oven dried for one hour at approximately 70°C.

Development

Two types of chromatography jars were used for the two size plates. The development of the twenty centimeter square TLC plates was carried out in a ten liter jar using six hundred milliliters of developing solvent. The development of the five centimeter plates used a two liter jar containing one hundred fifty milliliters of developing solvent.

The plates were spotted by means of a micropipette about two and one-half centimeters from the bottom of all plates which was about one and one-half centimeters above the liquid level.

Selection of a developing solvent is the most important aspect of thin layer chromatography. The rule is that the polarity of the solvent should be about equal to that of the substance to be separated[5]. See Figure 7, page 20, for developing solvents tested, their composition, and effectiveness.

Two of these deserve special attention. Solvent number 32 in Figure 7, page 20, is composed of equal parts by volume of ethyl acetate, methanol and cyclohexane.

Figure 7

COMPOSITION OF DEVELOPING SYSTEMS USED FOR THIN LAYER CHROMATOGRAPHY

Liquids were combined on a v/v ratio.

Developing System No.	3% NH_4OH Solution	Glacial Acetic Acid	Benzene	n-butanol	Chloroform	Citric Acid	Cyclohexane	Diethylamine	Ethanol	Ethyl Acetate	Methanol	Methyl Acetate	Petroleum Ether	Deionized Water	Resolution
CS2									1		9				Some
CS3									1	9					Some
CS4										2	3				Poor
CS11		1%					1				3				Poor
1		1		1											Poor
3				*50		lg.								50	Poor
5	1			1											Some
6				1			1								Poor
8					all										Poor
11									all						Poor
13					1		1		1						Poor
29				1						1					Poor
30	sat.									1					Poor
31									1	1					Poor
32							1			1	1				Good
33					1		1				1				Good
34			4										1		Poor
36					1					1					Poor
37							1				1	1			Good
38							1				3	1			Good
39							1			1	3				Fair
40					1		1			1	1				Poor
41					4		5	1							Poor

*butanol phase

Solvent number 33 is composed of equal parts by volume of chloroform, methanol and cyclohexane. Both of these developing media furnish excellent separations; however, solvent number 33 was used most extensively. For consistent results these solvents should be freshly prepared.

All chromatographs were run at room temperature.

Spotting

Amounts of spotting solutions used varied with their concentration. For the unfractionated alkaloidal material the same concentrations were used for the TLC work as were used for paper chromatographs (page 8). Five to ten microliter quantities were used for spotting. Five or six spots could be placed on the large plates and two on the smaller ones.

Identification

The plates were removed, allowed to air dry and the distance the material had advanced was ascertained by observing under ultraviolet light or by staining with Dragendorff's Reagent. Ultraviolet light gave more detail for most of the alkaloids except pilocereine which does not fluoresce.

There is one blue fluorescent spot observed under ultraviolet light that does not stain with Dragendorff's Reagent. Figure 8, page 22, points out the difference in the two methods of detection.

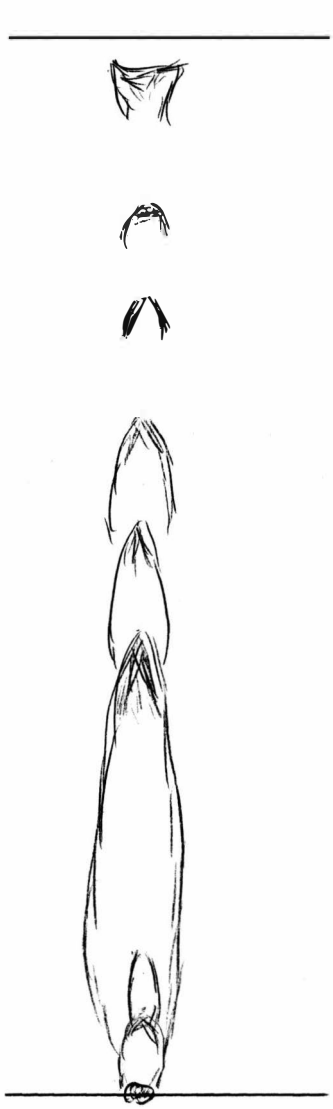
These cactus alkaloids fluoresce light yellow and show a less intense fluorescence in solution than in the dry finely divided state with the exceptions noted above.

The color and intensity of fluorescence depends to a large extent on the alkaloidal salt and frequently the salt gives a more intense color than the base. The cactus alkaloids in this investigation were the bases.

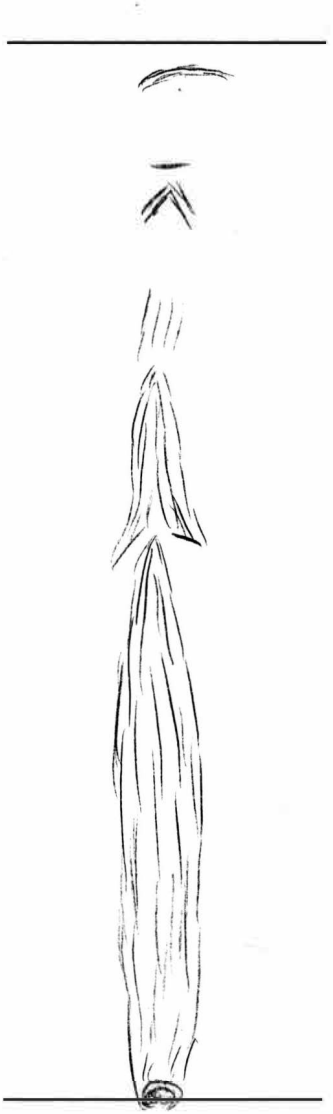
Figure 8

TWO DETECTION METHODS

Used for the same Thin Layer Chromatogram



Ultraviolet Light



Dragendorff's Reagent

Solvent Front

Developing
Solvent 3

3/9/63

(See page 21)

Results on a Solution of the Unfractionated Alkaloids

Seven of the twelve plates developed using either solvent number 32 or 33 had seven or eight distinct alkaloidal spots. These plates were spotted with solutions containing ninety-one milligrams of crude base per milliliter. Figure 9, page 24, shows the distribution of material on a typical two dimensional thin layer plate. This chromatoplate used solvent number 32 and was stained with Dragendorff's Reagent. Seven alkaloids are readily observed and the eighth is questionable. Additional drawings of chromatograms are illustrated in Figure 10, page 25. These drawings are self explanatory and reflect what was observed with ultraviolet light.

Based on these and other chromatoplates it is probable that there are eight alkaloids in addition to pilocereine in the cactus extract sample. On the same bases, R_F values have been established and recorded in Table 3 which follows.

Table 3

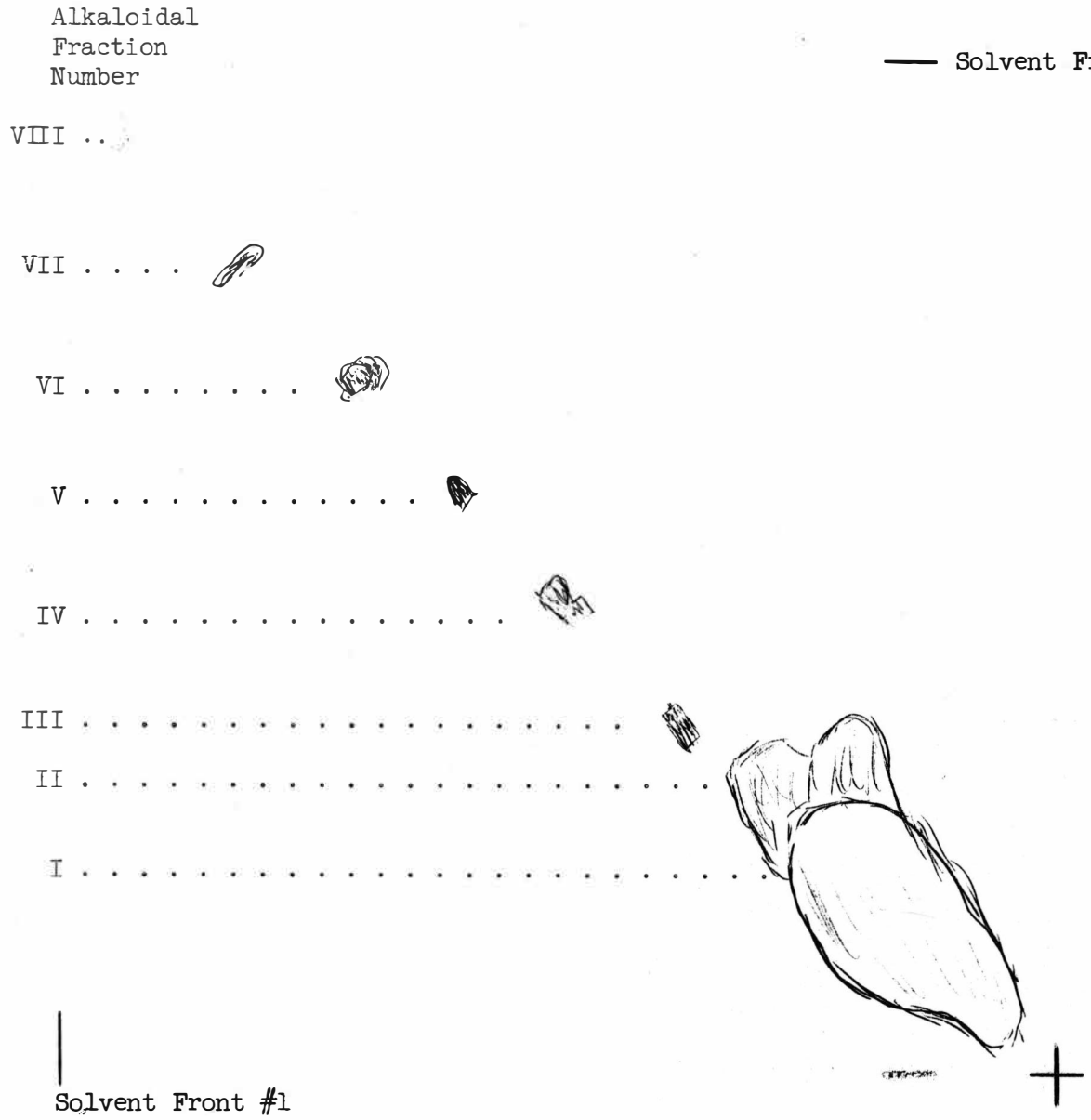
	R_F Values in Solvent 33
Alkaloid I	0.00 - 0.05
Alkaloid II	0.08 - 0.2
Alkaloid III	0.23 - 0.35
Alkaloid IV	0.6 - 0.65
Alkaloid V	0.65 - 0.7
Alkaloid VI	0.7 - 0.75
Alkaloid VII	0.85 - 0.95
Alkaloid VIII	0.95 - 1

Satisfactory reproducibility of absolute R_F values can only be obtained by the observation of rather difficult experimental conditions

Figure 9

TWO DIMENSIONAL THIN LAYER CHROMATOGRAM

OF THE UNFRACTIONATED ALKALOIDAL SOLUTION



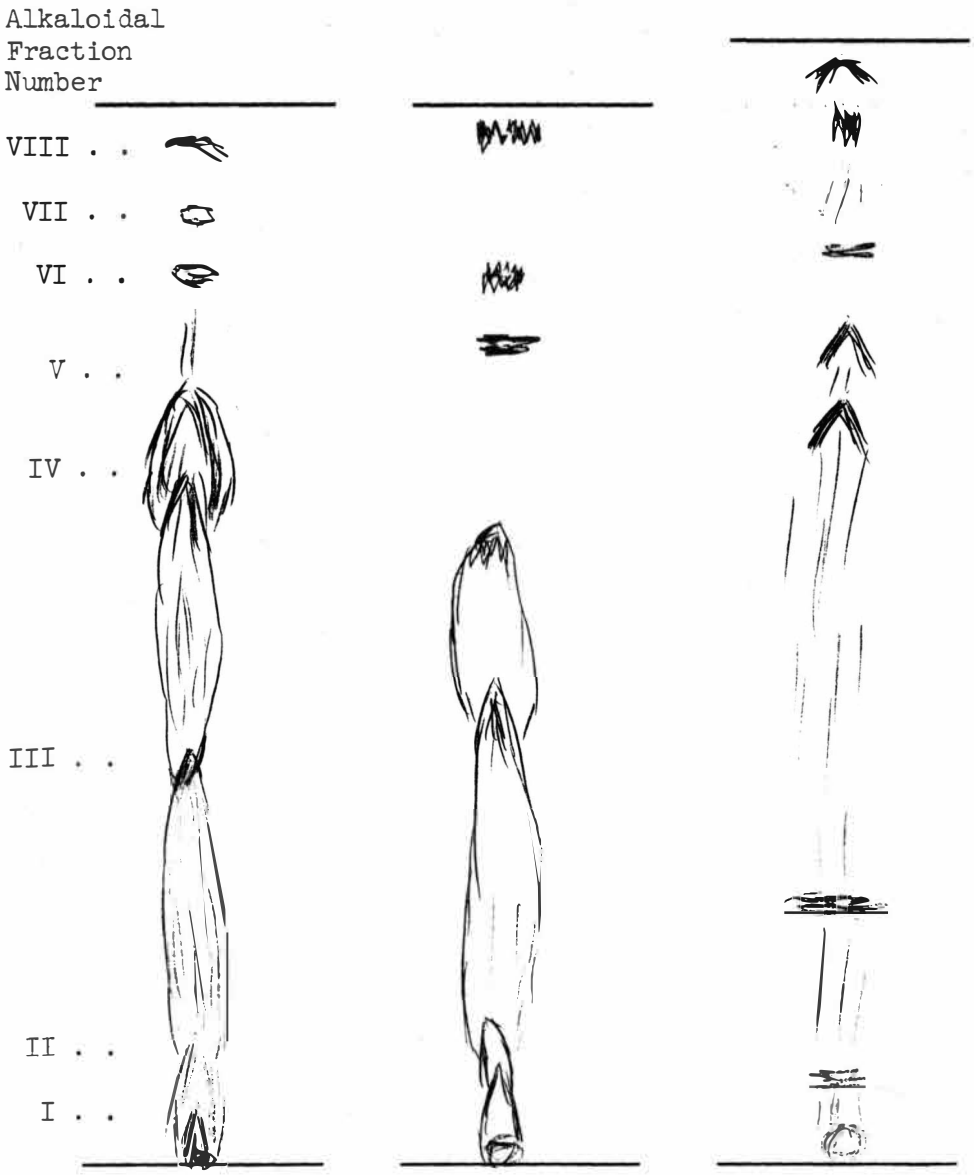
2/28/63

Developing Solvent 32

(See page 19)

Figure 10

THIN LAYER CHROMATOGRAMS OF UNFRACTIONATED ALKALOIDAL SOLUTION



Developing Solvent 33
(See page 21)

(manipulation of the plates under total absence of humidity, constant and equal temperature, elimination of "edge effect," etc.). Given optimum condition, R_F values show an approximate $\pm$ 0.05 accuracy[5].

A sample of pilocereine received from Dr. Carl Djerassi was developed with solvent number 33, stained with Dragendorff's Reagent, and has an R_F value of 0.48.

Results of Thin Layer Chromatographic Separations on Countercurrent Distribution Fractions

Two types of solvent systems were employed in countercurrent distribution experiments and these will be described separately.

Countercurrent Distribution using Solvents Type 1 (See page 11)

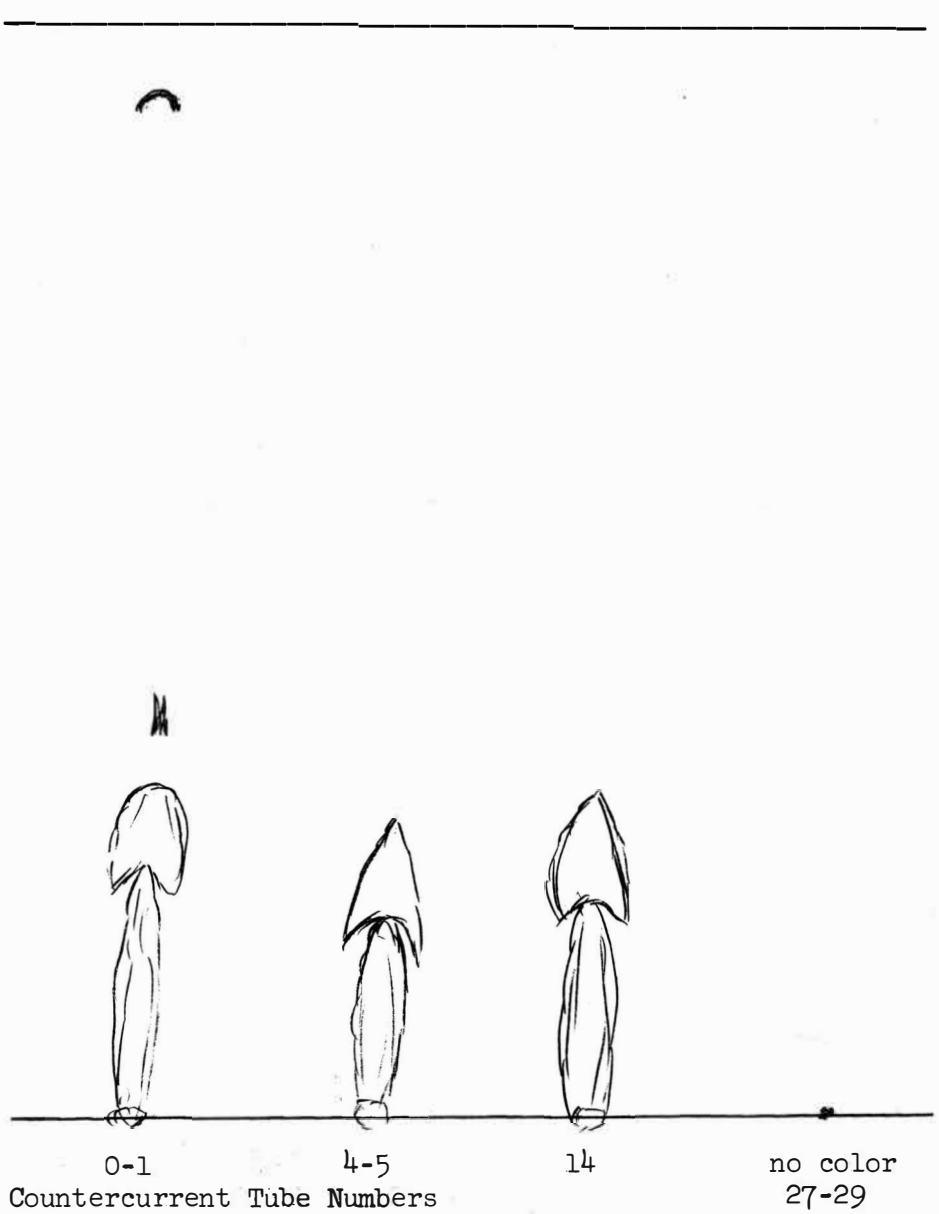
As written earlier in this thesis considerable effort was expended in the continued separations of countercurrent fractions. Since these solutions were dilute, they were concentrated by heating on a water bath. Inasmuch as developing solvents number 32 and 33 were not used in all instances, the chromatographic results of countercurrent distribution experiments 1 and 2 can not be compared easily with those of later separations.

Solutions from tube numbers 15 and 23 from countercurrent distributions experiment number 1 were tested both on paper and TLC. The results of the TLC, as might be expected, from the total solids graph Figure 4, page 13, indicated very little separation accomplished by countercurrent distribution.

Distribution of solids in countercurrent experiment 3 is shown in Figure 5, page 14. Alkaloids were found in the first sixteen of the twenty-nine tubes; however, most of the material was in the first three tubes. Figure 11, page 27, shows a thin layer chromatograph of the

Figure 11

THIN LAYER CHROMATOGRAM OF COUNTERCURRENT DISTRIBUTION EXPERIMENT #3



(See page 19)

2/23/63

Dragendorff's Reagent used for staining

distribution of material in the third countercurrent experiment. Little separation was obtained from the countercurrent experiment; however, Alkaloid VIII appeared exclusively in tubes number 0 and 1.

Countercurrent Distribution using Solvent Type 2 (See page 11)

In countercurrent distribution experiment number 6 (190 transfers) alkaloids are found in all tubes except those from fifty-five to eighty-five. Tubes 160 to 180 contained most of the solids.

Thin layer chromatography work showed (Table 4, page 29) that Alkaloid I and Alkaloid II moved with the aqueous upper phase; Alkaloid III, Alkaloid V, and Alkaloid VI, spread throughout all the tubes and Alkaloid VIII stayed in the lower numbered tubes. These separations seem to varify the fast and slow moving group found in paper chromatography. Typical drawings of chromatograms are found in Figure 12, page 30.

Table 4

ALKALOIDAL DIVISION IN

COUNTERCURRENT DISTRIBUTION EXPERIMENT 6 AS

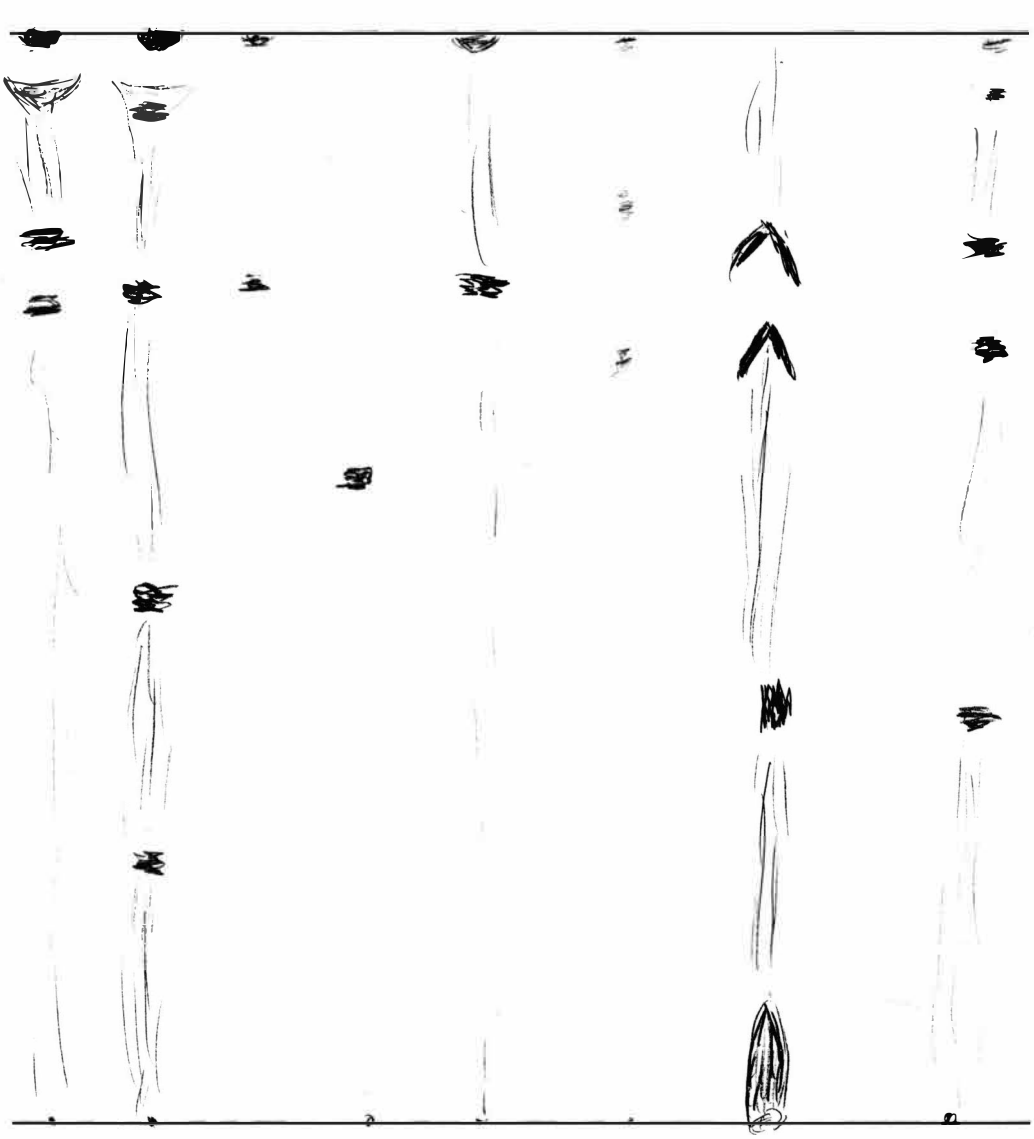
INDICATED BY THIN LAYER CHROMATOGRAPHY

Alkaloid Number	Located Tube Number	Most Concentrated In	Approx. R_F Value
I	160-180		0.05
II	160-170		0.1
III	0-55 and 80-180	7-11	0.25
IV	0-55 and 80-180	161-166	0.6
V	0-55 and 80-180	161-166	0.65
VI	0-55 and 80-180	174-176	0.7
VII			0.9
VIII	0-55	1-11	1

Figure 12

TYPICAL THIN LAYER CHROMATOGRAMS OF

COUNTERCURRENT DISTRIBUTION EXPERIMENT #6



0-6 7-11 20 90 109-114 140 161-166 174-176 Developing Solvent 33 (See page 21)

Countercurrent Tube Numbers

5/15 8/3 5/3 5/15 8/2 5/3 8/2 7/14

1963 dates that TLC was developed

ISOLATION OF THE ALKALOIDS

Two methods were tried: (thick) thin layer chromatography (open column) and regular column chromatography.

THIN LAYER CHROMATOGRAPHY FOR ISOLATION OF ALKALOIDS

The procedure used, as previously described (page 19) for plate preparation and development, was the same except that the thickness of the silica gel was increased to either 1 mm. or 0.75 mm. and approximately 0.05 ml. of spotting material was used per spot—six to eight spots per plate. In these instances the spotting material was obtained from countercurrent distribution experiment number 6.

After the plates were dry, spots in the same relative position from two plates were brushed off into a filter. The fluorescent solid, containing the silica gel and the alkaloid was washed with three five milliliter portions of chloroform. If this was unsuccessful, washing was continued with three five milliliter portions of methanol. If either dissolved some of the fluorescent alkaloid, large amounts (approximately 100 ml.) of solvent were used. If neither one nor the other was successful, methanol was acidified to pH 3 with concentrated hydrochloric acid and used as a wash. In several instances all of the alkaloid could not be completely recovered from the gel.

The wash was evaporated to dryness in vacuo. If the resultant microscopic bit of material appeared crystalline under a hand magnifying lens, melting points, thin layer chromatograms, and infrared curves were obtained. If the material appeared amorphous, it was redissolved in chloroform, and petroleum ether (30 - 60 B.P.) was added to precipitate

the alkaloid. After centrifuging the resulting mixture, the liquid was pipetted off and the precipitate dried and used to check physical constants.

No attempt was made to weigh the dry solids, but the amounts obtained were in milligram quantities.

A control was run by brushing the silica gel from a developed plate into a filter and washing it with about twenty-five to thirty milliliters each of chloroform and methanol. The chloroform-methanol solution was allowed to evaporate. There was no residue.

Results

Data that was secured is recorded in Table 5, page 33.

Explanation of Table 5

The thin layer spots that corresponded to the various fractions that were isolated often were spread. The numbers of the alkaloidal fractions were assigned on the basis of the above spots from the thin layer chromatogram. These numbers may denote mixtures of alkaloids rather than a pure compound. The foregoing assumption is based on the range of melting points indicated in column 3 of Table 5, page 33.

The "Source Tube #" column is the tubes from countercurrent distribution experiment number 6 that were used to spot the thin layer plates. Melting points were run on a melting point block made by Fisher Scientific Co.

The R_F values are those obtained using the isolated material and solvent number 33.

Infrared curves were run on Beckman Infrared Spectrophotometer IR 8, made by Beckman Instrument, Inc., Fullerton, California. Three are illustrated in Figures 13, 14, and 15, pages 34, 35, and 36.

Table 5

EXPERIMENTAL DATA ON ALKALOIDAL FRACTIONS

Alkaloidal Fraction No.	Source Tube #	MP °C.	R_F of Isolated Alkaloid	IR	Description
VIII	0-6	44-45	----	----	Semi crystalline, white but darken to a brown
VIII	7-11	42-47 45-50	1	----	White non-crystalline
VII	0-6	49-51	.82	----	White to buff small crystals
VII	174-176	63-64	.93	----	Light brown crystals
VI HCl	7-11	over 290	.48 .65	----	----------
V or VI HCl	161-166	-----	.55 .88	Yes Fig. 13 page 34	Brown non-crystalline
V or VI	161-166	about 100	.74	----	Amber amorphous
III	174-176	over 300	.91	----	Cream colored small crystals
III	7-11	decomposes 225-230	.1	Yes Fig. 14 page 35	Light brown powder
III HCl	7-11	-----	----	----	----------
I	0-6	42-45	----	----	Amber amorphous
I	161-166	43-46 41-42	0	----	Straw colored amorphous
I HCl	161-166	32-42	0	Yes Fig. 15 page 36	Amber amorphous

Figure 13

INFRARED ABSORPTION SPECTRUM FOR ALKALOID V OR VI HYDROCHLORIDE

In chloroform

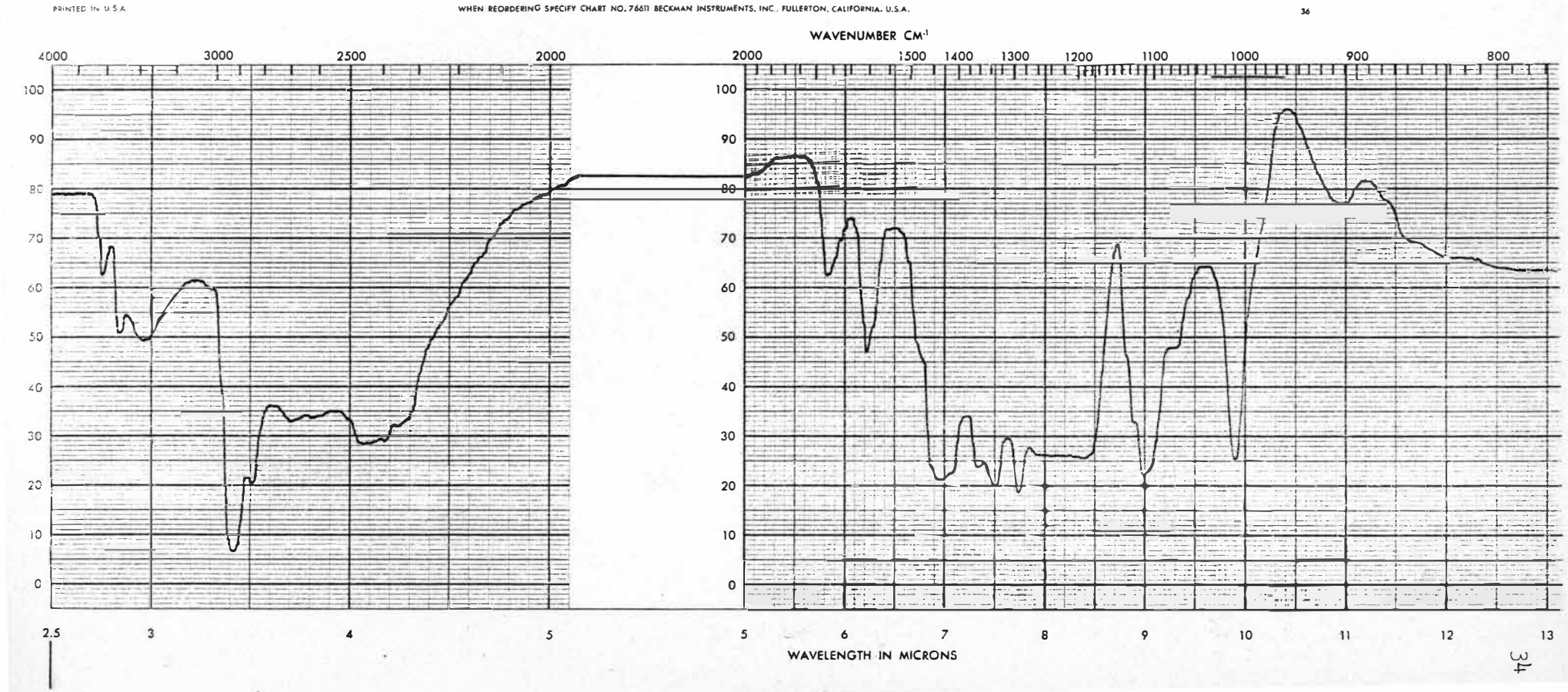


Figure 14

INFRARED ABSORPTION SPECTRUM FOR ALKALOID III

In chloroform

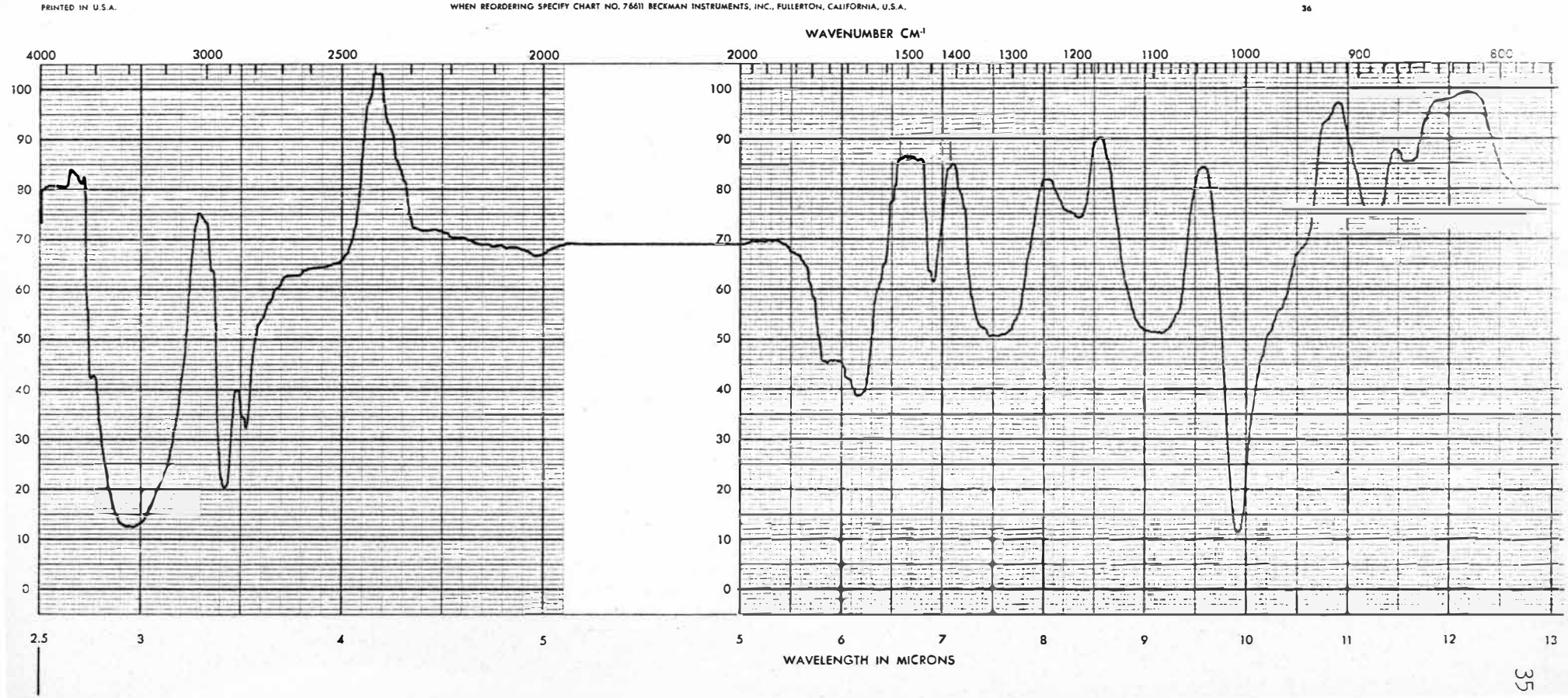
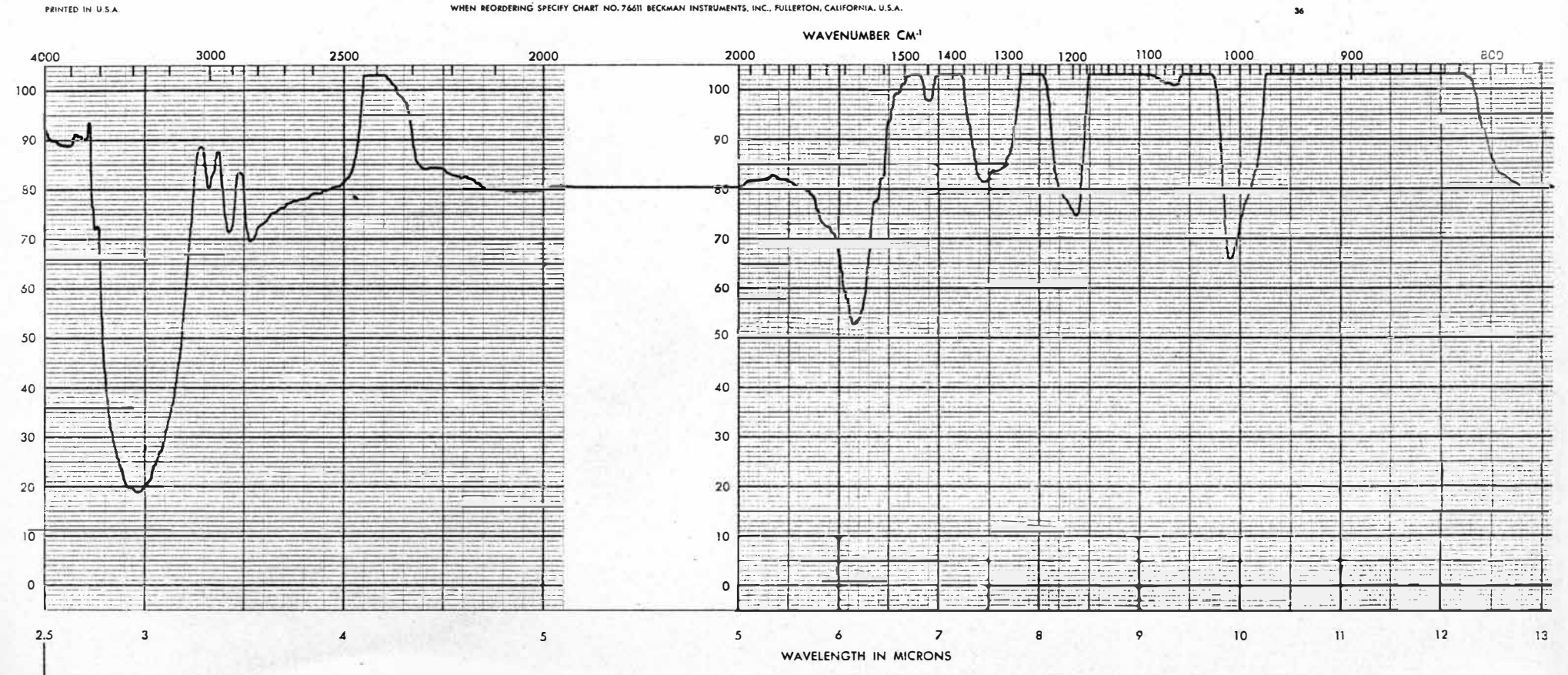


Figure 15

INFRARED ABSORPTION SPECTRUM ALKALOID I HYDROCHLORIDE

In chloroform



USE OF COLUMN CHROMATOGRAPHY

Because Alkaloid I remained stationary on thin layer plates developed with solvent 33 (page 21), it was thought a larger amount could be obtained by column chromatography if silica gel were used in the column and if solvent 33 were used as the eluate. Alkaloid I should remain at the top of the column and be washed free of the other alkaloids if a large volume of eluate were used. Accordingly, a column was packed using approximately one hundred grams of twenty-eight to two hundred mesh silica gel made by Fisher Scientific Co. The column was approximately one inch in diameter. The sample consisted of forty milliliters of chloroform extract from countercurrent distribution experiment number 6, tube numbers 161-166 representing approximately one gram of solids. To the sample was added forty milliliters each of cyclohexane and methanol before starting it through the column. The eluate used was solvent 33. Total volume of eluate passed through the column was about 2200 milliliters. The top inch of the silica column was very fluorescent. This portion of the column was removed and extracted with five hundred to six hundred milliliters of methanol acidified with concentrated hydrochloric acid. Even after this extraction process, the silica was still very fluorescent.

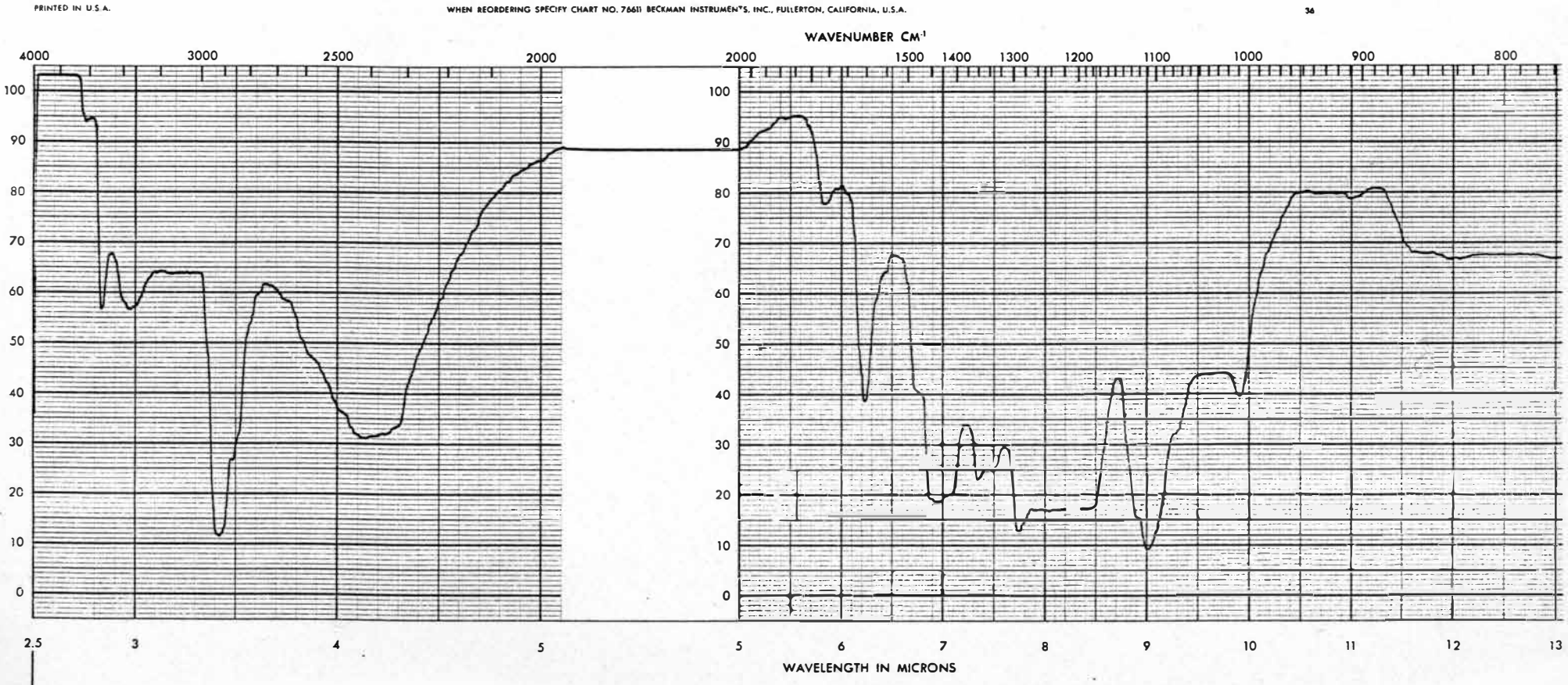
The methanol solution was evaporated. The dry amber, non-crystalline alkaloidal material was thought to be Alkaloid I Hydrochloride. An infrared absorption spectrum of this material in chloroform, is shown in Figure 16, page 38.

It was not Alkaloid I Hydrochloride as thought, since it had a different melting point than Alkaloid I Hydrochloride isolated from thin layer chromatography.

Figure 16

INFRARED ABSORPTION SPECTRUM ALKALOIDAL MATERIAL FROM COLUMN CHROMATOGRAPHY

In chloroform



This alkaloidal material was redissolved in methanol, made basic with ammonium hydroxide, and evaporated. The resulting solid melted at 210° to 220°C. It was dark brown and gave a strong alkaloidal spot test.

DISCUSSIONS AND CONCLUSIONS

Paper Chromatography

Early experimental work consisted of an extensive effort using paper chromatography to separate the alkaloids of Pachycereus marginatus. The best results obtained were three spots for which the predominate two were located at the origin and the solvent front. These results were obtained using treated paper and ethyl acetate or chloroform as developing solvents. The results, as shown by later thin layer chromatograms, were incomplete.

When an acetic acid-butanol-water developing solvent is used, the Pachycereus marginatus alkaloids have R_F values of 0.90 to 0.95 and correspond closely to piperine, a C_{17} alkaloid[2].

The best conclusions that can be made from the accumulated data from paper chromatography are:

1. There are two distinct groups of alkaloids present which differ in polarity.
2. A general idea of the types of solvent systems to use in countercurrent experiments.

Thin Layer Chromatography

When thin layer chromatography was used the material was separated. The use of ultraviolet light or Dragendorff's Reagent revealed there were eight spots, each of these was assumed to be an individual alkaloid. R_F values were determined as shown in Table 3, page 23, using a solvent of equal volumes of methanol, cyclohexane, and chloroform. As can be seen by the table none of these alkaloids is pilocereine, known to be in P. marginatus and to have an R_F value in the methanol-cyclohexane-chloroform solvent of 0.48.

The basic alkaloids of P. marginatus under ultraviolet light fluoresce a pale yellow color with a low intensity. The fact that pilocereine, an isoquinoline derivative[6], does not fluoresce under ultraviolet light is not unusual[13], as shown in Table 6 below:

Table 6

Isoquinoline Alkaloids	Color of Fluorescence	Intensity
Cortarnine	None	0
Cortarnine hydrochloride	Yellow	35
Hydrastinine	None	0
Hydrastinine hydrochloride	------	4
Hydrastine	Green	55
Hydrastine hydrochloride	Blue	36

Attempts were made to isolate the alkaloids by removing the silica gel from developed thin layer plates containing the alkaloidal spots. Various alkaloidal materials were isolated by this means although frequently the alkaloid had to be converted to the hydrochloride before it could be removed from the silica.

Three of the alkaloids isolated from the above experiments were subjected to thin layer chromatography. The solvents and conditions used were similar to those used for the initial isolation. This chromatogram, however, yielded different R_F values or two spots for each of the three alkaloids. Obviously the alkaloid molecule was split, oxidized, or in some manner altered by the isolation procedure. For this reason thin layer chromatography does not seem to be a satisfactory method of isolation of these alkaloids. Perhaps this difficulty can be circumvented by developing and drying the thin layer plates in an inert gaseous atmosphere.

Countercurrent Distribution

Two phase solvent systems used for the countercurrent experiments were chosen on the bases of their partition coefficients which were determined experimentally. As can be seen from the three countercurrent experiments shown in Figures 4, 5, and 6, pages 13, 14, and 17, good fractionation was not obtained. However, in the 190 plate distribution the weight balance curve may be misleading because there is more alkaloidal material in tubes 1 through 11 than the total solids curve indicates. This conclusion was reached on the bases of the results obtained from thin layer chromatography because when solutions in tubes 1 through 11 were concentrated to equal volume they gave spots of equal intensity regardless of the amounts of total solids.

Discussion About Isolated Alkaloidal Material

An infrared absorption spectrum of pilocereine in chloroform is given in Figure 18, page 43. Pilocereine is known to be one of the alkaloids of P. marginatus and it has the structure illustrated in Figure 17 below.

Figure 17

Pilocereine Structural Formula[7]

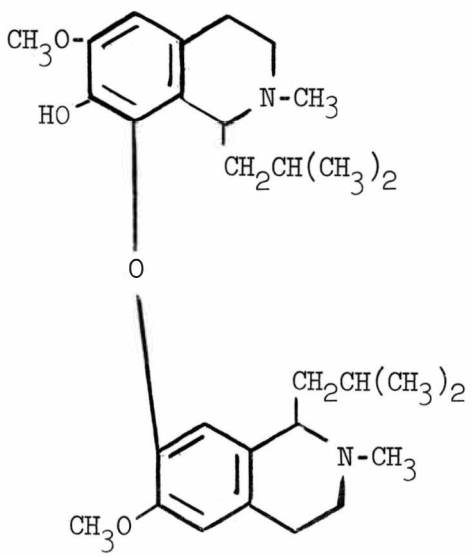
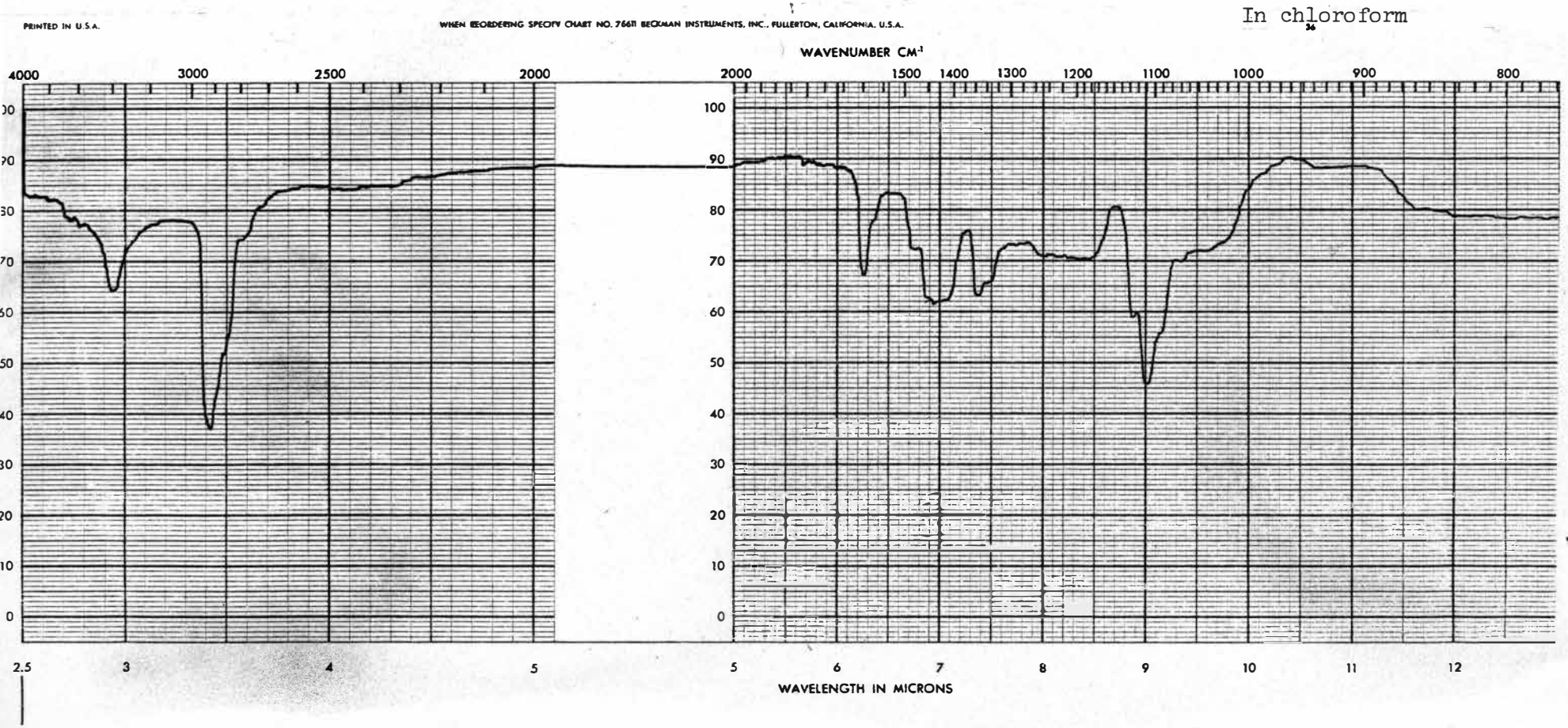


Figure 17

INFRARED ABSORPTION SPECTRUM FOR PILOCEREINE

In chloroform[36]



The spectrum and structure of pilocereine are given as a reference.

Table 7, page 45, is a comparison of the peaks and shoulders of the various infrared absorption spectra. All the alkaloids have spectra reasonably similar to pilocereine, considering that the infrared spectra of some were run on the hydrochlorides of these alkaloids. The major differences are in the 2850 to 1600 $cm.^{-1}$ region.

Comparison of the absorption spectra of Alkaloid V or VI Hydrochloride (Figure 13, page 34) and alkaloidal material from column chromatography (Figure 16, page 38) indicate that they are the same. Both also gave two spots on a thin layer chromatogram.

Table 7

PEAKS AND SHOULDERS OF INFRARED ABSORPTION SPECTRA

Alkaloid I HCl Page 36	Alkaloid III Page 35	Alkaloid V or VI HCl Page 34	Pilocereine Page 43	Column Alkaloid Page 38
cm.$^{-1}$	cm.$^{-1}$	cm.$^{-1}$	cm.$^{-1}$	cm.$^{-1}$
3625	3625	3625		3625
		3550		3550
3400	3400	3400	3400	3400
3000				
2925	2925	2925	2925	2925
2840	2840	2840		2850
		2460		2450
	1710	1710		1710
1620	1620			
		1600	1600	1600
			1490	
1450	1450			
		1440	1440	1440
		1370		1365
			1350	
1340				
	1335	1335	1335	1335
		1290		1290
1195	1195		1195	
			1120	
		1110	1110	1110
	1090			
1010	1010	1010		1010
	888			
	860			

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VITA

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The author is married and the father of two daughters and a son.

APPROVAL OF EXAMINING COMMITTEE

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(Chairman)

Date _____