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A phytochemical investigation of liverwort Frullania franciscana Howe

Timothy Ta-E Huang
Portland State University

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APPROVED BY MEMBERS OF THE THESIS COMMITTEE:

[redacted]

Alfred S. Levinson, Chairman

[redacted]

Raymond P. Lutz

[redacted]

Norman C. Rose

Liverwort Frullania franciscana Howe was found high on the allergic test scale by J. Mitchell of the Medical School of the University of British Columbia and co-workers. Frullanolide was isolated from Frullania tamarisci by J.D. Connolly and by G. Ouisson and his co-workers.

The plant sample, collected in Oregon, was hand separated, confirmed, air dried and milled before the extractions were done. A Soxhlet extraction with ether and cold extractions with n-hexane and with ether were carried out. Ether is a better extraction solvent than n-hexane for cold extraction.

Column chromatography was used for the separation of the extract based on gradient elution from silica gel or aluminum oxide. Non-polar hydrocarbon sesquiterpenes and other hydrocarbons usually were eluted first. Frullanolide then was eluted.

Studies by thin layer chromatography on silica gel showed that none of the fractions was a pure component; therefore, further separations were done by thin layer chromatography and small-scale column chromatography. However, no satisfactory separation systems were found, except that frullanolide was isolated from the silica gel plate with cyclohexane/ethyl acetate 80/20, Rf' = 25.8, which was very close to the reported value. An infrared spectrum of this was taken and used as supporting evidence.

An infrared spectrum of the first fraction from an aluminum oxide column showed that this was a reasonably pure fraction of hydrocarbons. By gas chromatography, eleven components were found in this fraction, and the percentage of the major component was calculated to be 97% by weight. Nuclear magnetic resonance and infrared spectra showed an exo-double bond with a 6-membered ring or larger. (I.R.: 3080 cm^{-1}, 1643 cm^{-1}, 888 cm^{-1}, NMR δ 4.73) This fraction was then studied by means of a gas chromatography-mass spectrometer, and the molecular weight of the main component of this fraction was 204. Kovats' indices of the main component were taken by co-injection with α-cedrene and β-bourbonene; these data, however, did not match any of the compounds listed.

Infrared analysis of the other fractions from the columns and from thin layer chromatography showed the presence of carbonyl groups. One of the carbonyl groups absorbed at about 1770 cm^{-1}, characteristic of a γ-lactone.

A PHYTOCHEMICAL INVESTIGATION OF LIVERWORT FRULLANIA franciscana HOWE

by

Timothy Ta-E Huang

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TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

The members of the Committee approve the thesis of Timothy Ta-E Huang presented 26 August 1974.

[redacted]
Alfred S. Levinson, Chairman

[redacted]
Raymond F. Lutz

[redacted]
Norman C. Rose

APPROVED:

[redacted]
Gary L. Gard, Chairman, Department of Chemistry

[redacted]
David T. Clark, Dean of Graduate Studies and Research

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This thesis is dedicated to my mother,

Mrs. Chin-len Y. Huang

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INTRODUCTION

The liverworts (Hepaticae) form a unique family in the plant kingdom. About 8,500 species of the plants are distributed throughout the world (1). They are taxonomically and phylogenetically placed between vascular plants and thallophtes (algae). The liverworts contain, in the cells of the gametophytes, several oil bodies which are characteristic for species of their shape and distribution density and which are used as a useful factor in taxonomical diagnosis (2).

Chemical investigation of the essential oil from liverworts was first made by Müller in 1905 (2). Relatively little additional work was done, however, until the advent of modern analytical tools. The essential oils are obtained from liverworts as very complicated mixtures characterized by relatively low volatility (3). It has been assumed that the main components were sesquiterpenes, hydrocarbons or alcohols (4), frequently of azulenogenic character (3).

In recent years there has been much interest in the natural products of liverworts. A literature search of Chemical Abstracts from January 1st, 1960 to April 8, 1974, under the topic "liverworts" was carried out. A number of different classes of natural products have been isolated from liverworts: for instance; n-alkanes and ester waxes (4,5); alkaloids (6); azulenes and indenes (7,8,9); alcohols and acids (10,11); flavonoids (12-18); steroids (11,19,20); mono-, di-, and triterpenoids (11,21-24); and sesquiterpenoids (1,2,4,10,11,25-41) etc., (see Appendix I). By examination of Appendix I, the following conclu-

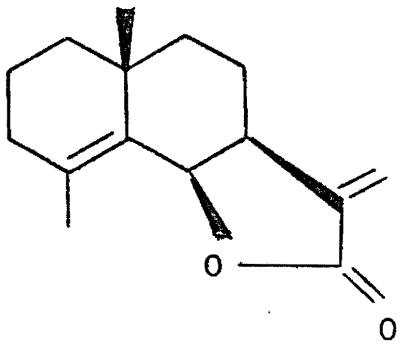
sions were reached:

(1) Flavonoids and steroids which were thought to be absent in lower plants are present in liverworts (11-20).

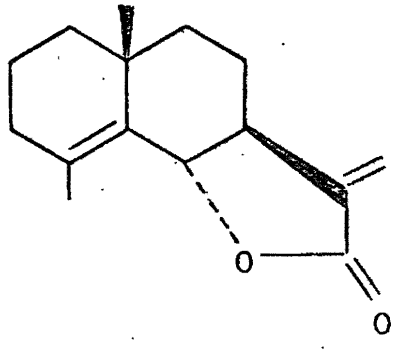
(2) The same compounds could be present in different genera of liverworts: for example, β-sitosterol in Aneurs pinguis (L) Dum., Riccardia sinnata (Hook) Trev., Conociphalum conicum (L) Underw., and Scapania parvitexta (11,19).

(3) Liverworts of the same genus could contain the same compounds, such as: friedelin in both Frullania tarmarisci and F. dilatata (21); isolongifolene in both Scapania subalpina, S. uliginosa, and S. undulata (30); and β-barbatene in both Barbilophozia barbata, B. floerkei, B. lycopodioides, and B. attennata (34).

This research project was undertaken to gain information on the natural products in the liverwort Frullania franciscana Howe. Earlier work indicated that several species of the genus Frullania contain an allergenic α-methylene-γ-lactone sesquiterpenoid, frullanolide (I).

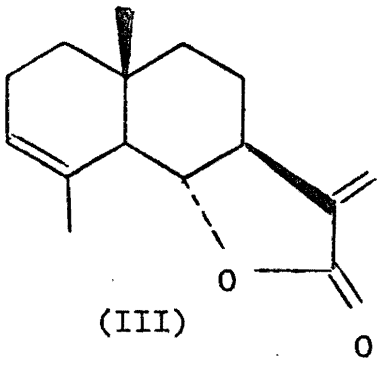


(I)



(II)

Frullanolide was reported in F. nisquallensis Sull. (37)., F. dilitata (L) Dum. (21), and F. tamarisci (L) Dum. (34). F. tamarisci (L) Dum. also yielded the α-methylene-γ-lactone sesquiterpenoids, γ-cyclocostunolide (II), α-cyclocostunolide (III), and costunolide (IV). γ-Cyclocostunolide (II) is also known as arbusculin-B (25,26).



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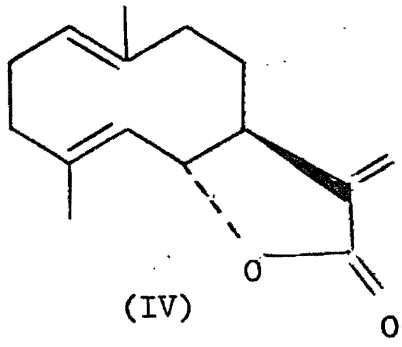

(IV)

Several other species of Frullania yielded extracts which are active on sensitive patients, but frullanolide could not be detected in these extracts (42). This observation suggests the possible existence of allergens besides frullanolide in these plants. Dr. John C. Mitchell, a dermatologist at the University of British Columbia Medical School, has studied the allergenic properties of liverworts extensively. He reports (42),

> Investigation of Frullania nisquallensis Sull. for its allergenic fractions indicates that sesquiterpene lactones are probably the common denominators of allergic contact sensitization by Frullania and by some species of compositae.

On patch testing on sensitive patients, Dr. Mitchell found that Frullania franciscana Howe was as active as the frullanolide-containing F. tamarisci (L) Dum. and F. nisquallensis Sull. but less active than F. tamarisci (L) Dum. (42).

Under a comprehensive program of screening plant products for anti-tumor activity by Kupchan, some sesquiterpene-α-methylene-γ-lactones showed significant in vivo inhibitory activity against P-388 lymphocytic leukemia in the mouse and against Walker carcinosarcoma-256 in the rat (44).

The dermatological activity of Frullania franciscana Howe suggests that the α-methylene-γ-lactones and other compounds in that class might be of considerable interest as possible anti-tumor agents. Thus, we hoped to gain information on the natural products of this liverwort to extend the knowledge on causative agents of dermatitis and possibly to find new anti-tumor agents. For the preliminary stage of the examination of this plant, we compared various methods for the isolation of natural products and estimated how many components might be present in the crude extracts. Finally, the identification of some components was attempted.

DISCUSSION

The general approach to the problem of resolving the mixture of natural products isolated from the liverwort Frullania franciscana Howe was as follows:

(a) The liverwort Frullania franciscana Howe was hand separated from other plants, air dried, and milled.

(b) The sample was then extracted by means of a Soxhlet extractor with ether or by cold extraction with n-hexane and ether; the solvents were evaporated with a rotary evaporator. Crude extracts were obtained.

(c) Column chromatography was used to separate the crude mixture into fractions. This separation was based on gradient elution from silica gel or alumina.

(d) The column fractions were examined by thin layer chromatography, and their infrared spectra were determined. Similar fractions were combined.

(e) Further separations of the fractions were attempted by both column chromatography and preparative thin layer chromatography.

Column I

The preliminary examination of several fractions from silica gel column chromatography of crude extract was carried out using thin layer chromatography and infrared spectroscopy. The data indicated that all of the fractions were mixtures. Although some fractions (for example, number 7) gave quite well defined infrared spectra, thin layer chromatography showed that with more polar solvents the fractions themselves could be fractionated. The infrared spectra were consistent

with the presence of γ -lactones as well as double bonds in the mixtures. This preliminary work demonstrated that the general approach to the separation was reasonable.

Attempted Resolution of Fraction I-18*

Fraction I-18 was the second largest fraction from column I and showed three distinct carbonyl peaks in the infrared. It was re-chromatographed on silica gel into twelve fractions (A,B,C,. . .K). Examination of the fractions that contained reasonable quantities of material showed each fraction to be impure. This technique did not appear to be successful in purifying the components of the mixture. The purer fractions were eventually combined with similar material from column II (see below).

Column II

The infrared spectra of the fractions from column II (a silica gel column, basically following the same solvent system of column I) showed that fraction II-1 was apparently a hydrocarbon. All the rest of the fractions contained carbonyl group(s) with or without double bond(s) and with or without hydroxyl groups. Thin layer chromatography results showed fraction II-3 through fraction II-32 to be impure compounds, and the number of spots varied from two to seven. Fraction

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* Numbering system was done as Column I, Column II, . . . and the fraction I-18, (fraction 18 of Column I), fraction II-44B, (fraction B of fraction 44 of Column II), fraction III-1920, (the combination fraction of fraction 19 and 20 of column III).

II-33 through fraction II-48 could not be resolved effectively by thin layer chromatography with five solvent systems.

Column Chromatography of Fraction II-44

Fraction II-44 was the third largest fraction of column II and showed three distinct carbonyl peaks in its infrared spectrum. It was rechromatographed on silica gel and gave seventeen fractions (A,B,C, . . .Q, and T). Infrared spectra suggested that fractions C,D and E were reasonably "pure". At this time an adequate thin layer chromatographic system was not at hand. Later, however, further thin layer chromatographic experiments demonstrated that fractions C,D, and E were not pure (see below).

Fraction II-44B was the largest fraction among all the fractions obtained by rechromatography of fraction II-44. It had three distinct carbonyl peaks in the infrared and, thus, was probably not pure.

Further Treatment of Fraction II-44B

Separation of fraction II-44B was attempted thrice, but the data from infrared and thin layer chromatography indicated that the attempted separations were not successful.

Attempted Separation of Fractions I-18D and II-44C, D,E, and F

The fractions I-18D and II-44C, D, E and F had similar infrared spectra and thin layer chromatography characteristics, and hence were combined. The combination of fraction I-18D with II-44C, D, E, and F showed at least five components, based on the thin layer chromatography data. No further separation of this combination was attempted, the reason being the unsuccessful separation of the similar fraction III-

2131* on 2 mm precoated TLC silica gel F-254 (EM) (see below).

Column III*

Column III was a silica gel column chromatograph attempted with the same crude extract as used previously. We obtained from Mr. Brad A. Halverson green fractions believed to be similar to our green fractions from column II. Mr. Halverson's fractions were fractions III-19 through III-31. Thin layer chromatography indicated that these fractions contained from four to twelve components.

Both the infrared data and thin layer chromatography results suggested the similarity of fractions III-19 and III-20, and III-21 through III-31, so they were combined, respectively, and numbered as fraction III-1920, and fraction III-2131. Separation of these two combination fractions was attempted by thin layer chromatography. The conditions, which were used satisfactorily for the thin layer chromatography of the single fractions, did not give satisfactory results in the case of the separation of combined fraction III-1920 and did not cause any separation at all in the case of combined fraction III-2131.

Second Extractions and Separations

In order to study the occurrence of sesquiterpenes and α-methylene-γ-lactones in liverwort Frullania franciscana Howe, the extrac-

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* Column III in this paper was same as column V of Mr. Brad A. Halverson.

tion method and column chromatography were modified. By use of a non-polar solvent, the less polar components in the liverwort F. franciscana Howe were extracted; then by use of a polar solvent, ether, the more polar components hopefully were removed by extraction.

The air-dried, milled sample was extracted by n-hexane at room temperature, and a crude extract of 1.15% by weight from the original sample was obtained. The extracted (by n-hexane) residue was then re-extracted with ether at room temperature, and a crude extract of 1.25% by weight from the original sample was obtained. The over all yield was 2.4% by weight. Two-dimensional thin layer chromatography was then carried out for both extracts on silica gel plates and, after concentrated sulfuric acid development, the number of the spots was counted (n-hexane extract: fourteen spots, ether extract: twenty spots). The Rf' values were also calculated and compared for each plate. It was found that all spots in the n-hexane extract appeared in the ether extract, the ether extract showing an additional six spots. One of these six spots was deep green before and gray-brown after the concentrated sulfuric acid development, in which the thin layer chromatography plate was kept at 120°C for 90 seconds. It was thought that the presence of this colored material was the main reason for the color difference between the two extracts. The color of n-hexane extract was green, and ether extract was dark green. These six spots were thought to be polar substances, and it was quite obvious that the ether was better for the extraction than the n-hexane at room temperature.

Column IV

The ether extract described above was column chromatographed on an aluminum oxide (activity I) column, and ten fractions were collected. The percentage recovery was 66%. Two-dimensional thin layer chromatograms were taken for fractions IV-1 (one spot), IV-7 (two spots), and IV-8 (two spots). An infrared spectrum was taken for each fraction except fraction IV-2, and fraction IV-1 was shown to apparently be a hydrocarbon. The other fractions showed carbonyl group(s). Fraction IV-8 showed a γ-lactone, double bond, and hydroxyl group.

Fraction IV-1

Gas chromatograms for fraction IV-1 were then obtained (see below). Nuclear magnetic resonance and infrared spectroscopy of fraction IV-1 suggested that it was a hydrocarbon, with the following data: 3080 cm^{-1} ($\rangle C{=}CH$), 1643 cm^{-1} (C=C), 888cm^{-1} ($\begin{matrix} R \\ R \end{matrix}\rangle C{=}CH_2$, or exo $\rangle C{=}CH_2$); nuclear magnetic resonance: δ 4.73 ppm, (exo double bond with the ring numbered six or larger, $\rangle C{=}CH_2$). Because of the small amount of sample and since a good nuclear magnetic resonance spectrum was not available, the rest of the nuclear magnetic resonance data could not be used for the study of the structure of the main component of fraction IV-1. The mass spectrum (gas

chromatography-mass spectrometer connection) showed that the molecular weight of the main component was 204, meaning that the number of double bond(s) plus the number of ring(s) was equal to four. Based on this information alone, the structure of this unknown could not be determined, and further experiments such as silver nitrate/silica gel thin layer chromatography to verify the purity and time averaging nuclear magnetic resonance spectrum would be recommended. The reduction of the double bond(s) of the compound would give the saturated structural skeleton, and the infrared spectra and other spectra of the saturated products would help in the final structure determination. For the identification of the rest of the components in fraction IV-1, a gas chromatography-mass spectrometer analysis would be suggested.

Gas Chromatography of Fraction IV-1

Temperature programmed gas chromatograms (SE-30 and Apiezon-L) showed eleven peaks in fraction IV-1, and isothermal gas chromatograms showed that peak number 3 was the largest peak, with 97% of the total area. Since flame ionization detectors were used, the area under the peak was assumed to be proportional to the amount of the component, and the calculations were based on the area under the peaks.

The Kovats' indices of sesquiterpenes could then be used to identify the sesquiterpene hydrocarbons. Niels H. Andersen and Mark S. Falcone state (43):

> At present, the less numerous, and more thoroughly studied, mono-terpene hydrocarbons can be identified unambiguously by GLC on one or two column. The retention data from such studies have generally been given in Kovats' indices or as retention times relative

> to a standard terpene. We felt that the more numerous sesquiterpenes could be identified in a similar manner if retention data were obtained on a sufficient number of distinct selective phases.

They also point out that in all cases, the Kovats' indices increase with increasing temperature and the temperature dependence variation in Kovats' indices within the sesquiterpenes is small ($\Delta I / \Delta T = 1.1 \pm 0.25$ for the entire group) and thus Kovats' indices can be reproduced to $\pm$ 1 unit by using sesquiterpenes as standards rather than using n-alkanes, as is usually done. These authors had fifty-five sesquiterpene hydrocarbons listed with their Kovats' indices on different columns at different temperatures. Examination of the data presented in their table quickly showed that all of these sesquiterpenes can be distinguished by the use of only two to three different phases and that the identify of these substances could be confirmed by co-injection with two identified sesquiterpenes appearing in their table.

The Kovats' indices were taken for the main component (peak number 3) of fraction IV-1 by using β-bourbonene and α-cedrene as two standards. β-Caryophyllene was used to check the two standards. The Kovats' indices of the main component of fraction IV-1 were $I^{155°}_{AP-L}$ 1530, $I^{130°}_{SE-30}$ 1480.7, $I^{132°}_{Carbw-20M}$ 1732.4, $I^{165°}_{Carbw-20M}$ 1752.6, $I^{205°}_{Carbw-20M}$ 1841.9, and $I^{160°}_{DEGS}$ 1976.7. These indices did not match any of the compounds listed in Andersen's table.

Fraction IV-8

The infrared spectrum of fraction IV-8 suggested that this fraction was reasonably pure. Fraction IV-8 was rechromatographed on a

silica gel plate. A band centered at Rf' = 25.7 was extracted with ether at room temperature and showed an infrared spectrum identical to that of the frullanolide recrystallized by Mr. Brad A. Halverson from the fractions of the previous columns. Recrystallization of this pure component was not done because of the small quantity. Thin layer chromatography of the component showed Rf' = 25.8, which was very close to Mr. Halverson's result of 25.7. The component showed the same blue color after development with concentrated sulfuric acid, in which the thin layer chromatography plate was kept at 120°C for 90 seconds.

Silica Gel Vs. Aluminum Oxide for Column Chromatography

Aluminum oxide (activity I) is a more strongly polar column chromatography material than silica gel. Hence, the aluminum oxide might be expected to retain the more polar components of the extract from the liverwort Frullania franciscana Howe. In agreement with this expectation, the recovery of material from the aluminum oxide column was less than that from the silica gel column even after elution with ethyl alcohol and ethyl ether. For example, column II (silica gel) gave 99.8% recovery, whereas column IV (aluminum oxide column) gave 66%.

Also, aluminum oxide should hold the more polar components strongly and permit elution only of the less polar components, resulting in purer fractions. Indeed, fraction IV-1 was about 97% pure sesquiterpene hydrocarbon, and fraction IV-8 was reasonably pure frullanolide. In contrast, the first fraction from the silica gel column contained several components, for example, fraction I-1A had eight components.

The polar components of the extract could not be eluted from the aluminum oxide column, and this was the disadvantage of that type of column. The polar components can be eluted from the silica gel column, but there is less separation of the components using this column. Therefore, liquid-liquid column or dry column chromatography on silica gel or deactivated alumina would be suggested for future experiments.

Attempts to Detect Alkaloids in Frullania franciscana

About 2-4 grams of both fresh dried, milled sample and extracted sample residue were examined by Mayers' reagent to test for the presence of alkaloids. Negative results suggested that no alkaloids were present in the liverwort Frullania franciscana Howe.

Summary and Suggestions for Future Investigations

It is obvious that the liverwort Frullania franciscana Howe contains frullanolide and an undetermined sesquiterpenoid hydrocarbon which could be separated by the methods described in this paper. For future researchers, the following based on the experience of previous experiments would be suggested:

(1) About 300 grams of dried, milled sample must be used for the extraction.

(2) The extraction could be done with ethyl ether at room temperature to obtain the crude extract.

(3) The crude extract should then be chromatographed by liquid-liquid or by dry column on silica gel or deactivated alumina.

(4) The hydrocarbon fraction(s) should then be separated by silver nitrate/silica gel thin layer chromatography or by preparative gas chromatography. The purified components should then be examined by infrared, nuclear magnetic resonance and mass spectroscopy, hopefully making possible the determination of the structures of the

compounds. The Kovats' indices should also be obtained for the identification of the sesquiterpene hydrocarbons.

(5) The remaining fractions should be examined mainly by infrared spectroscopy and thin layer chromatography. Further purification could be carried out with preparative thin layer chromatography or small column chromatography. Similar fractions should be combined before any separation is carried out. The purified components should then be examined by infrared, nuclear magnetic resonance and mass spectroscopy and their physical properties determined. The structure of the components would hopefully be elucidated.

EXPERIMENTAL SECTION

The Frullania franciscana Howe used in the following experiments (column I, II and III) was collected by Mr. Michael Clement in Lane County, Oregon, in September, 1972. The identity was confirmed by comparison with a herbarium specimen, through the courtesy of Professor Kenton L. Chambers, Curator of the Department of Botany and Plant Pathology at Oregon State University.

An ether extract* of the milled plant material had been chromatographed on a silica gel column (1.22 g crude/15 g silica gel) by Dr. Levinson (column I). The preliminary phase of this work consisted of the examination of several of these fractions. The results are summarized in Table I.

All solvents were reagent grade and were distilled before use. The ratios given for the mixed solvent systems used in this work were by volume.

These fractions were subjected to thin layer chromatography with the following solvent systems using Bakerflex IB-F Silica Gel. Rf' equals to Rf x 100. (Refer to Table II.)

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* The extraction was done by Dr. Levinson.

TABLE I

COLUMN CHROMATOGRAPHIC DATA
FOR SEVERAL FRACTIONS
OF COLUMN I**

Fr. No.	Weight (g)	Solvent for Elution
1A***	0.20	Benzene
3	0.05	Benzene
5	0.05	Benzene
7	0.05	4% Chloroform in Benzene
9	0.02	12% Chloroform in Benzene
11	0.01	20% Chloroform in Benzene
13	0.01	20% Chloroform in Benzene

** Only several fractions were chosen from column I.

*** Ether soluble part of fraction I-1.

TABLE II

THIN LAYER CHROMATOGRAPHY RESULTS
OF SEVERAL FRACTIONS
OF COLUMN I

Fr. No.	No. of TLC Dimension	Solvent Used	No. of Spots	Rf'
1A	1	Benzene	3	67/22.4/13.2
	1	Benzene/Et Ac* 1/1	2	66.4/57.6
	1	Benzene/Acetone 1/1	Polarity of solvent was too high to give a good separation.	
	2	I. Cyclohexane/Et Ac 80/20 II. Benzene/Ethyl Ether 60/40	8	92.2,90.5/81.3, 85.7/59.4,80.3/ 50.7,73.2/40.6, 54.4/15.6,26.8/ 10.9,15.7/4.7, 7.9
	2	I. $CHCl_3$/Ethyl Ether 80/20 II. Benzene/Et Ac 50/50	7	87,75.8/75,78.3/ 66,74.1/62,54/ 29,48.3/16.9, 36.7/7.1,20.8
3	1	Carbontetrachloride	--	Did not move at all
5	1	Carbontetrachloride	--	
7	1	Carbontetrachloride	--	

* Et Ac = ethyl Acetate

Fr. No.	No. of TLC Dimension	Solvent Used	No. of Spots	Rf'
3	1	Toluene	--	Did not move at all
5	1	Toluene	--	
7	1	Toluene	1	7.09
9	1	Toluene	1	7.09
11	1	Toluene	1	7.09
13	1	Toluene	1	4.87
9	1	Benzene	1	7.4
11	1	Benzene	1	7.6
13	1	Benzene	1	4.8
9	1	Benzene/Ethyl Ether 6/4	1	63.2**
11	1	Benzene/Ethyl Ether 6/4	2	66.6/46.8**
13	1	Benzene/Ethyl Ether 6/4	3	82.7/72.7/46.3
3	2	I. Cyclohexane/Et Ac 80/20 II. Benzene/Ethyl Ether 60/40	1	86.1,94.2
3	2	I. Benzene II. Cyclohexane/Et Ac 80/20	3	89.8,95.3/79, 30.4/64,90.4
5	2	I. Cyclohexane/EtAc 80/20	2	88,91.7/58.6,80

** Considerable tailing noted.

Fr. No.	No. of TLC Dimensions	Solvent Used	No. of Spots	Rf'
		II. Benzene/Ethyl Ether 60/40		
5	2	I. Benzene	1	79.3,92.8
		II. Cyclohexane/Et Ac 80/20		
7	2	I. Cyclohexane/Et Ac 80/20	5	78.2,84.1/83, 78.8/71,81.8/
		II. Benzene/Ethyl Ether 60/40		75,87.4/29.1, 65.2

Infrared Analysis

All the spectra were taken between salt plates on a Perkin-Elmer Model 467 (grating) spectrophotometer. Crude Extract: 3500 cm^{-1} b, OH; 3075 cm^{-1} w, 1660 cm^{-1}, 1640 cm^{-1} double bond; 1700-1770 cm^{-1} carbonyl.

Fraction 3: 3000 cm^{-1}, 1640 cm^{-1} double bond; 1740 cm^{-1} carbonyl; 1705 cm^{-1} w, carbonyl

Fraction 5: 3000 cm^{-1}, 1660 cm^{-1}, 1640 cm^{-1} double bond; 1760 cm^{-1} carbonyl;

Fraction 7: 3500 cm^{-1} OH(?); 3090 cm^{-1}, 1660 cm^{-1} double bond; 1775 cm^{-1} carbonyl;

Fraction 9: 1755 cm^{-1} carbonyl; 1660 cm^{-1} double bond;

Fraction 11: 3300-3600 cm^{-1} b, OH; 1760 cm^{-1} carbonyl ; 1660 $^{-1}$ double bond;

Fraction 13: poorly resolved spectrum.

b: broad, w: weak

The largest fraction (excepting number one) from column I was fraction 18. This was eluted with 50% chloroform/benzene. Material resulting was dark colored and had a mass of 119 mg. The infrared spectrum showed a double bond (3000 cm^{-1} and 1610 cm^{-1}) and three carbonyl peaks: 1770 cm^{-1} w, 1735 cm^{-1} s, 1700 cm^{-1} m (where w: weak, s: strong, and m: medium intensity). Attempted thin layer chromatography on silica gel IB-F of fraction I-18 with chloroform/ether 80/20 and cyclohexane/ethyl acetate 50/50, did not give good resolution.

Column Chromatography of Fraction I-18

About 100 mg of fraction I-18 was put on the top of a 1 cm OD column packed with 5 g of silica gel Woelm 0.05-0.2 mm in benzene, and

then eluted using the conditions shown in Table III and Figure 1.

Analysis of Fractions from Above Column

Attempted thin layer chromatography on Bakerflex IB-F silica gel and aluminum oxide plates gave little in the way of useful results. The results are summarized in Table IV, and IR spectrum in Appendix II.

Column II

The column was packed with 30 g of 0.05--0.2 mm silica gel (Woelm) in benzene, column diameter OD 2 cm, and 2.1043 g of the ether extract (same material as was used in column I) was placed in the column, and developed as shown in Table V and Figure 2.

Thin Layer Chromatography Analysis of the Fractions of Column II

Several fractions were subjected to thin layer chromatography on silica gel Bakerflex IB-F, as shown in Table VII-A and Table VII-B. Also see the reproduction of chromatogram, Figure 3.

Fraction II-33 through fraction II-48 were analyzed with silica gel and the following solvent systems: (a) benzene/ethyl acetate 50/50, (b) benzene/ether 60/40, (c) chloroform/ether 60/40, (d) benzene/acetone 50/50, and (e) acetone, but no good separations were obtained.

Fraction II-44 was the third largest fraction. It was dark colored and had a mass of 205.7 mg. A small column was used to resolve fraction II-44, OD 1 cm, 3 g of 0.050-0.2 mm silica gel (Woelm) in benzene, and gradient elution was performed as shown in Table VIII and Figure 4.

TABLE III

COLUMN CHROMATOGRAPHIC DATA
OF FRACTION I-18

Fr. No.	Solvent Used	Volume (ml)	Weight (mg)	Infrared (cm^{-1})
A	Benzene	34	4.9	1738--carbonyl
B	Benzene/Et Ac 9/1	25	18.4	1710 & 1738--carbonyl
C	Benzene/Et Ac 9/1	75	16.9	3300-3600 b,OH 1710,1738, & 1770--carbonyl
D	Benzene/Et Ac 8/2	50	34.8	3300-3600 b,OH 1700-1780 s,b --carbonyl
E	Benzene/Et Ac 8/2	50	5.5	poor spectrum
F	Benzene/Et Ac 3/2	100	1.2?	no spectrum taken
G	Benzene/Et Ac 1/9	50	5.2	3300-3600 b, OH 1700-1780 s,b--carbonyl
H	Isopropyl Ether	50	3.2	no spectrum taken
I	CH_2Cl_2	50	1.1	no spectrum taken
J	$CHCl=CCl_2$	50	1.1	no spectrum taken
K	Ethyl Alcohol	50	28.1	3300-3600 b, OH 1700-1780 s,b--carbonyl
L	Dioxane	80	---?	no spectrum taken

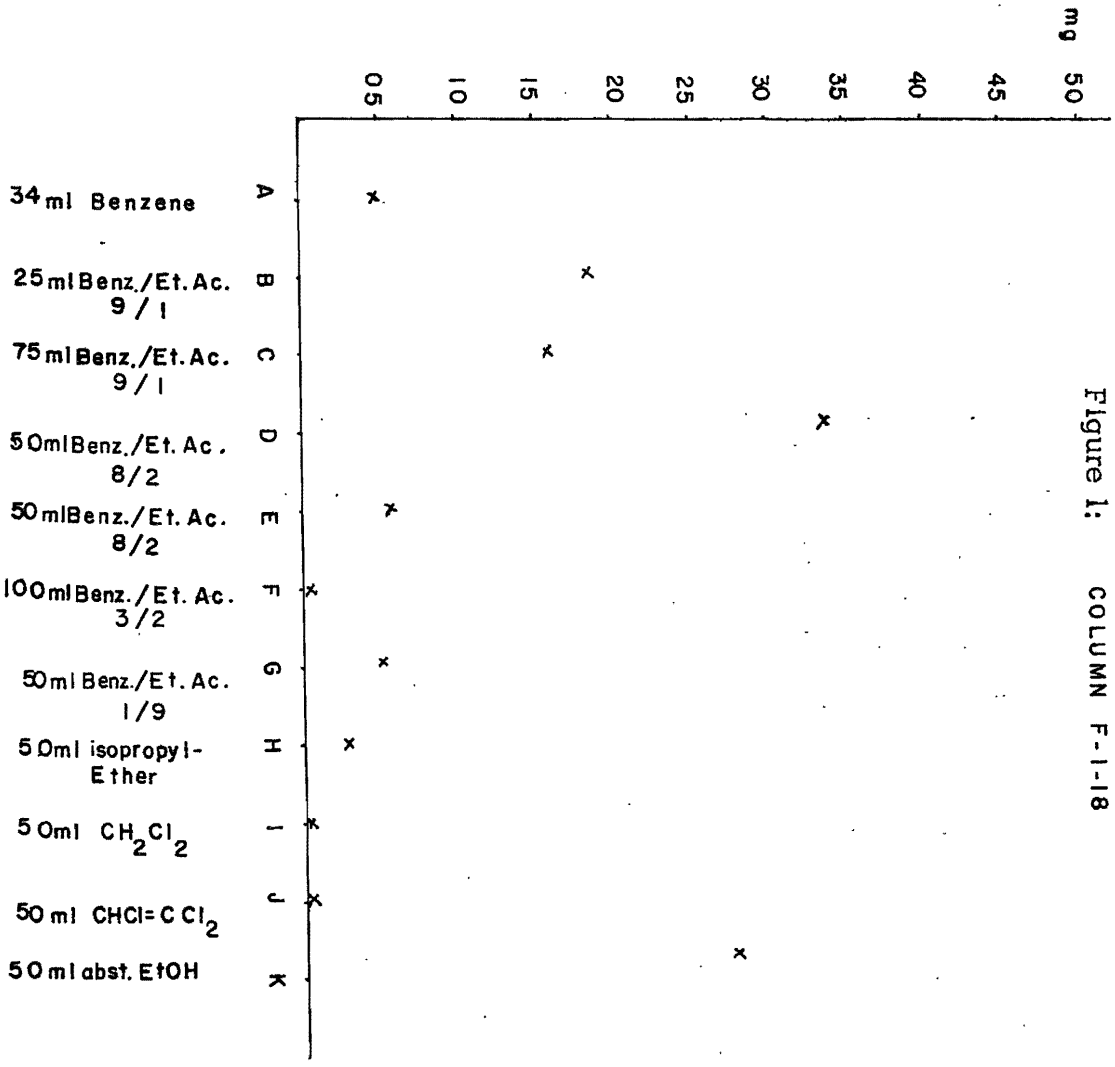


Figure 1: COLUMN F-1-18

TABLE IV

THIN LAYER CHROMATOGRAPHY RESULTS
OF THE FRACTIONS OF
COLUMN F-I-18

Fr. No.	Solvent Used	TLC Plate	Rf' Value
B	Isopropyl Ether	Silica Gel	60.3/49.6
C	Chloroform 80/20	Silica Gel	49.6
B	Chloroform 80/20	Aluminum Oxide	30.6*
C	Chloroform 80/20	Aluminum Oxide	30.6
G	Chloroform 80/20	Aluminum Oxide	88.4
B	Acetone/$CHCl_3$ 3/7	Silica Gel	74.8
C	Acetone/$CHCl_3$ 3/7	Silica Gel	74.8
A	Acetone/$CHCl_3$ 3/7	Aluminum Oxide	94.8/88.9
B	Acetone/$CHCl_3$ 3/7	Aluminum Oxide	94.8/88.9
C	Acetone/$CHCl_3$ 3/7	Aluminum Oxide	88.9
D	Acetone/$CHCl_3$ 3/7	Aluminum Oxide	88.9/52.6*
E	Acetone/$CHCl_3$ 3/7	Aluminum Oxide	95.6
G	Acetone/$CHCl_3$ 3/7	Aluminum Oxide	88.2
B	Benzene/Ethyl Ether 10/90	Silica Gel	82.3
C	Benzene/Ethyl Ether 10/90	Silica Gel	75.2
D	Benzene/Ethyl Ether 10/90	Silica Gel	70.8/32.7
A	Butyl Acetate	Silica Gel	87
B	Butyl Acetate	Silica Gel	75.6
C	Butyl Acetate	Silica Gel	69.9
D	Butyl Acetate	Silica Gel	69.9/27.6**

* Strong haloing and tailing observed.

** May have higher value due to the difficulty of fixing the real position of spot.

TABLE V

COLUMN CHROMATOGRAPHIC DATA
OF COLUMN II

Fr. No.	Solvent Used	Volumn (ml)	Weight (mg)	Remarks
1	Benzene	50	19.2	
2	Benzene	50	207.0	
3	Benzene	50	48.0	
4	Benzene	50	42.4	
5	Benzene	100	65.2	
6	Benzene	100	90.6	
7	2% $CHCl_3$/Benzene	50	24.1	
8	2% $CHCl_3$/Benzene	50	24.1	
9	4% $CHCl_3$/Benzene	50	23.5	
10	4% $CHCl_3$/Benzene	50	21.6	
11	8% $CHCl_3$/Benzene	50	29.2	
12	8% $CHCl_3$/Benzene	50	19.8	
13	12% $CHCl_3$/Benzene	50	13.6	
14	12% $CHCl_3$/Benzene	50	9.0	
15	16% $CHCl_3$/Benzene	50	17.9	
16	16% $CHCl_3$/Benzene	50	15.3	
17	20% $CHCl_3$/Benzene	50	15.2	
18	20% $CHCl_3$/Benzene	50	15.5	
19	20% $CHCl_3$/Benzene	100	29.0	
20	20% $CHCl_3$/Benzene	100	25.3	
21	30% $CHCl_3$/Benzene	50	8.7	
22	30% $CHCl_3$/Benzene	50	9.3	
23	40% $CHCl_3$/Benzene	50	6.9	
24	40% $CHCl_3$/Benzene	50	7.4	
25	50% $CHCl_3$/Benzene	50	6.9	
26	50% $CHCl_3$/Benzene	50	7.0	

Fr. No.	Solvent Used	Volumn (ml)	Weight (mg)	Remarks
27	50% $CHCl_3$/Benzene	50	5.5	
28	50% $CHCl_3$/Benzene	50	5.2	
29	50% $CHCl_3$/Benzene	50	6.8	
30	50% $CHCl_3$/Benzene	50	5.2	
31	50% $CHCl_3$/Benzene	100	10.5	
32	50% $CHCl_3$/Benzene	200	18.6	
33	60% $CHCl_3$/Benzene	100	17.3	green
34	60% $CHCl_3$/Benzene	50	15.0	green
35	60% $CHCl_3$/Benzene	50	19.5	green
36	60% $CHCl_3$/Benzene	100	39.4	green
37	60% $CHCl_3$/Benzene	100	40.5	green
38	70% $CHCl_3$/Benzene	50	16.6	green
39	70% $CHCl_3$/Benzene	100	19.3	green
40	70% $CHCl_3$/Benzene	100	15.3	green
41	$CHCl_3$	50	6.5	green
42	$CHCl_3$	100	20.8	green
43	$CHCl_3$	100	54.2	green
44	$CHCl_3$	100	205.7	Second largest green fraction
45	$CHCl_3$	100	85.2	green
46	2% Ether/$CHCl_3$	100	41.0	green
47	2% Ether/$CHCl_3$	100	37.6	green
48	10% Ether/$CHCl_3$	100	64.2	green
49	10% Ether/$CHCl_3$	50	27.0	green
50	10% Ether/$CHCl_3$	50	12.1	green
51	20% Ether/$CHCl_3$	50	8.0	green
52	20% Ether/$CHCl_3$	50	22.3	green
53	20% Ether/$CHCl_3$	100	42.0	green
54	30% Ether/$CHCl_3$	100	23.4	green
55	30% Ether/$CHCl_3$	100	21.6	green
56	30% Ether/$CHCl_3$	100	9.6	green
57	40% Ether/$CHCl_3$	100	11.4	green

Fr. No.	Solvent Used	Volumn (ml)	Weight (mg)	Remarks
58	60% Ether/$CHCl_3$	100	16.5	green
59	80% Ether/$CHCl_3$	100	14.3	green
60	Ether	100	8.0	green
61	Ether	100	1.3	green
62	50% $CHCl_3$/MeOH	50	---	Fire destroyed part of sample
63	50% $CHCl_3$/MeOH	50	303.1	Dark colored
64	50% $CHCl_3$/MeOH	50	10.0	Dark colored
65	50% $CHCl_3$/MeOH	50	4.8	Dark colored
66	50% $CHCl_3$/MeOH	50	3.5	Dark colored
67	50% $CHCl_3$/MeOH	50	5.2	Dark colored
68	50% $CHCl_3$/MeOH	50	6.6	Dark colored
69	50% $CHCl_3$/MeOH	50	5.0	Dark colored
		Total	2,100.7	99.8% recovery

Figure 2 : COLUMN II

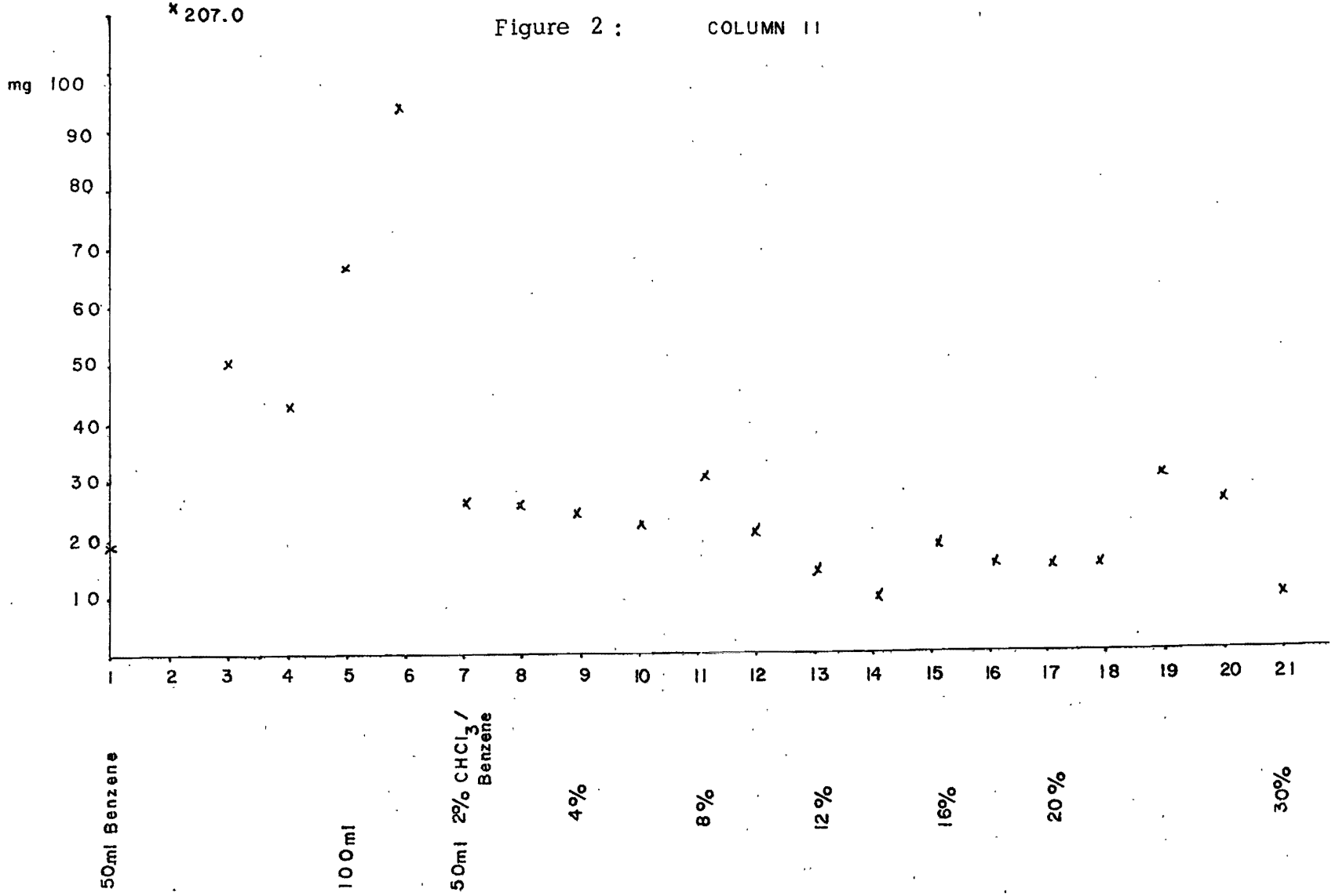
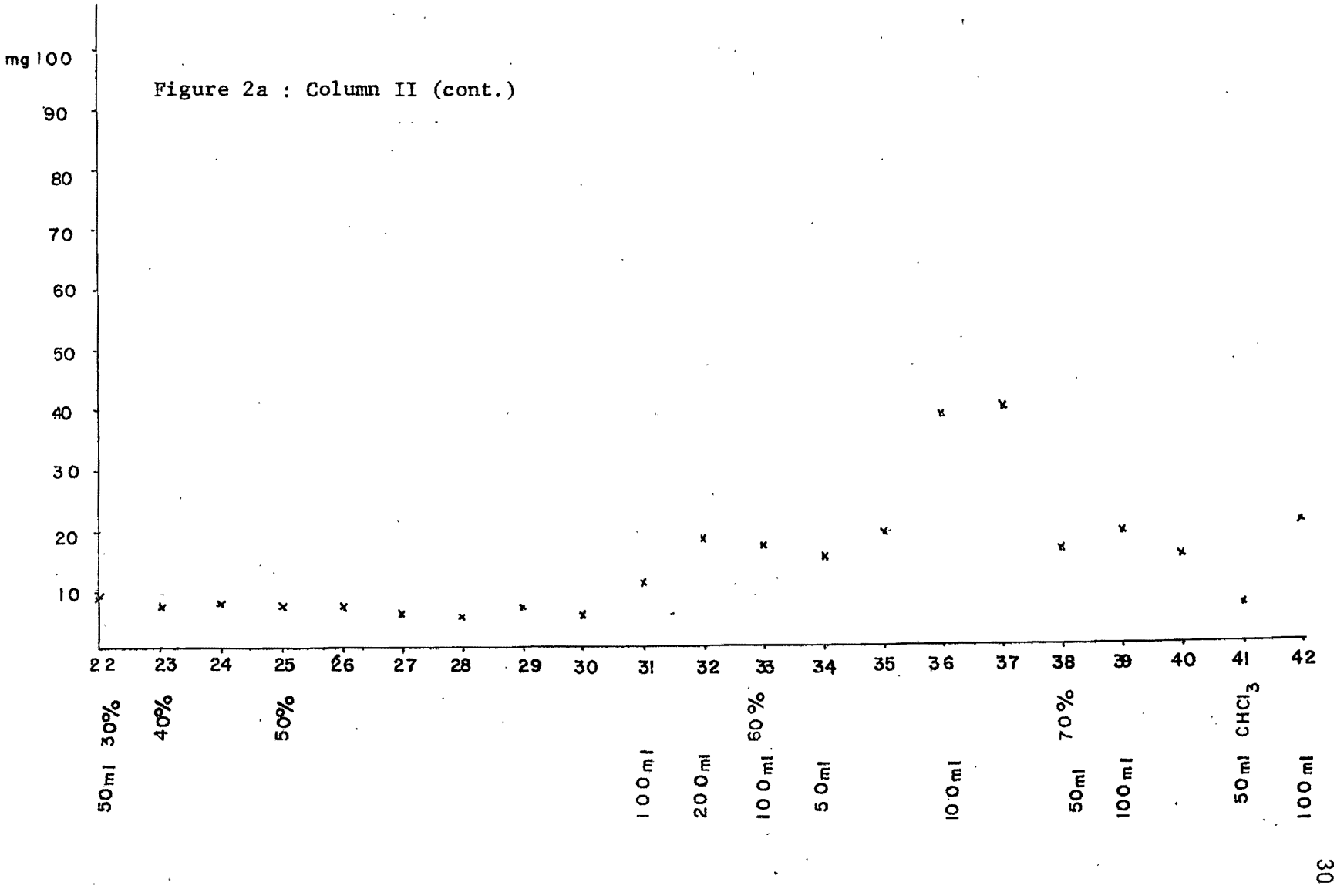


Figure 2a : Column II (cont.)



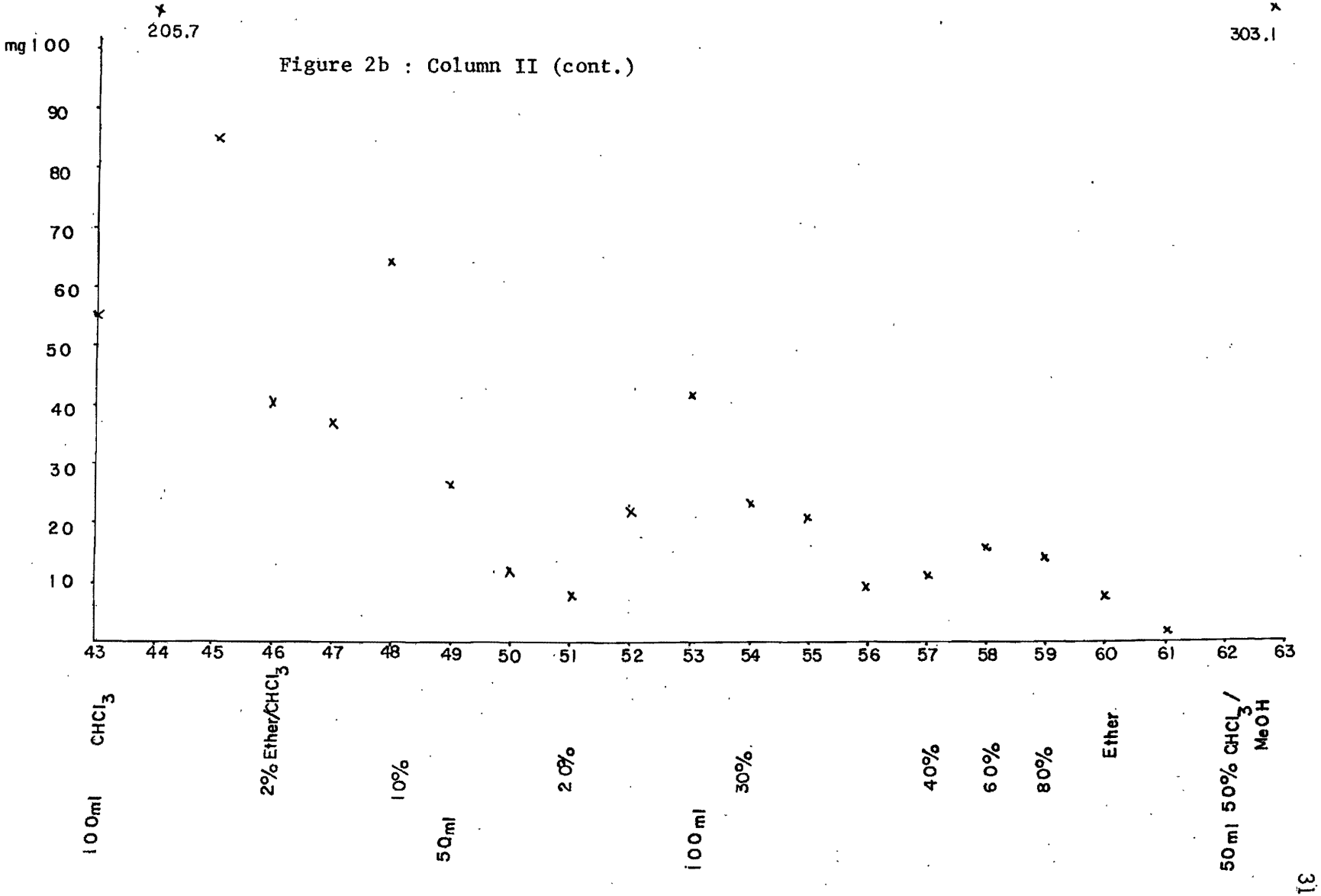


Figure 2b : Column II (cont.)

Figure 2c: Column II (cont.)

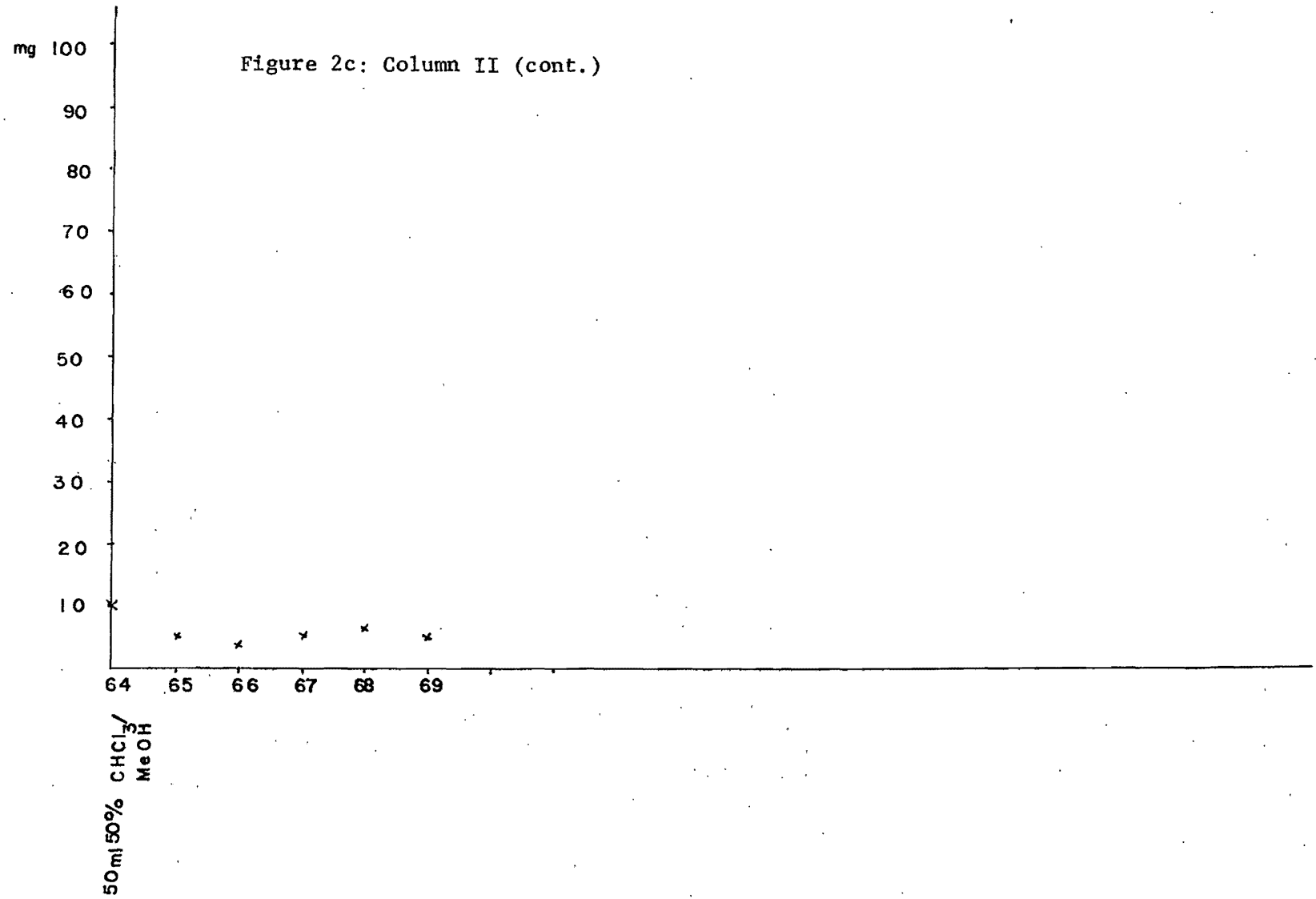


TABLE VI

INFRARED ANALYSIS OF THE FRACTIONS OF COLUMN II

Fr. No.	Wave Number (cm^{-1})	Remarks
1	3080,1640,890	Double bond
2	3080 m, 1640 s, 890s	Double bond
	1730 s, 1770 w	Carbonyl
3	3080 w, 1640 w, 880 m	Double bond
	1740 s, 1700 w	Carbonyl
4	3080 w, 1640 w	Double bond
	1740 s, 1705 m	Carbonyl
5	3075 w, 1640 w, 3000 m	Double bond
	1770 sd, 1742 s, 1705 m	Carbonyl
6	3000 m, 1640 w	Double bond
	1768 s, 1740 s, 1700 m	Carbonyl
7	3000 sd, 1660 w	Double bond
	1770 s, 1740 s	Carbonyl
8	3000 sd, 1660 w	Double bond
	1770 s, 1740 s	Carbonyl
9	3300-3600 b	OH
	3000 m, 1660 m, 1640 sd	Double bond
	1770 s, 1740 s	Carbonyl
10	3300-3600 b	OH
	3000 m, 1660 m, 1640 sd	Double bond
	1770 s, 1740 s	Carbonyl
11	3300-3600 b	OH
	3000 sd, 1660 w, 1640 sd	Double bond
	1770 s, 1740 sd	Carbonyl
12	3300-3600 b,w	OH
	1660 w, 1640 sd	Double bond
	1770 s	Carbonyl

Fr. No	Wave Number (cm^{-1})	Remarks
13	3300-3600 b,w	OH
	3080 w, 1660 w, 1640 sd	Double bond
	1770 s	Carbonyl
14	3300-3600 b,w	OH
	1660 w, 1640 sd	Double bond
	1760 s	Carbonyl
15	1660 w, 1640 sd	Double bond
	1756 s	Carbonyl
16	16600 m, 1640 sd	Double bond
	1763 s	Carbonyl
17	3300-3600 b,w	OH
	1664 m, 1640 sd	Double bond
	1760 s	Carbonyl
18	1665 m	Double bond
	1760 s	Carbonyl
19	3300-3600 b,w	OH
	1660 m	Double bond
	1760 s	Carbonyl
20	3300-3600 b,w	OH
	1660 m	Double bond
	1760 s	Carbonyl
21	1660 w	Double bond
	1760 s	Carbonyl
22	3300-3600 b,w	OH
	1660 w	Double bond
	1760 s	Carbonyl
23	1660 w	Double bond
	1760 s	Carbonyl
24	1660 w	Double bond
	1765 s	Carbonyl
25	3300-3600 b,w	OH
	1660 m	Double bond
	1760 s	Carbonyl

Fr. No.	Wave Number (cm^{-1})	Remarks
26	1765 s	Carbonyl
	poor spectrum	
27	3300-3600 b,w	OH
	1760 s, 1700 w	Carbonyl
28	poor spectrum	
29	poor spectrum	
30	poor spectrum	
31	3300-3600 b,m	OH
	1770-1700 s,b	Carbonyl
32	3300-3600 b,w	OH
	1775-1700 s,b	Carbonyl
33	3300-3600 b,m	OH
	1775-1700 b,m	Carbonyl
34	poor spectrum	
35	3300-3600 b,m	OH
	1770-1600 b,s	Carbonyl
36	3300-3600 b,m	OH
	1700-1775 s,b	Carbonyl
37	3300-3600 b,m	OH
	3050 m, 1660 m	Double bond
	1700-1775 b,s	Carbonyl
39	poor spectrum	
40	poor spectrum	
41	poor spectrum	
42	3300-3600 b,s	OH
	1770-1700 s,b	Carbonyl
43	3300-3600 b,m	OH
	1775-1700 b,s	Carbonyl
44	3300-3600 b,m	OH
	1775-1700 b,s	Carbonyl
45	3300-3600 b,m	OH
	1775 s, 1770 m	Carbonyl
	3080 w, 1640	Double bond

Fr. No.	Wave Number (cm^{-1})	Remarks
46	3300-3600 b,m	OH
	1770 s, 1700 sd	Carbonyl
47	3300-3600 b,s	OH
	1700-1775 b,s	Carbonyl
48	3300-3600 b,s	OH
	1700-1775 b,s	Carbonyl
49	3300-3600 b,m	OH
	1700-1775 b,s	Carbonyl
50	poor spectrum	
51	3300-3600 b,s	OH
	3075 w, 1640 sd	Double bond
	1700-1775 b,s	Carbonyl
52	3300-3600 b,s	OH
	1700-1775 b,s	Carbonyl
53	3300-3600 b,m	OH
	1700-1778 b,s	Carbonyl
54	poor spectrum	
55	poor spectrum	
56	3300-3600 b,s	OH
	1700-1780 b,s	Carbonyl
57	3300-3600 b,m	OH
	1775-1770 b,s	Carbonyl
58	3300-3600 b,m	OH
	1700-1775 b,s	Carbonyl
59	3300-3600 b,s	OH
	1700-1775 b,s	Carbonyl
60	poor spectrum	
61	3300-3600 b,s	OH
	1700-1775 b,s	Carbonyl
62	poor spectrum	
63	3300-3600 b,s	OH
	1700-1750 b,s	Carbonyl
64	poor spectrum	

Fr. No.	Wave Number (cm^{-1})	Remarks
65	poor spectrum	
66	poor spectrum	
67	poor spectrum	
68	poor spectrum	
69	poor spectrum	

s: strong, m: medium, w: weak, b: broad, sd: shoulder

TABLE VII-A

THIN LAYER CHROMATOGRAPHY RESULTS OF FRACTION II-1
THROUGH FRACTION II-26

Solvent used: Cyclohexane/Ethyl Acetate 80/20

Development: Iodine vapor

Fr. No.	Rf'					
1	62.1					
2	56.9					
3	51.8	63.5				
4	50.9	58.8				
5	43.9	52.7				
6	23.5	32.8	41.2	58.8		
7	23.2	32.2	41.1	63.4		
8	24.8	33.1	---*	65.2		
9	23.6	31.8	---*	63.6		
10	3.5	27.2	35.9	42.1	47.4	71.9
11	3.5	30.4	40.0	45.2	50.4	71.3
12	4.4	29.8	38.6	44.7	50.9	70.2
13	26.9	34.3	---*	63.9		
14	23.5	30.4	---*	59.1		
15	3.4	19.7	29.9	39.3	46.2	51.3
	70.9					
16	3.4	20.5	28.2	37.6	45.3	50.4
	70.9					
17	2.6	19.8	28.4	37.9	45.7	50.0
18	3.4	18.5	26.1	34.5	42.9	
19	3.5	21.9	29.8	39.5	50.9	
20	3.5	20.9	28.4	38.8		
21	2.6	20.7	28.4	38.8		
22	2.5	21.0	26.9	36.1		
23	2.5	20.8	28.3	37.5		
24	3.4	21.4	28.2	39.3		
25	2.6	21.9	28.9	39.5		
26	3.4	21.0	27.7	38.7		

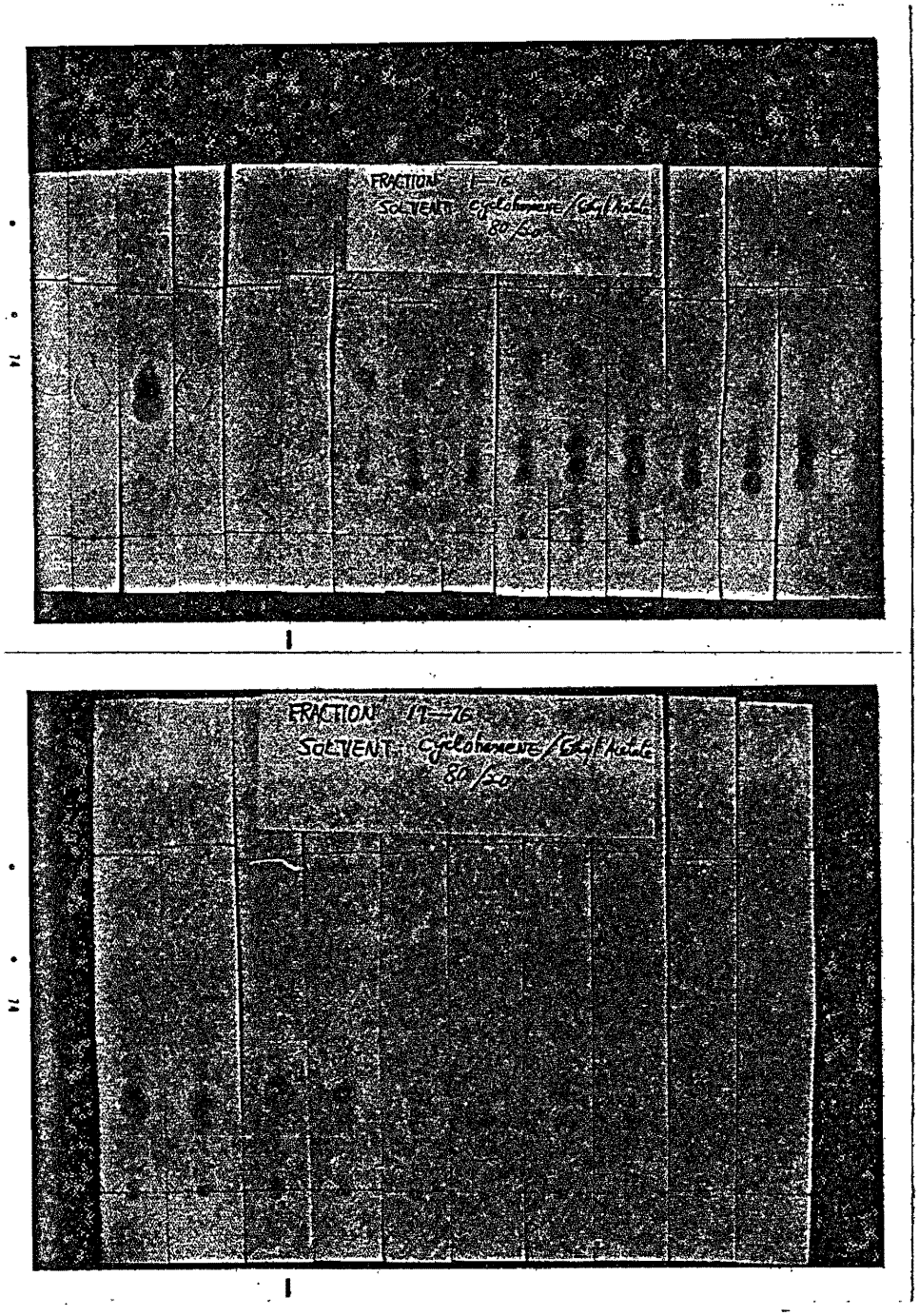
* Haloing observed

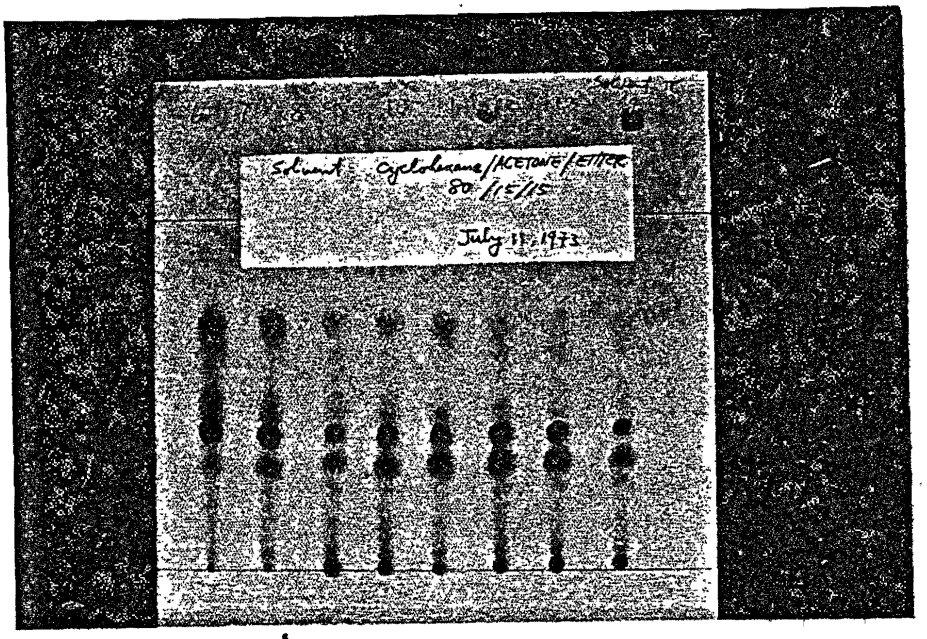
TABLE VII-B

THIN LAYER CHROMATOGRAPHY RESULTS OF FRACTION II-6 THROUGH FRACTION II-32

Fr. No.	Rf'					
6	0.0	29.2	39.1	46.5	63.4	68.9
7	0.0	39.2	39.1	46.5	63.4	68.9
8	0.0	29.2	39.1	46.5	63.4	68.9
9	0.0	29.2	39.1	46.5	63.4	68.9
10	0.0	29.2	39.1	46.5	63.4	68.9
11	0.0	29.2	39.1	46.5	63.4	68.9
12	0.0	29.2	39.1	46.5	63.4	68.9
13	0.0	29.2	39.1	46.5	63.4	68.9
14	0.0	29.2	39.1	46.5	63.4	68.9
15	5.1	31.2	39.5	45.2		
16	5.1	31.2	39.5	45.2		
17	5.1	21.7	31.2	39.5	45.2	
18	5.1	21.7	31.2	39.5	45.2	
19	5.1	21.7	31.2	39.5	45.2	
20	5.1	21.7	31.2	39.5	45.2	
21	5.1	21.7	31.2	39.5	45.2	
22	5.1	21.7	31.2	39.5	45.2	
23	5.1	21.7	31.2	39.5	45.2	
24	5.4	23.2	30.4	38.8		
25	5.4	23.2	30.4	38.8		
26	5.4	23.2	30.4	38.8		
27	5.4	23.2	30.4	38.8		
28	5.4	23.2	30.4	38.8		
29	5.4	23.2	30.4	38.8		
30	5.4	23.2	30.4	38.8		
31	5.4	23.2	30.4	33.9	38.8	47.0?
32	5.4	23.2	30.4	33.9	38.8	47.0?

Figure 3 : Thin Layer Chromatograms of Fractions II-1 Through II-32




Solvent: Cyclohexane/ACETONE/ETHER
80: 15/15
July 11, 1973

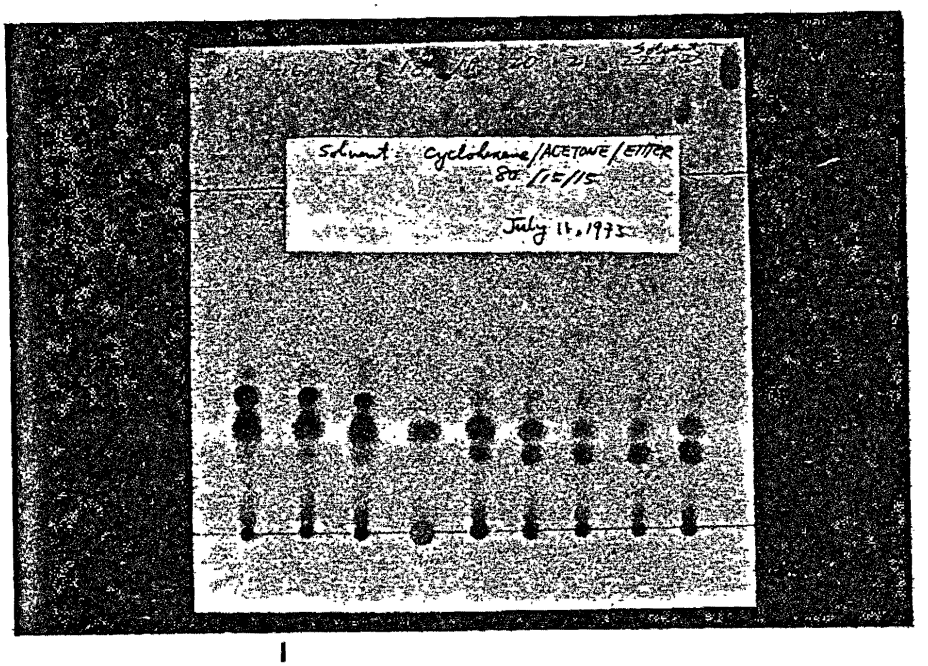
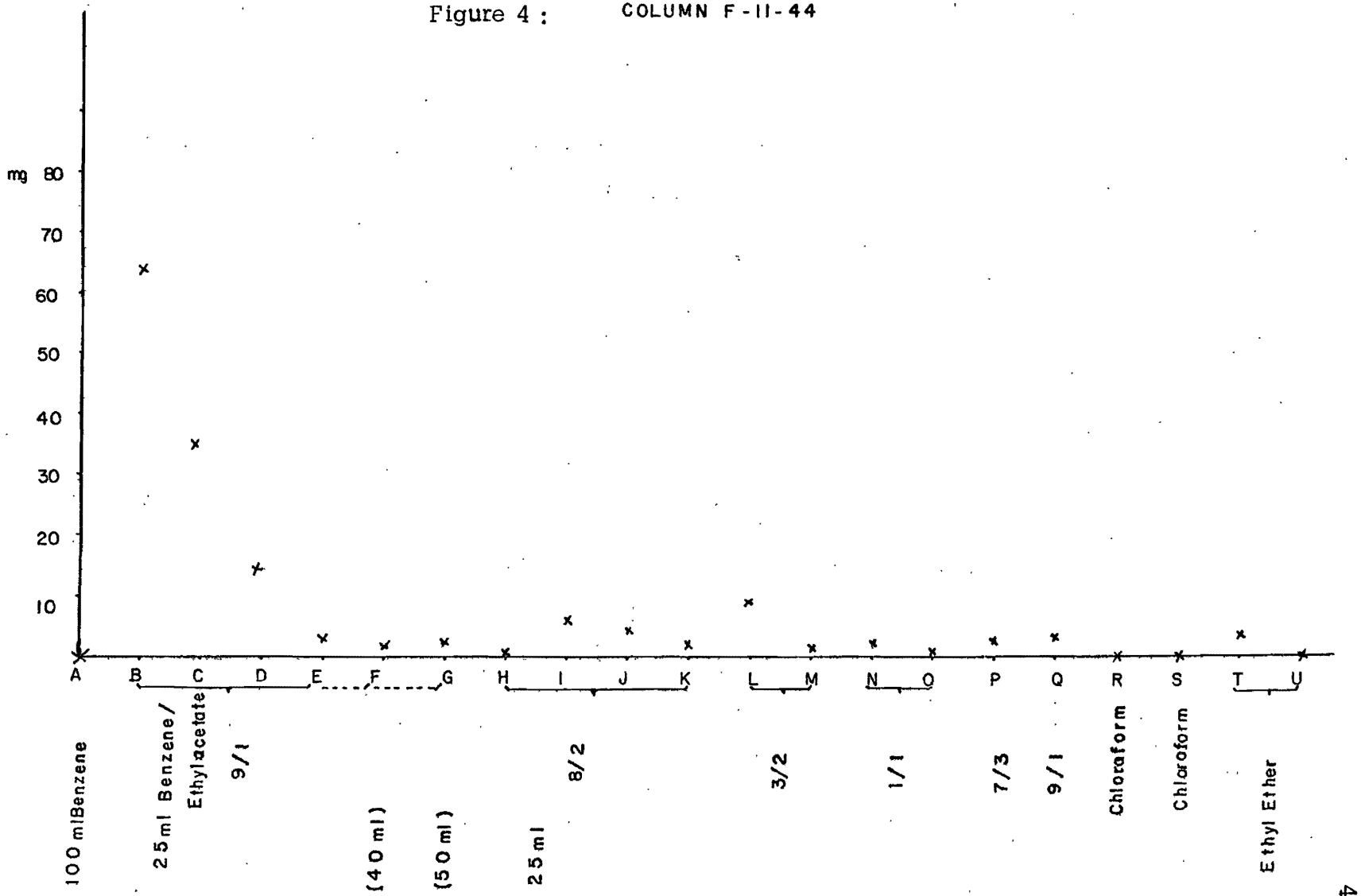

Solvent: Cyclohexane/ACETONE/ETHER
80: 15/15
July 11, 1973

TABLE VIII

COLUMN CHROMATOGRAPHIC DATA
OF COLUMN F-II-44

Fr. No.	Solvent Used	Volumn (ml)	Mass (mg)
A	Benzene	100	---
B	10% Ethyl Acetate/Benzene	25	63.8
C	10% Ethyl Acetate/Benzene	25	34.2
D	10% Ethyl Acetate/Benzene	25	14.5
E	10% Ethyl Acetate/Benzene	25	3.1
F	10% Ethyl Acetate/Benzene	40	2.0
G	10% Ethyl Acetate/Benzene	50	2.1
H	20% Ethyl Acetate/Benzene	25	0.9
I	20% Ethyl Acetate/Benzene	25	5.1
J	20% Ethyl Acetate/Benzene	25	4.4
K	20% Ethyl Acetate/Benzene	25	2.2
L	40% Ethyl Acetate/Benzene	25	9.3
M	40% Ethyl Acetate/Benzene	25	1.6
N	50% Ethyl Acetate/Benzene	25	2.0
O	50% Ethyl Acetate/Benzene	25	1.2
P	70% Ethyl Acetate/Benzene	25	2.6
Q	90% Ethyl Acetate/Benzene	25	2.7
R	Chloroform	50	----
S	Chloroform	25	----
T	Ethyl Ether	100	2.6
U	Ethyl Ether	50	----

Figure 4 : COLUMN F-II-44



Infrared Analysis of Fractions from Column F-II-44

Fraction B: 1705 cm^{-1}, 1735 cm^{-1}, 1770 cm^{-1} three strong carbonyl peaks;

Fraction C: one 1770 cm^{-1} strong carbonyl peak with a shoulder at 1640 cm^{-1} double bond;

Fraction D: strong carbonyl peak at 1770 cm^{-1} with a weak 1640 cm^{-1} double bond peak;

Fraction E: strong carbonyl peak at 1770 cm^{-1} and a stronger 1640 cm^{-1} double bond peak than that in fraction D;

Fraction F through T: Strong and broad carbonyl peak from 1700-1775 cm^{-1} with poor resolution.

(See Appendix II.)

Thin Layer Chromatography Analysis of Fractions II-44's

Two-dimensional thin layer chromatography on silica gel Bakerflex IB-F was attempted with different solvents (systems I: chloroform/ether 90/10; II: benzene/acetone 90/10) for fraction II-44B. Satisfactory resolution was not obtained. Fraction II-44B through fraction II-44E were subjected to thin layer chromatography with benzene/acetone 90/10, and once more satisfactory resolution was not obtained.

Fraction II-44B, the largest fraction from column F-II-44, was rechromatographed on silica gel Bakerflex IB-F. See the following chart. Each development was done twice with the solvents shown. All fractions were recovered from the silica gel by removing the silica gel from the plate in bands observed with a UV lamp. The silica gel was extracted in a small Soxhlet extractor with ether. Evaporation of the ether gave the recovered material.

II44B (63.8 mg) —$CHCl_3$/Ether 8/2, then Cyclohexane/Et Ac 1/1→

- II44BI, 26.8 mg, Rf'=82.5
- II44BII, 8.3 mg, Rf'=69.3
- II44BIII, 2.6 mg, Rf'=59.9
- II44BIV, 1.8 mg, Rf'=49.6
- II44BV, 2.8 mg, Rf'=40.9
- II44BVI, 4.1 mg, Rf'=16.8
- II44BVII, 3.9 mg, baseline

II44BI (26.8 mg) —Cyclohexane/Ethyl Acetate 50/50 twice→

- II44BIA*, 25.3 mg, Rf'=69.6
- II44BIB, Rf'= 8.8
- II44BIC, Baseline

II44BIA(25.3mg) —$CHCl_3$/Ether 80/20 twice→

- II44BIAα **, 6.4 mg, Rf'=83.3
- II44BIAβ **, 7.1 mg, Rf'=73.4
- II44BIAγ **, 2.6mg, Rf'=53.2
- II44BIAδ **, Rf'=10.5
- II44BIAϵ **, Baseline

———— Infrared spectrum taken (see Appendix II)

---

* Thin layer chromatography: cyclohexane/Et Ac 1/1, Rf'=44.5, tailing and haloing observed.

II Thin layer chromatography under long wave length UV light:

II44BIAα : yellow; II44BIA β: blood-red; II44BIAγ : red-brown; II44BIA$^{\delta}$ &$^{\epsilon}$: dark yellow

Under normal light: all green;

Analysis of sub-Fraction II44BIA α, β and γ

One-dimension thin layer chromatography on aluminum oxide Bakerflex IB-F was attempted with these fractions. With the solvent system chloroform/ether 80/20 no distinct spots were obtained.

Thin Layer Chromatography Comparison of Fraction I-18 and Fraction II-44

Thin layer chromatography analysis of fraction I-18 and II-44 on pre-coated TLC plate silica gel F-254 (EM) gave the results shown in the Table IX. All the chromatograms were developed with concentrated sulfuric acid and then kept at 120°C for 90 seconds.

With Bakerflex IB-F silica gel the following results were obtained: (same developing procedure as above)

	Butyl acetate	Benzene/ether 10/90
I-18D	53.9/27.3	57.1/26.9
II-44C	53.9/27.3	57.1/26.9

The results showed that fraction I-18D was similar to fraction II-44C,D,E and F, and hence combined. The combination fraction of I-18D and II-44C,D,E and F was subjected to thin layer chromatography on a pre-coated TLC plate silica gel F-254 (0.5 mm) (EM) with combination fractions I-17-21*, I-22-25*, and I-26-28*. Butyl acetate did not give good resolution, but petroleum ether **/chloroform/ethyl acetate 4/4/2 gave a nice separation as shown in Table X.

---

* These fractions of Column I were combined by Mr. Brad Halverson.
** Petroleum ether bp. 30-60°C.

TABLE IX

THIN LAYER CHROMATOGRAPHY RESULTS OF FRACTION I-18 AND FRACTION II-44

Solvent used: Ether/Benzene 90/10

Fr. No.	Rf'	Remark
II-44C	61.7/32.5/5.0	haloing and tailing
D	61.7/32.5	haloing and tailing
E	6.17/32.5	haloing and tailing
F	32.5	haloing and tailing
I-18B	82.3	
C	75.2	
D	70.8/32.7	

Solvent used: Butyl acetate

II-44C	71.4/36.1/8.4/4.2	
D	71.4/36.1/8.4/4.2	
E	7.14/36.1/8.4/4.2	
F	36.1/8.4/4.2	
I-18B	75.6	
C	69.9	
D	69.9/27.6*	

* May be higher

TABLE X
THIN LAYER CHROMATOGRAPHY OF COMBINATION FRACTIONS

Solvent: pet. ether/$CHCl_3$/Et Ac 4/4/2, conc. H_2SO_4 spray at room temperature, then 90 seconds, 120°C.

Fr. No.	Rf'									
Combination	64.7			38.2^G		33.1^G	29.4^P		18.4^P	
I-17-22	64.7	52.9	46.3	38.2^G		33.1^G	29.4^P		18.4^P	5.9
I-22-25	64.7	52.9	46.3		35.3		29.4^P		18.4^P	5.9
I-25-28		52.9	46.3		35.3		29.4^P	23.5	18.4^P	5.9

G: green

P: purple

Column III

This column was run mainly by Mr. Brad Halverson. One gram of the crude extract of F. franciscana Howe used in this work was chromatographed on silica gel. Fractions 19 through 31* were subjected to thin layer chromatography on 0.5 mm precoated TLC silica gel F254 (EM). Butyl acetate did not give an acceptable resolution, but pet ether*/ chloroform/Et Ac 4/4/2 gave a very good separation, as shown as the following Table XI.

Combination of Certain Fractions from Column III

Based on both infrared spectra and thin layer chromatographic data, fraction III-19 was combined with III-20 and called fraction III-1920. Fractions III-21 through 31 were combined and called III-2131.

Separation of III-1920

Fraction III-1920 (mass 104.3 mg) was placed on a 0.5 mm precoated TLC plate silica gel F 254 and developed with pet ether**/ chloroform/Et Acetate 4/4/2. Several bands were removed from the plate by scraping, and the silica gel was extracted first by ether and then by ethanol at room temperature. Infrared spectra were taken for the subfractions. See Table XIII.

These subfractions of III-1920 were subjected to thin layer chromatography on 0.5 mm precoated TLC silica gel F-254 plate. (Same solvent system and developing system)

---

* Solvent: Fraction 19-24, 50% $CHCl_3$/Benzene; Fraction 25: 60% $CHCl_3$/ Benzene; Fraction 26: 70% $CHCl_3$/Benzene; Fraction 27-31: 80% $CHCl_3$/ Benzene.

** Pet. ether: b.p. 30-60°.

TABLE XI

THIN LAYER CHROMATOGRAPHY OF FRACTION III-19 THROUGH FRACTION III-31

Fr. No.	Rf'											
19	86.9	79.6	71.5	64.2	52.6	42.3	34.3	16.8	7.3	3.6		
20	86.9	79.6	71.5	64.2	52.6	42.3	34.3	27.7	23.4	16.8	7.3	3.6
21	71.5	64.2	52.6	42.3	34.3	27.7	23.4	16.8	7.3	3.6		
22	64.2	57.7	52.6	42.3	34.3	32.1	27.7	23.4	16.8	7.3	3.6	
23	64.2	57.7	52.6	42.3	34.3	32.1	23.4	16.8	7.8	7.3	3.6	
24	86.9	71.6	71.5	64.2	57.7	52.6	34.3	32.1	23.4	16.8	7.3	
25	86.9	71.5	64.2	57.7	52.6	34.3	32.1	23.4	16.8	7.3		
26	86.9	71.5	64.2	57.7	52.6	34.3	32.1	23.4	16.8	7.3		
27	64.2	57.7	52.6	34.3	32.1	23.4	16.8	7.3				
28	65.4	49.6	37	18.5	13.3	7.4						
29	65.4	49.6	42.9	37	18.5	13.3	7.4					
30	65.4	34.1	22.2	18.5	13.3	7.4						
31	34.1	22.2	13.3	7.4								

TABLE XII

INFRARED ANALYSIS OF FRACTIONS
III-19 THROUGH 31

Fr. No.	Wave Number (cm)	Remarks
19	3300-3600 b,m 1765 s, 1735 s, 1709 s	OH carbonyl
20	3300-3600 b,m 1765, 1735, 1705 s	OH carbonyl
21	3300-3600 b,m, 1765 s, 1705 sd	OH carbonyl
22	3300-3600 b,m, 1740-1780 s,b, 1705 s, sd	OH carbonyl
23	3300-3600 b,s 1740-1780 s,b, 1705 s, sd 1645 w, 1670 w	OH carbonyl double bond
24	3300-3600 b,m, 1768 s, 1705 m, sd 3080 w, 1645 w	OH carbonyl double bond
25	same as 24	
26	poor spectrum	
27	3300-3600 b,m, 1770 s, 1740 sd, 1700 sd	OH carbonyl
28	3300-3600 b,m, 3075 w, 3050 w, 1660 w, 1640 sd 1700-1780 b,s,	OH double bond carbonyl
29	3300-3600 b,m, 3075 w, 1662 m, 1640 w 1765 s, 1740 sd, 1700 sd	OH double bond carbonyl
30	same as 29	
31	same as 30	

b=broad, m=medium, s=strong, w=weak, sd=shoulder

TABLE XIII

THIN LAYER CHROMATOGRAPHY OF FRACTION III-1920

Fr. No.	Rf'	Mass of Extracted Material
A	93.3	5.5
B	88.7	?
C	81.3	6.4
D	69.3	11.7
E	60.0	5.5
F	56.7	3.9
G	53.3	5.7
H	49.3	9.9
I	32.0	14.1
J	21.3	4.4
K	12.0	3.5
L	7.3	5.4
M	baseline	5.5

Infrared Data of Subfractions of III-1920

A: 1738 cm^{-1} carbonyl, B: 3300-3600 b*,w*, OH, 1738 s*, 1770 w, sd* carbonyl, C: 3300-3600 b,w, OH, 1770 w,sd, 1738 m*, 1765 m, carbonyl, D: 3300-3600 b,m, OH, 1770 m, sd, 1700-1740 b,s, carbonyl, 3055 w, 1640 w, sd, double bond, E: 3300-3600 s,b, OH, 1770 s, 1700 m, sd, carbonyl, 1640 w, double bond, H: poor spectrum, I: 3300-3600 m,b, OH, 3055 w, 1640 sd, m, double bond, 1770 s, 1710 s, carbonyl, J,K,L,M: poor spectrum. (Unit : Cm^{-1})

Analysis of the Separation of Fraction III-1920

Thin layer chromatographic data suggest that most of the subfractions were not pure. The number of components varied from 2 to 8, and infrared data confirmed the thin layer chromatography data.

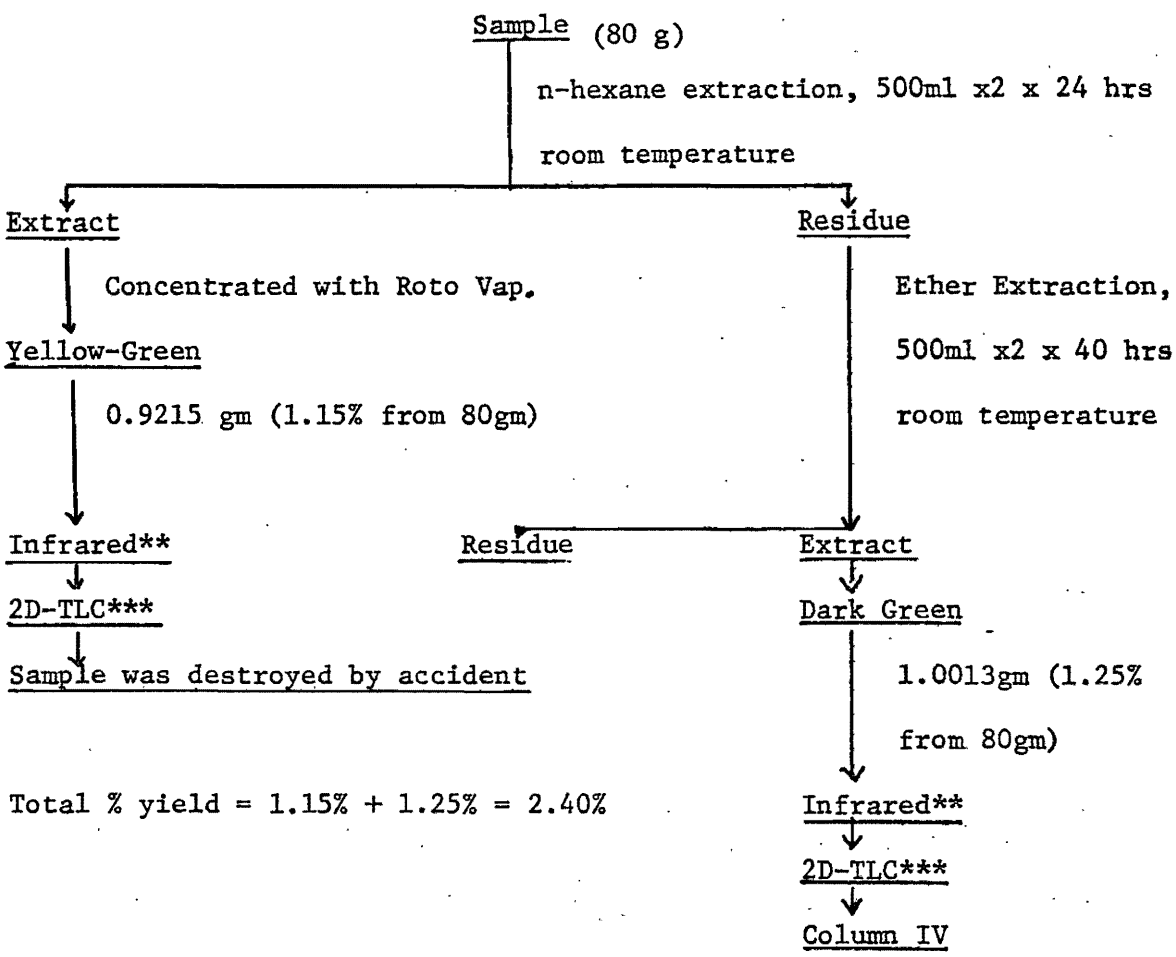
Separation of III-2131

Fraction III-2131 mass 156.8 mg, was placed on 2 mm pre-coated TLC plate silica gel F-254 (EM) and developed by (1) pet. ether**/ chloroform/ethyl acetate 4/4/2(2) ether. The entire sample stayed at the origin.

The liverwort Frullania franciscana Howe (identified by Dr. Lippert, Department of Biology, Portland State University) used in the following experiment was the second collection* by Mr. Brad Halverson and myself during the spring of 1974 in Lincoln and Polk County, Oregon. The sample was hand separated from other plant material, air dried, and milled. Eighty grams of the milled sample were treated according to the following flow chart.

---

* sd = shoulder, b = broad, m = medium, w = weak, s = strong
** pet. ether: b.p. 30-60°C



** Infrared spectra data: n-Hexane extract; 3080 cm^{-1} w, 1662 cm^{-1} w, 1640 cm^{-1} s, double bond; 3300-3600 cm^{-1} b,s, OH; 1768 cm^{-1} s, 1740 cm^{-1} s, 1705 cm^{-1} m, sd, Carbonyl
Ether Extract: 3300-3600 cm^{-1} s,b, OH; 3080 cm^{-1} w, 3050 cm^{-1} w, 1662 cm^{-1} w, 1640 cm^{-1} w, double bond; 1765 cm^{-1} s, 1740 cm^{-1} s, 1700 cm^{-1} m, sd, carbonyl

*** Two-dimensional thin layer chromatography: Solvent: Direction I: Cyclohexane/Ethyl Acetate 80/20: Direction II: Benzene/Ether 60/40 on Pre-coated TLC plate silica gel F-254 (EM) 0.5 mm, developed by concentrated sulfuric acid spray and heated for 90 seconds at 120°C.

TABLE XIV

THIN LAYER CHROMATOGRAPHY OF FRACTION III-1920

Fraction	Rf[1]							
A	92.6							
B	86.8	14.7						
C	82.4	73.5	14.7					
D	73.5	61.0	57.4	52.2	48.5	14.7	11.0	7.4
E	61.0	57.4	48.5	14.7	11.0	7.4		
F	57.4	52.2	14.7	11.0	7.4			
G	57.4	48.5	14.7	11.0	7.4			
H	46.7	40.1	30.7	29.2[g]				
I	46.7	40.1	30.7	29.2[g]				
J	29.2[g]	10.9	7.4	5.8				
K	7.4	5.8						
L	7.4	5.8						
M	No separation							

g : green

TABLE XV

RF' OF 2D-TLC OF N-HEXANE EXTRACT AND ETHER EXTRACT

Spot No.	Rf' in Solvent I	II	Present in n-Hexane and/or Ether Extract: n-hexane	ethyl ether	Color
A	65.96	86.15	yes	yes	gray
B	61.70	78,46	yes	yes	brown
C	58.16	86.15	yes	yes	yellow
D	52.35	73.08	yes	yes	
E	33.33	61.54	yes	yes	
F	26.95	59.23	yes	yes	brown-gray
G	23.40	71.54	yes	yes	deep-green
H	21.28	47.69	no	yes	
I	20.57	32.31	yes	yes	purple
J	15.60	41.54	yes	yes	pink
K	12.06	53.08	yes	yes	
L	12.06	67.69	no	yes	
M	7.09	17.69	yes	yes	purple
N	5.67	60.00	no	yes	
O	2.84	21.54	yes	yes	
P	2.84	10.77	yes	yes	gray-brown
Q	1.42	5.39	yes	yes	
R	3.55	baseline	no	yes	
S	27.66	46.15	no	yes	brown-gray
T	5.67	32.31	no	yes	

Figure 5: 2-D Thin Layer Chromatogram

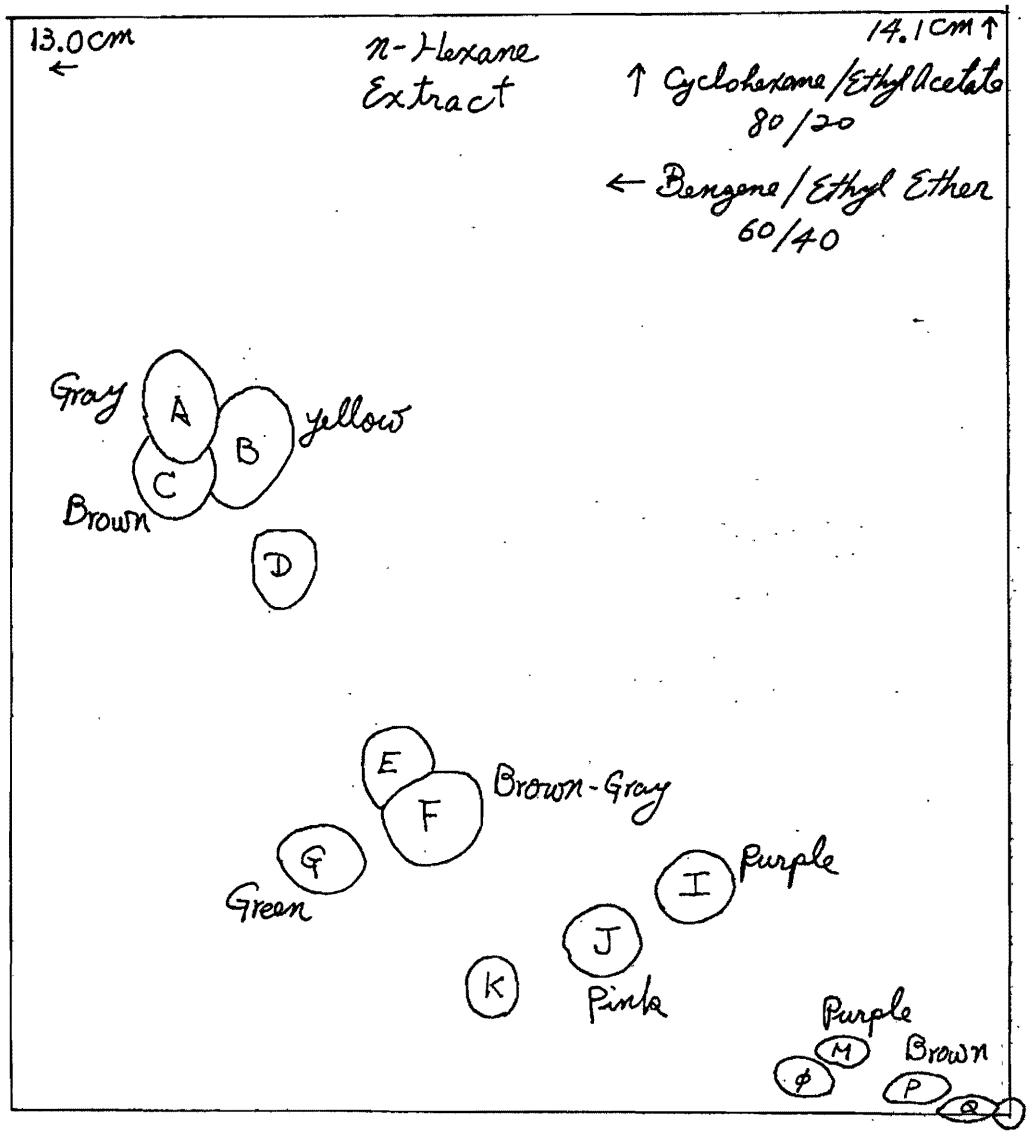
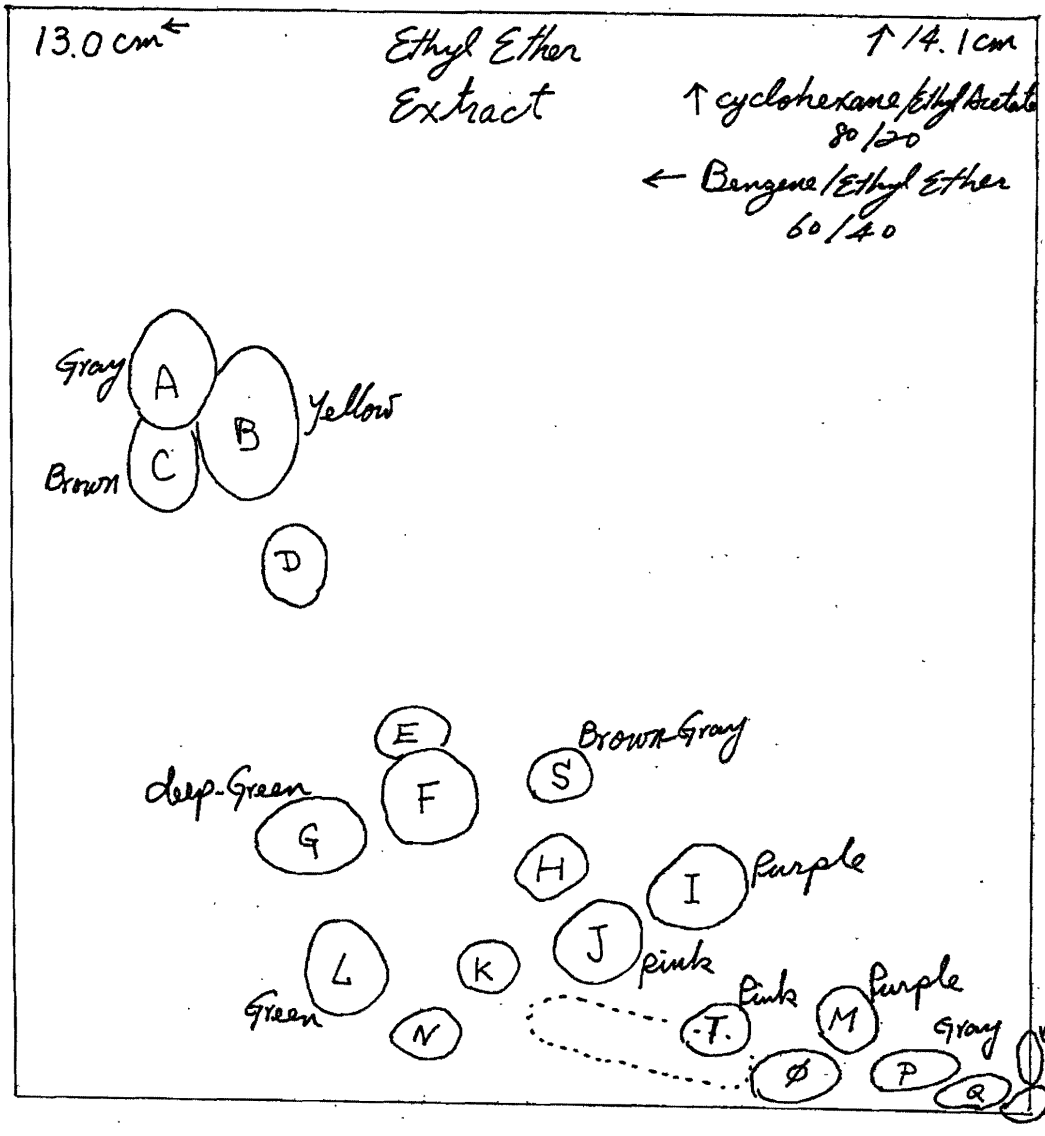


Figure 6 : 2-D Thin Layer Chromatogram



Column IV

Column IV was packed with 60 gm of aluminum oxide, Woelm neutral, (activity I) in n-hexane, ID of column 2 cm. An ether solution of 0.9176 gm of ether extract was absorbed by about 2 gm of aluminum oxide, Woelm neutral, (activity I) and placed in the top of the column. Then the column was eluted as shown in Table XVI. The recovery from this column was 0.6056 gm (66%).

Fraction IV-1

Infrared and thin layer chromatography data showed that this is a reasonably pure fraction of hydrocarbon. The nuclear magnetic resonance spectrum (Varian 60A) was taken, but due to the instrumental deficiency for the micro-sample, the following data should not be thought as a strong support for the structural determination. Solvent: Chloroform-d, 1% v/v tetramethylsilane, 99.8% D; 0.75 δ singlet; 0.88 δ singlet; 1.28 δ singlet; 1.72 δ singlet; 1.30--2.60 δ broad band; 4.42 δ and 4.73 δ broad band.

Preliminary Gas Chromatography of Fraction IV-1

The nuclear magnetic resonance sample was injected into a gas-liquid chromatograph (Hewlett-Packard 5750, flame ionization detector, nitrogen as carrier gas).

A. Column: Apiezon-L, 10% on 80/100 w, 6' x 1/8".
Temperature programmed: 150°C--2 min., then 6°C/min. upward to 255°C.
Four peaks were distinguishable, peak no. 3 being the biggest.

Temperature-isothermal: at 185°C

t_R, (min)	Peak Area %
5.35	1.4
9.55	98.6

TABLE XVI

COLUMN CHROMATOGRAPHIC DATA OF COLUMN IV

Fr. No.	Solvent	Volume (ml)	Mass (mg)	2D-TLC	IR
1	n-hexane	100	58.8	yes	yes
2	n-hexane	100	1.6	no	no
3	benzene	100	8.5	no	yes
4	benzene	100	4.0	no	yes
5	benzene	100	2.5	no	yes
6	benzene	100	4.2	no	yes
7	ether	100	66.9	yes	yes
8	ether	400	67.2	yes	yes
9	ethanol	100	243.0	no	yes
10	ethanol	100	148.9	no	yes

Figure 7 : COLUMN IV

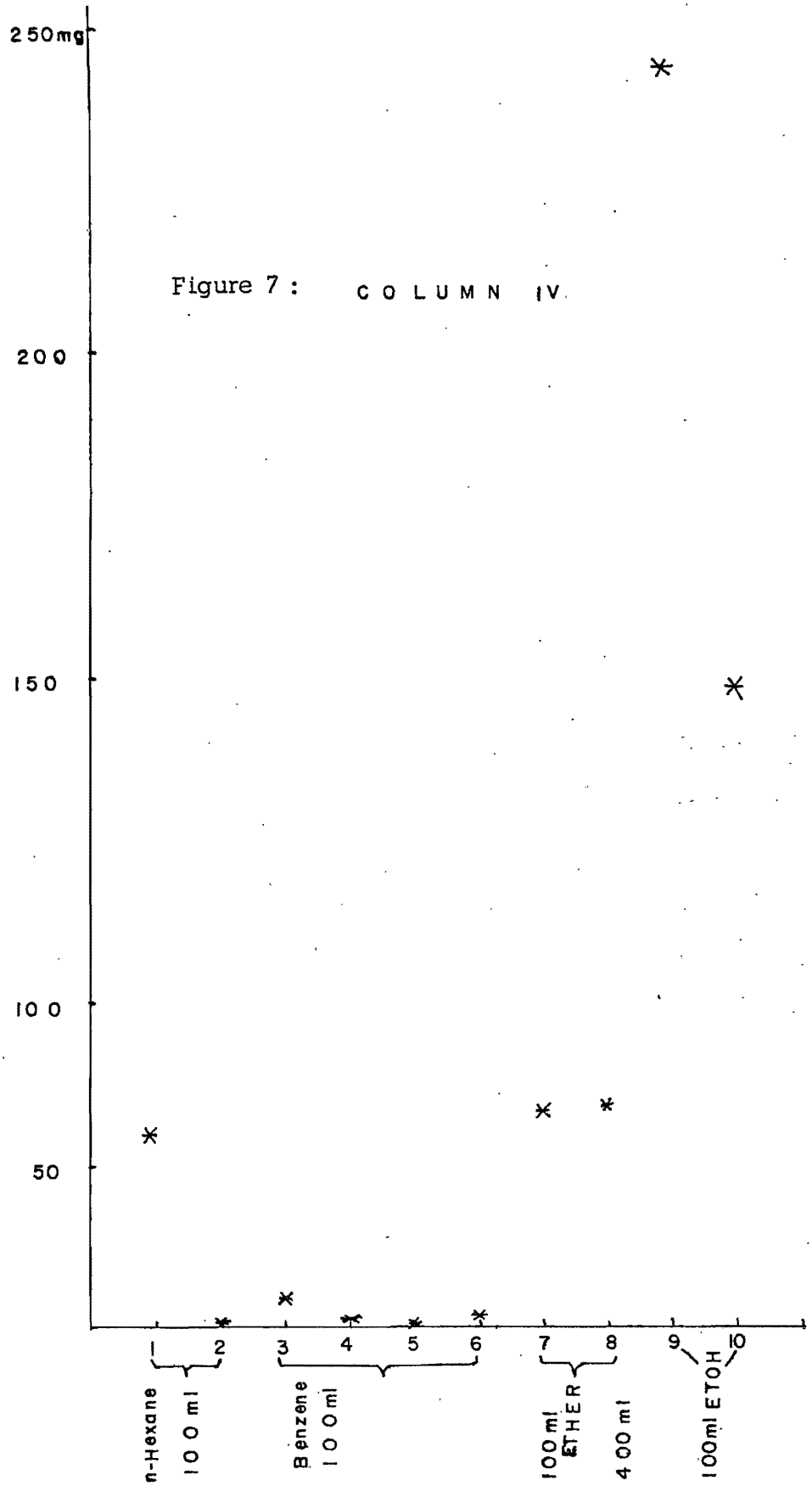


TABLE XVII

INFRARED DATA OF FRACTIONS FROM COLUMN IV

Fr. No.	Wave Number (cm^{-1})	Remarks
1	3080m, 1643s, 888s,	exo double bond
2	2930s, 2864s, 2848s, 1453m, 1441m, 1378m,	methyl, methylene
3	1729m, 1265m,	carbonyl (ester)
4	1735s, 1272-1285m,b,	carbonyl
5	poor spectrum	
6	1735m,	carbonyl
7	3005w, 1640w,	double bond
	1736s,	carbonyl
8	3300-3600w,b,	OH
	3510w, 1762s,b, 1290s, 1244s,	γ -lactone
	3060w, 1665m, 888m	double bond
9	3300-3600m,b,	OH
	3050w,	double bond
	1700-1780b,s,	carbonyl
10	3300-3600w,b,	OH
	1700-1780b,s,	carbonyl

TABLE XVIII

2D-THIN LAYER CHROMATOGRAPHY DATA

The solvents used were the same as those used for the two dimensional thin layer chromatography of the n-hexane and ether extracts. The same kind of silica gel (pre-coated TLC plate silica gel F-254 (EM) 0.5 mm) and the same developing method were used.

Fr. No.	Rf'				Similar to Spot of Table XV
	I	II	I	II	
1	67.5	85.3	----	----	A
7	59.3*	76.9*	55.5	74.4	B,D
8	58.0*	85.2*	31.4	63.8	C,E

*minor spots

There were two minor peaks less than the t_R' (5.35 min) peak. These minor peaks were neglected in the area calculations.

B. Column: SE-30, 5% GP 88 on Anakron ABS 100/110, 6' x 1/8"
Temperature programmed: 150°C--3 min., then 6°C/min. upward to 250°C--5 min.

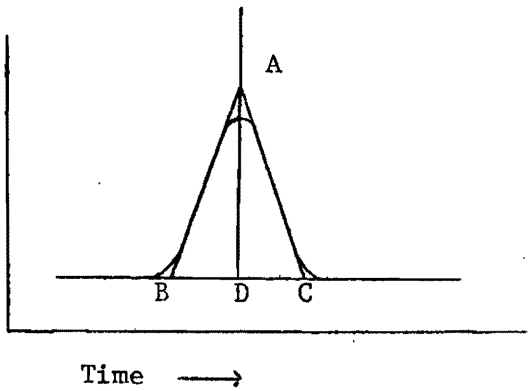
There were eleven peaks, with only peaks 1 and 3 being used in area calculations.

Temperature-isothermal: at 150°C

t_R' (min)	Peak Area %
2.33	2.35
2.35	0.32
3.63	97.33

Calculation Method

Since all the peaks were symmetrical Gaussian curves and a flame ionized detector was used, the quantities of the components in the sample would be assumed to be proportional to the areas of the peaks. The areas were estimated by triangulation. Tangents to the points of inflection on the peak sides were drawn and the area of the triangle formed with the base line computed.



$$\text{Area} = \tfrac{1}{2}\ AD \times BC$$

$$\text{The percentage of component A} = \frac{A}{\Sigma\ A_i} \times 100$$

Figure 8 a : Gas Chromatogram of F-IV-1 , Temperature Programmed

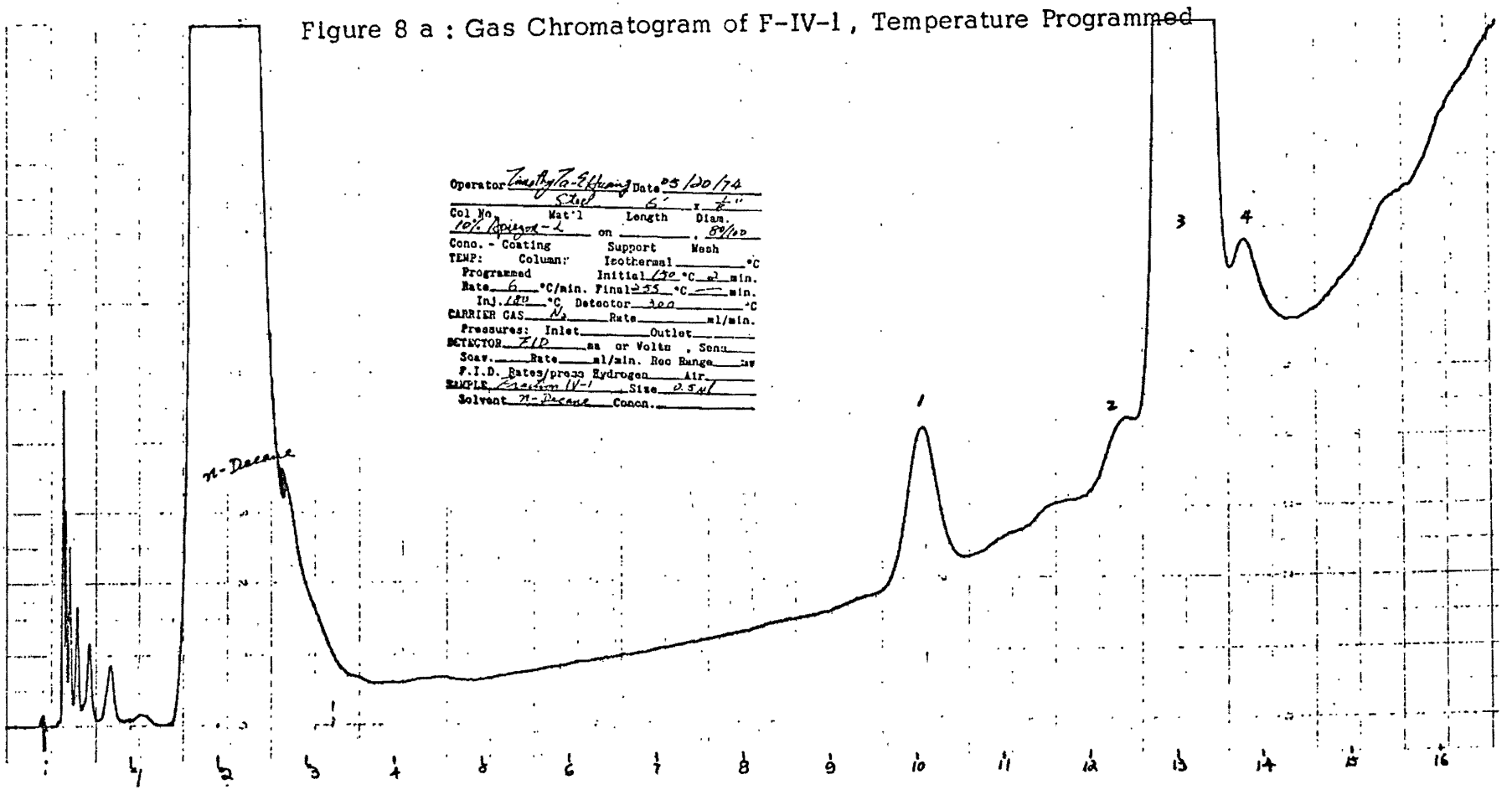


Figure 8 b : Gas Chromatogram of F-IV-1, Temperature Programmed

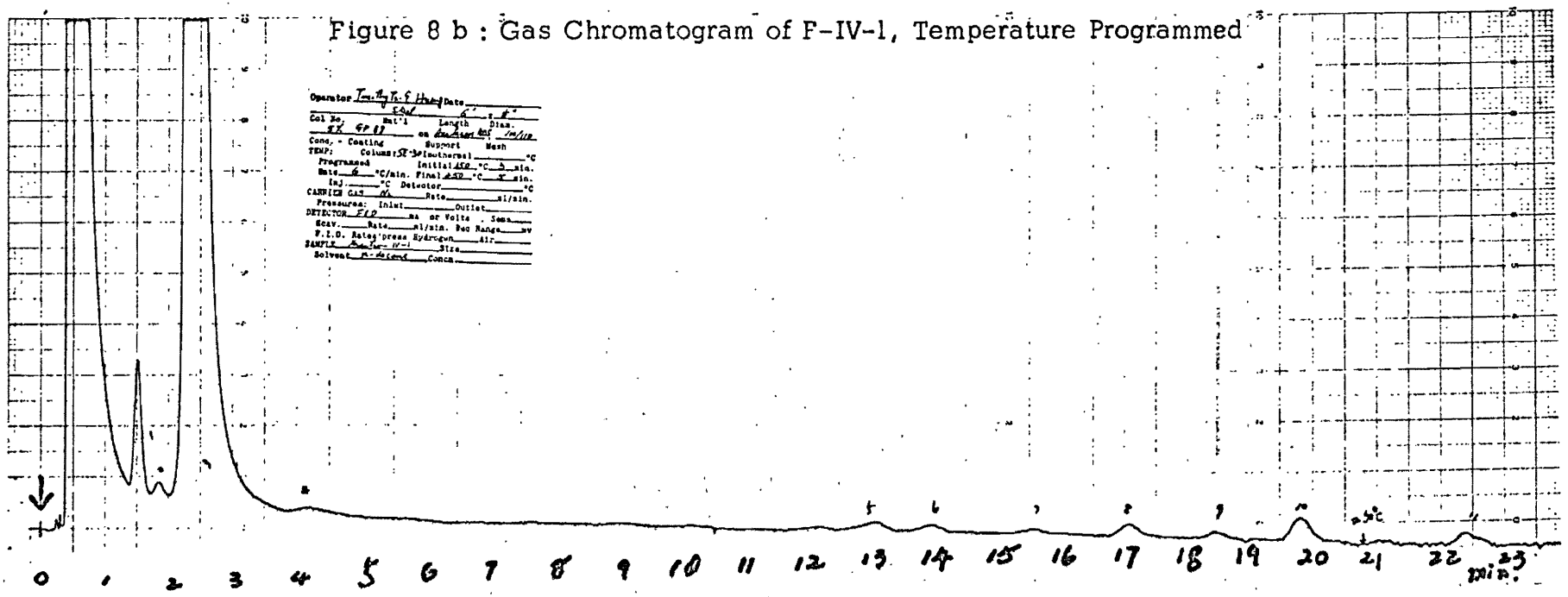


Figure 9 a : Gas Chromatogram of Fraction -IV-1, Temperature Isothermal

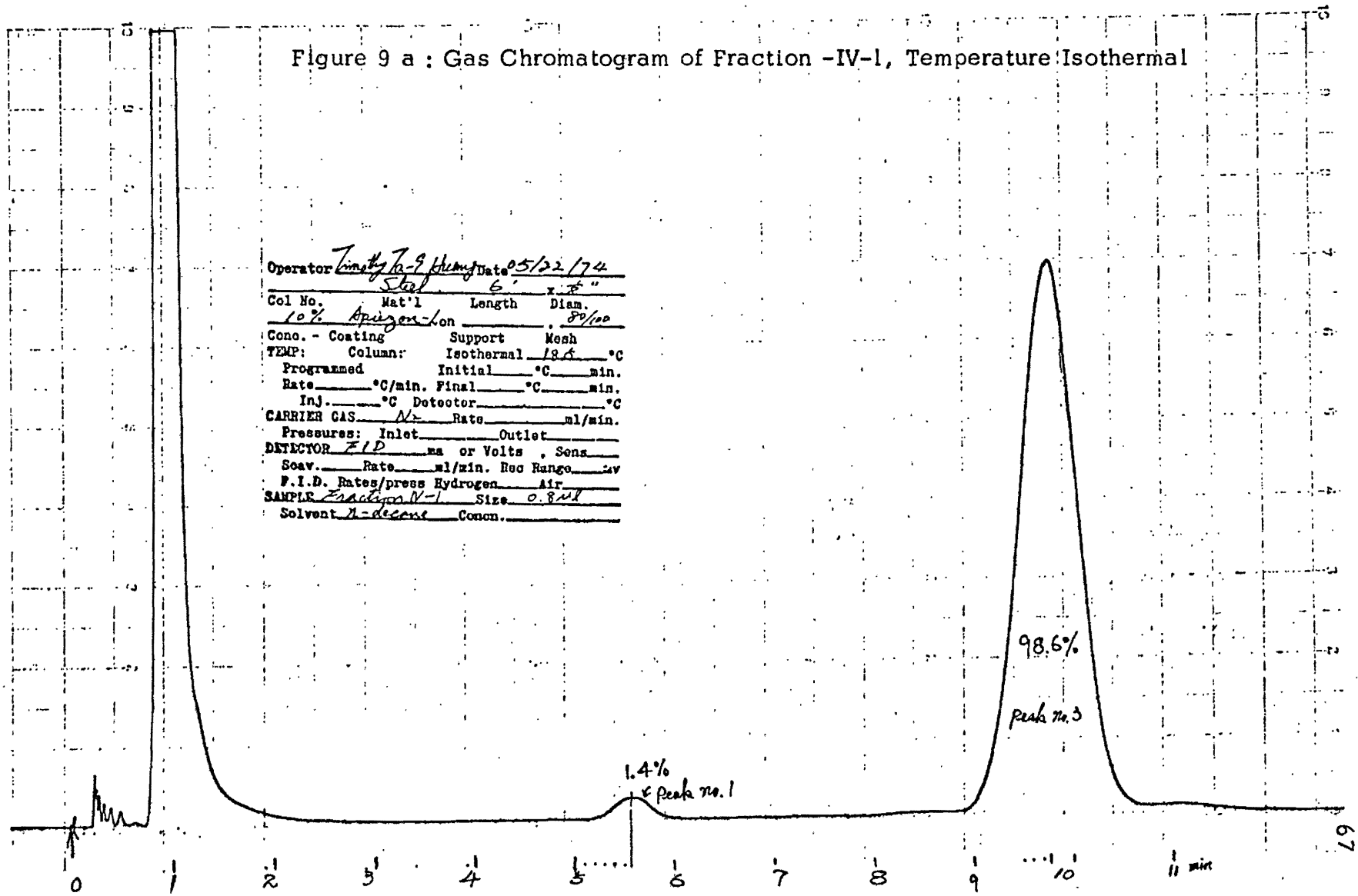


Figure 9 b : Gas Chromatogram of Fraction IV-1, Temperature Isothermal

Operator Timothy Ja-E Huang Date 05/22/74

Steel 6' x 8"

Col No. Mat'l Length Diam.

5% GP 88 on Anakrom ABS 100/110

Conc. - Coating Support Mesh

TEMP: Column: SE-30 Isothermal 150 °C

Programmed Initial ____ °C ____ min.

Rate ____ °C/min. Final ____ °C ____ min.

Inj. ____ °C Detector ____ °C

CARRIER GAS N2 Rate ____ ml/min.

Pressures: Inlet ____ Outlet ____

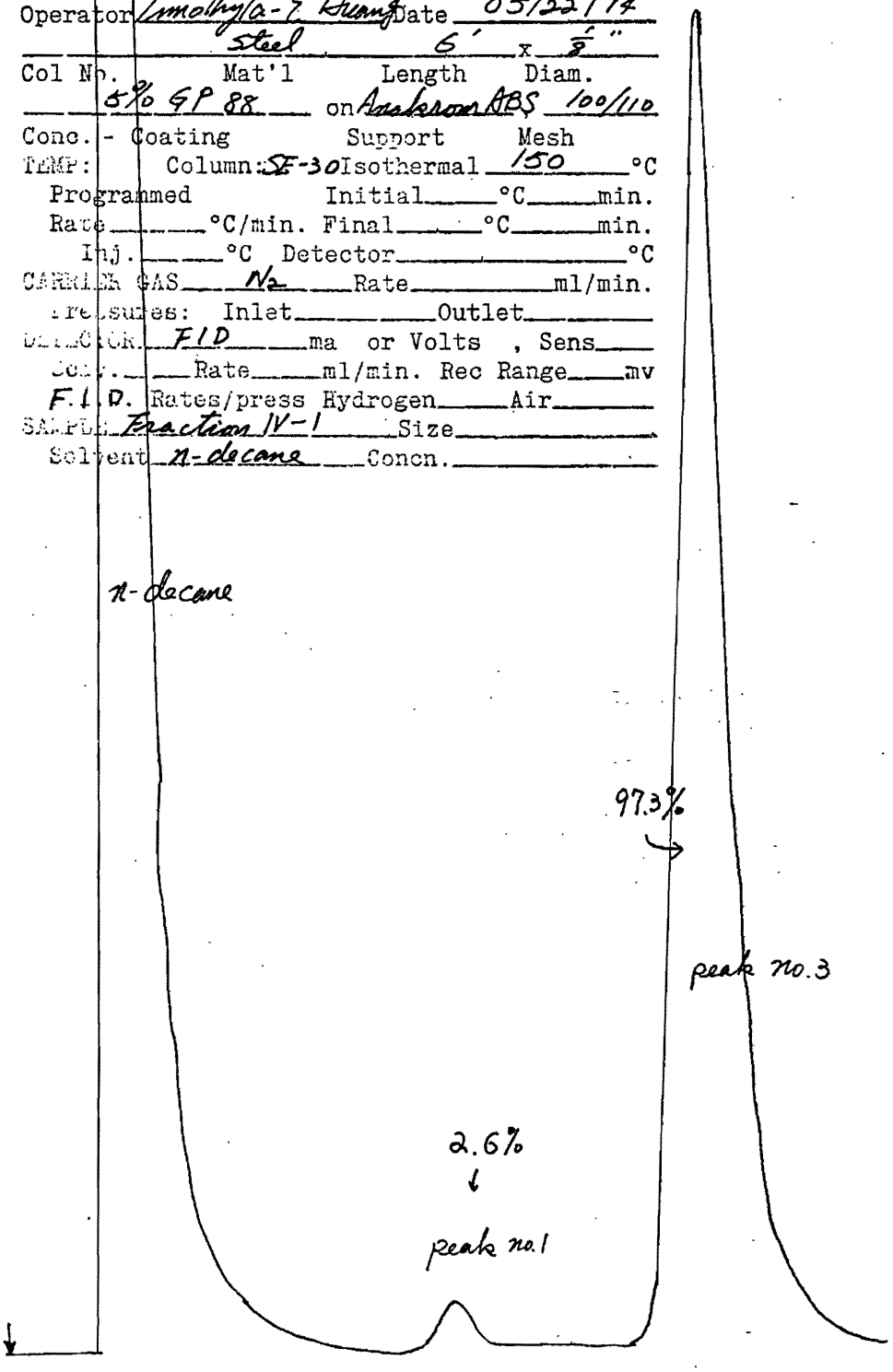
DETECTOR FID ma or Volts , Sens ____

Col. ____ Rate ____ ml/min. Rec Range ____ mv

F.I.D. Rates/press Hydrogen ____ Air ____

SAMPLE Fraction IV-1 Size ____

Solvent n-decane Concn. ____



where A was the area of peak A, and $\Sigma\ A_i$ was the sum of the areas of all the peaks.

Further Gas Chromatography of Fraction IV-1

Fraction IV-1 from the nuclear magnetic resonance tube was co-injected with 1% β-bourbonene in n-decane and 2% α-cedrene in n-decane. The β-bourbonene and α-cedrene were used as the standards for the calculation of the Kovats' indices.* β-Caryophyllene was used to check that data obtained for α-cedrene and β-bourbonene were consistent with that of Anderson and Falcone (43). The following results were obtained with a Perkin-Elmer Model 900 gas chromatograph, Carrier gas: He, flow rate 30 m./min., flame ionization detector.

---

* $I_x = I_{stdA} + \left(\frac{\log t'_{R\ (X)} - \log t'_{std\ (A)}}{\log t'_{std(B)} - \log t'_{std(A)}}\right) x\ \left(I_{std(B)} - I_{std(A)}\right)$

where:,

t'_{stdA} = corrected retention time of standard A

t'_{stdB} = corrected retention time of standard B

$t'_{R(X)}$ = corrected retention time of X

$I_{std(A)}$ = Kovats' indice of standard A

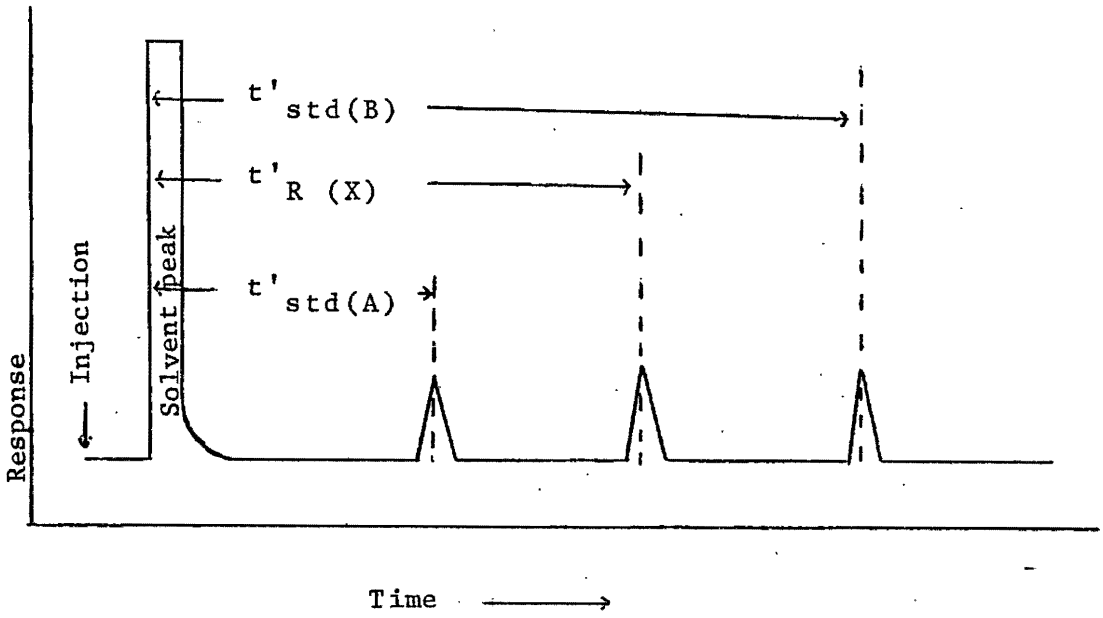
$I_{std(B)}$ = Kovats' indice of standard B

I_x = Kovats' indice of X

TABLE XIX

KOVATS' INDICES OF MAIN COMPONENT
OF FRACTION IV-1

Apiezon-L		SE-30	Carbowax-20M			DEGS
	155°C	130°C	132°C	165°C	205°C	160°C
β-bourbonene	1418.3	1386	1547	1586.5	1618	1714
β-caryophyllene (literature)	1451.7	1417.5	1618.5	1655.5	1695.5	1835.5
β-caryophyllene (calculation)	1451.3	1417.9	1622.6	1655.2	1701.3	1819.4
α-cedrene	1473.4	1414	1597.5	1640	1689	1788.5
peak no. 3	1530	1480.7	1732.4	1752.6	1841.9	1976.7



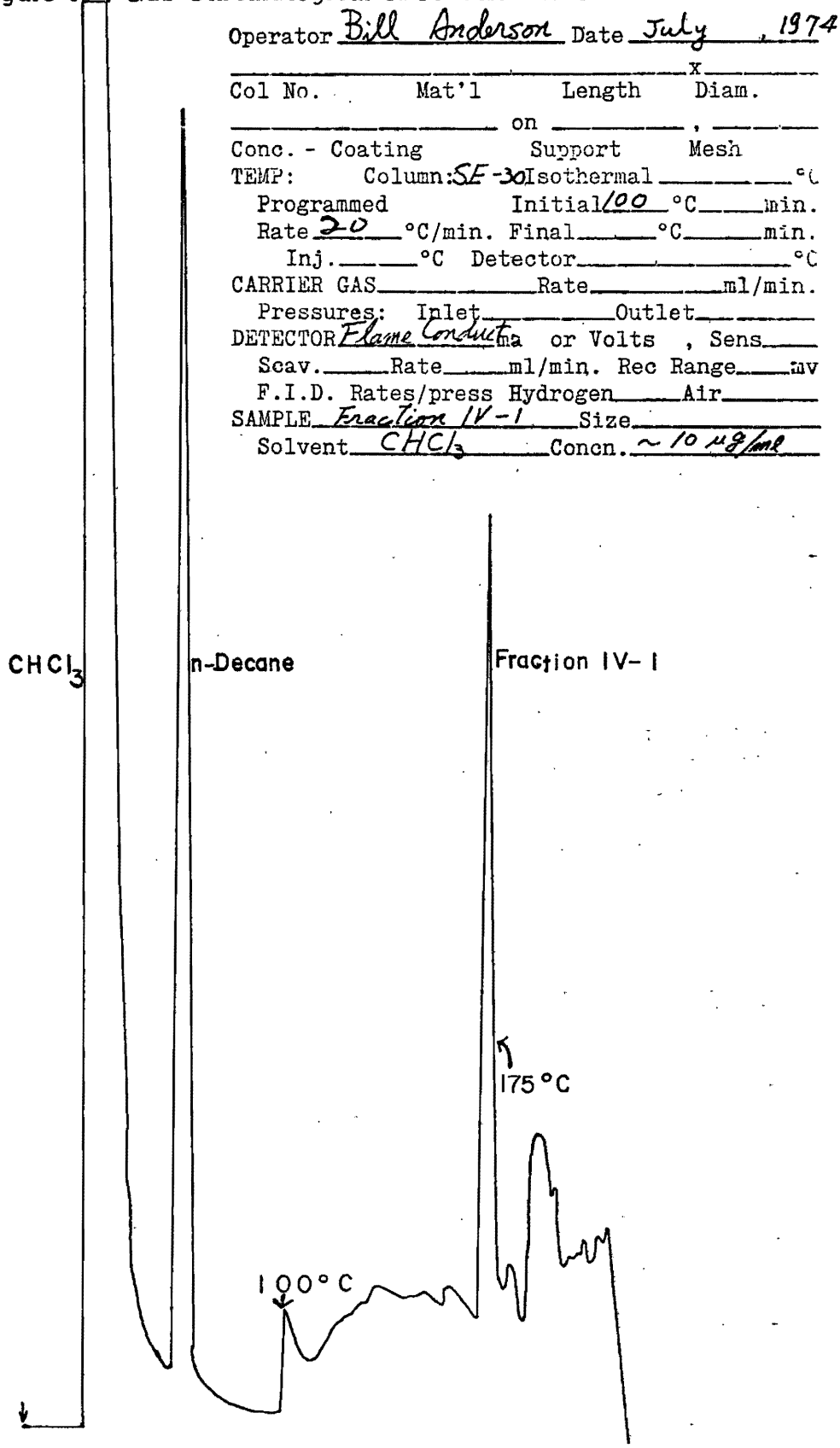
Analysis of These Kovats' Indices

The Kovats' indices obtained from the above experiment showed that peak no. 3 did not represent any of the compounds listed in the paper of Mr. Niels H. Andersen and Mark S. Falcone, and, thus was not identified.

The Mass Spectrum of the Main Component of Fraction IV-1

Fraction IV-1 was injected into a gas chromatography-mass spectrometer; column of gas chromatograph, SE-30; carrier gas, He; and temperature programmed from 100°C--175°C. The molecular weight of peak no. 3 was 204. Gas chromatograph: Varian Associate - 2440 with thermal conductivity detector; Mass Spectrometer: DuPont 21-491B. This work was done by the kind help of Mr. Bill Anderson and Dr. Doyle Daves of the Oregon Graduate Center.

Figure 10 : Gas Chromatogram of Fraction IV-1



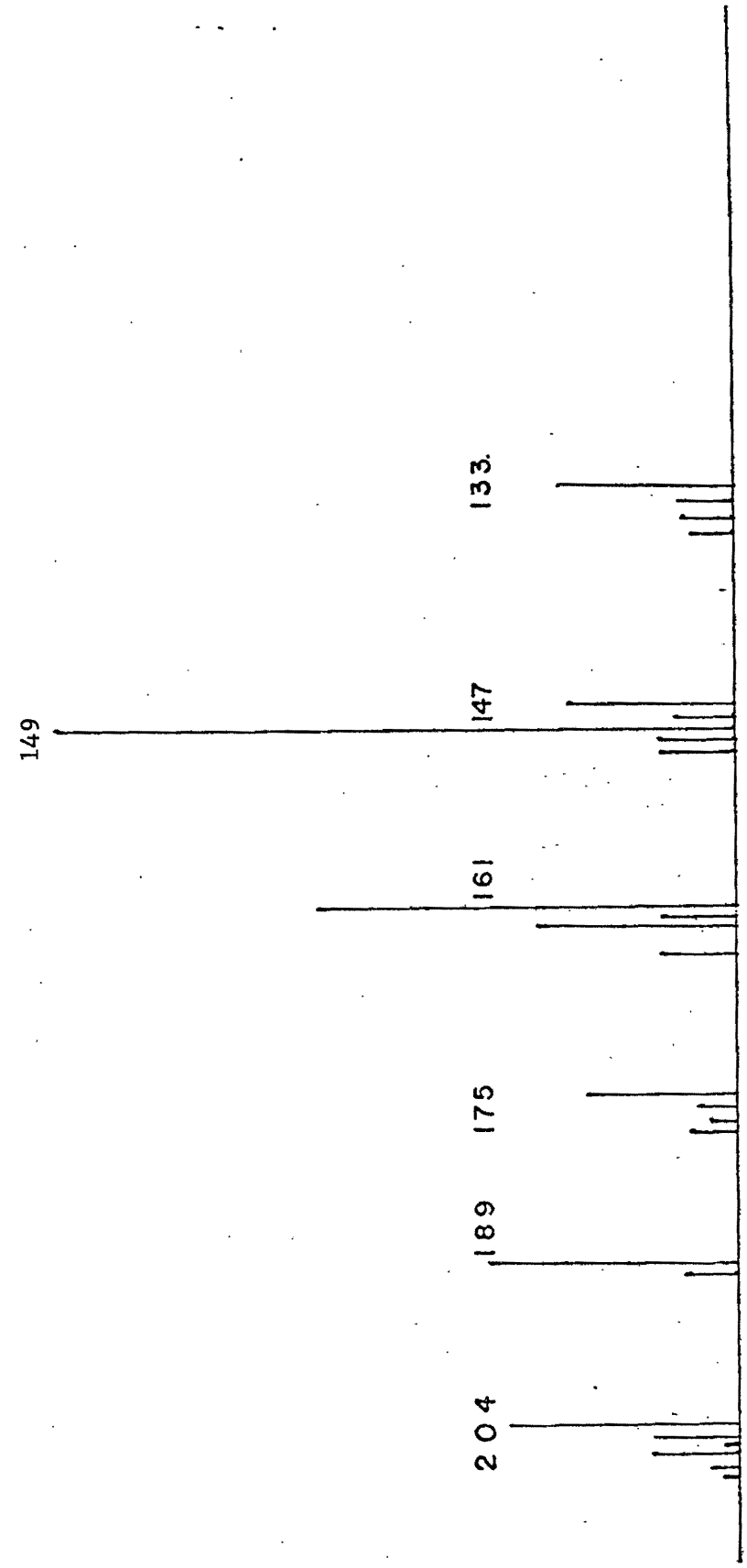


Figure 11: MASS SPECTRUM OF FRACTION- IV - 1

Fraction IV-8

Infrared data showed that fraction IV-8 was reasonably pure, and two-dimensional thin layer chromatography showed a major spot with a minor spot. Therefore, fraction IV-8 (mass 45.3 mg) was placed as a band on a 0.5 mm pre-coated TLC silica gel F-254 (EM) plate and developed in cyclohexane/sulfuric acid at 120°C for 90 seconds. The plate had two bands: Rf' = 48.6 and Rf's 25.7.

The band with Rf' = 25.7 was removed from the plate, and the silica gel was extracted by ether at room temperature, but only a small amount (5.25 mg) of material was recovered. Infrared spectrum: 3510 cm^{-1} w, 1762 cm^{-1} s, 1290 cm^{-1} s, 1244 cm^{-1} s, γ-lactone; 3060 cm^{-1} w, 1665 cm^{-1} m, 888 cm^{-1} m, double bond. These bands were identical to those of the spectrum of frullanolide purified and recrystallized by Mr. Brad Halverson from the fractions of previous columns.

Alkaloid Screening of Both Fresh and Extracted Samples

Two 2-4 g samples of Frullania franciscana Howe, one before and one after extraction, were ground in a 2-inch unglazed porcelain mortar with a small amount of clear sand and sufficient chloroform to yield a thick slurry. Ammoniacal chloroform (10 ml, N/20 with respect to ammonia) was added, and the mixture was stirred for about one minute, and filtered into a 5 x 1/2 inch test tube. Dilute sulfuric acid (2N, 0.5 ml) was added, the test tube shaken, and the phases allowed to separate. The aqueous layer was removed with a dropper in whose tip was a cotton wool plug for filtering and breaking emulsions.

After removing the cotton wool and any aqueous solution, the filtrates were placed in two 1-inch x 1/4-inch test tubes for testing with Mayers reagent. Negative results were obtained suggesting that there was not any alkaloid present in the Frullania franciscana Howe.

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44. Hand-out from Dr. Alfred S. Levinson

APPENDIX I

TABLE OF COMPOUNDS ISOLATED FROM LIVERWORTS

Compounds	From	Reference
A. N-alkanes and Ester Waxes		
N-alkanes (C_{15}-C_{35})	$C_{44e}C^{*}_{27a}$-*Calypogeia meylanii* (Buch)	(5)
	C_{27a}-*Jungermania aphaerocarpa* (Hook)	
esters (C_{34}-C_{54})	C_{48e} (Syn. *Solenostoma sphaerocarpa* (Hook) Steph.)	
	C_{44e}, C_{31a}-*Gymnocolea inflate* (Huds) Dum.	
	C_{48e}, C_{27a}-*Pellia fabbroniana* Raddi	
	$C_{48e}, C_{25a}, C_{31a}$-*Mylia taylorii* (Hook) Gray	
	Barbilophozia species	(4)
*C_{xxa} alkane C_{xxe} ester		
B. Alkaloids		
7-(3-methyl-2-bntenyl)-indole	*Riccardia sinnata* (Hook). Trev	(6)
6-(3-methyl-2-bentenyl)-indole	*Riccardia sinnata* (Hook). Trev	
C. Azulenes and Indenes		
1,4-dimethyl-azulene	*Calypogeia trichomanis* (L.) Corda	(7)(8)
4-methyl-1-methoxy-carbonyl-azulen	*Calypogeia trichomanis* (L.) Corda	(8)
3,7-dimethyl-5-methoxy-carbonyl-inden	*Calypogeia trichomanis* (L.) Corda	(9)

D. Alcohols and Acid

Nonacosan-10-ol	Bazzania Pompeana (Lac.) Mitt	(10)
Lignoceric Acid (Tetracosanoic acid)	Conocephalum conicum (L.) Underw	(11)
Triterpenic diols	Conocephalum conicum (L.) Underw	

E. Flavonoids

Saponarin (I)	Madotheca platyphylla	(12)
	Porella plortyphylla	(13)
Saponaretin (II)	Madotheca platyphylla	(12)
Pellepiphyllin	Pellia neesiana (Gettsche) Limpr.	(14)
7-O-β-D-glucuronides of Apigenin Chrysoeriol Tricin	Marchantia foliacea (Mitt) and M. berteroana (L.) and (L.)	(15)
Apigenin-6-8-di-C-glucoside (Vincenin-2)	Marchantia folliacea (Mitt) and M-berteroana (L.) and (L.)	(15)
Isovitexin	Porella platyphylla	(13)
Rhamnosyl-glucuronides of above flavones minor constituents	Marchantia foliacea (Mitt) and M. berteroana (L.) and (L.)	(13)
6,8,-di-C-glycosides of 5,7,4'-trihydroxyflavone	Hymmenophytum flabellatum	(16)
Acacetin 7-O-rhamnosyl-galacturonide O-glycoside of an acadetin 8-C-glycoside	Rebonlia hemispherica	(17)
8-methoxy-5,7,3',4'-tetrahydroxyflavone (bound to (a) water soluble polysacharide(s)	Monoclea forsteri	(18)
Apigenin-6,8-di-C-glycoside	Porella platyphlla	(13)

F. Steroids

β-sitosterol	Pingius (L.) Dum Sinuta (Hook) Trev Conicum (L.) Underw. Scapania Parvitexta	(11)(19)
Brassicasterol	"	"
Campesterol	"	"
Stigmasterol	"	"
Choesterol	"	"
Cholest-5-en-3β-ol (C_{27}, Δ^5)	Jungermannicaceae (J. thermarum J. torticalyx)	(20)
24-methylcholest-5 22-dien-3β-ol (C_{28}, $\Delta^{5,22}$)	Scapaniaceae {S. parvitexta S. Undulata Macrodiplophylum Plicatum	(20)
24-methylcholest-5-en-3β-ol (C_{28}, Δ^5)	Lophocoleaceae- {Chiloscyphus polyanthus Heteroscyphus bescherellei	(20)
24-ethycholest-5, 22-dien-3β-ol (C_{29}, $\Delta^{5,22}$)	Plagiochiclacea- {P. japonica P. ovalifolia	(20)
24-ethylcholest-5-en-3β-ol (C_{29}, Δ^5)		

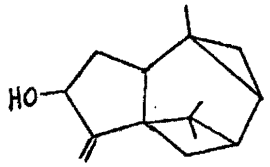
G. Mono-, di-, and Triterpenoids

Friedelin	Frullania tamarisci	(21)
	F. dilatata	
	C. Conicum (L.) Underw.	(11)
β-Carotene	Aneura Pinguis (L.) Dum Riccardia Sinuata (Hook) Trev. Conicum (L.) Underw.)	(11)
ent-Kaurene hydroxy acetate	Solenostoma triste (Nees) K. Mull	(22)

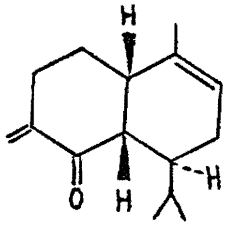
(-)-16α-Hydroxy-kauran	Anthelia julacea (L.) Dum Anthelia juratzkana (Limpr.) Trev.	(23)
(-)-Manool	Jungermannia torticalyx	(24)
ent-Kaurene hydroxy-ketone	Solenostoma triste (Nees) K. Mull	
ent-Kaurene unsaturated hydroxy-ketone	"	"
ent-kaurene diol	"	"

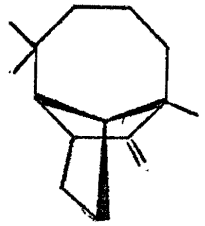
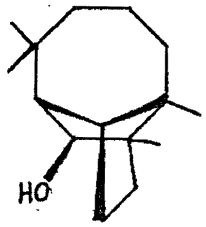
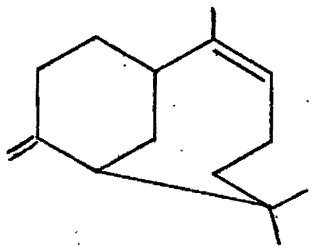
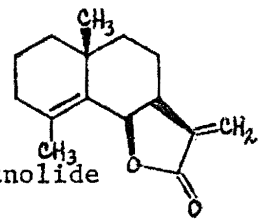
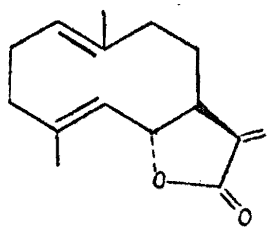
H. Sesquiterpenoids

Myliol	Mylia taylorii (Hook) Gray	(25,26)

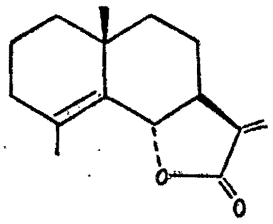


Chiloscyphone	Chiloscyphus polyanthus (L.) Corda	(27-29)

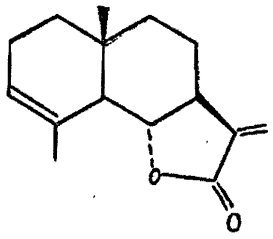


Isolongifolene	Scapania subalpina Scapania uliginosa Scapania undulata	(30)
(-) longifolene	Scapania undulate (L.) Dum	(31)
	S. nemorosa S. subalpina S. uliginosa Jungermania cordifolia	(30) " " (32)
(-) Longiborneol	Scapania subalpina Scapania undulata	(32) (30)
		
Bazzanene	Bazzania pompeana (Lac.) Mitt	(2,33)
	Scapania parvitexta (Steph.)	(11)
Frullanolide	Barbilophozia floerkei Bazzania trilofata Frullania tamarisci (L.) Dum F. dilata (L.) Dum	(4) (34) (21) (35,36,37)
		
Costunolide	Frullania tamarisci (L.) Dum	(35)
		

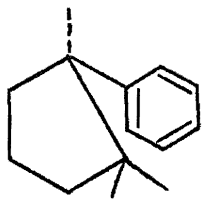
γ-cyclocostunolide — Frullania tamarisci (L.) Dum — (35)



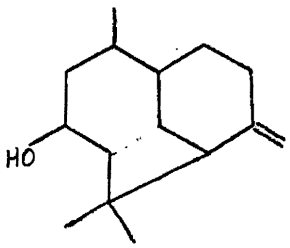
α-cyclocostunolide — Frullania tamarisci (L.) Dum — "



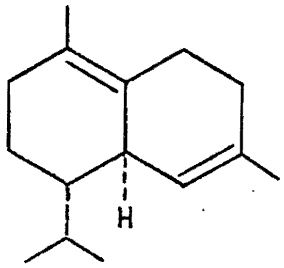
Cuparene — Bazzania pompeana (L.) Mitt — (10)



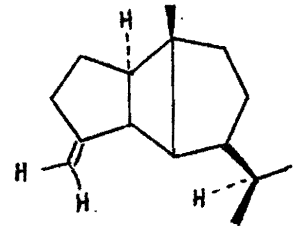
Bazzanenol — " — (38)



δ -Cadinene — Conocephalum Conicum (L.) Underw. — (11)



β-Bourbonene — Scapania parvitexta (Steph.) (11)



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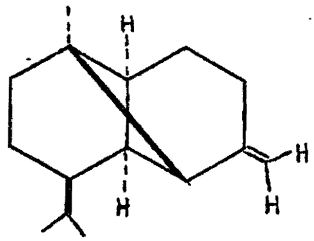
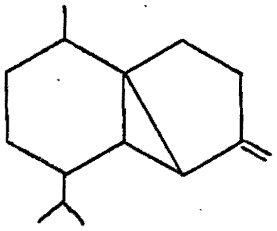
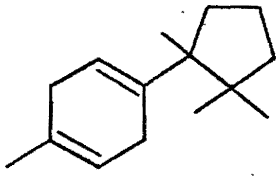


β-Cubebene — " "

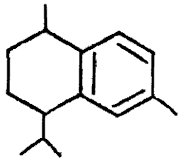


γ-Cuprenene — " "

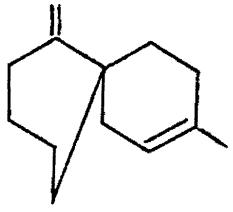


Calamenene

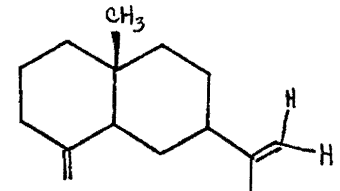
1. Scapania parvitexta (Steph.) (1)
2. Barbilophozia species (4)
3. Bazzamia Trilobata (37)



β-Chamigrene — Scapania parvitexta (Steph) (1)



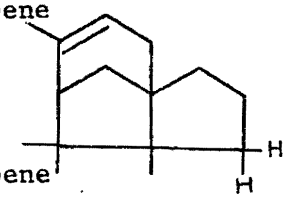
β-Selinene — Scapania parvitexta (Steph.) (1)



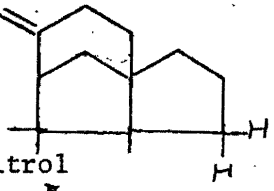
(-) Cuparene — " (1)

Bazzania pompeana (Lac.) Mitt

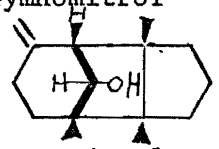
α-Pompene — Bazzania pompeana (L.) Mitt (39)



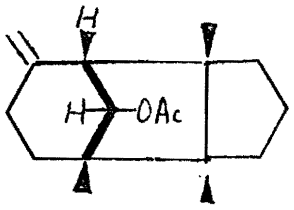
β-Pompene — Bazzania pompeana (L.) Mitt "



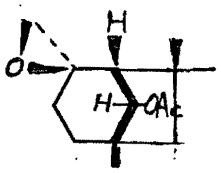
Gymnomitrol — Gymnomitrion obtusum (Lindb) Pears (40)



Gymnomitrol acetate — " (40)

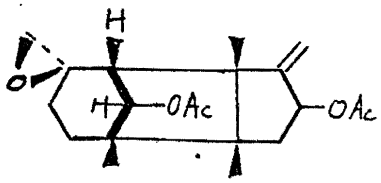


Epoxygymnomitrol acetate



Gymnomitrion obtusum (Lindb) Pears (40)

Eposygymnomitrol diacetate



"

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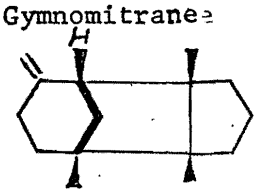
Gymnomitrane



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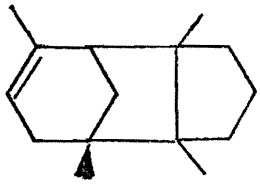
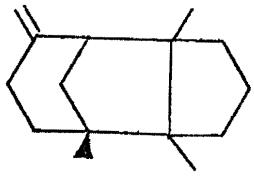
α- Barbatene



Barbilophozia barbata (4)

Barbilophozia floerkei
Bazzania tribobata (34)

β-Barbatene

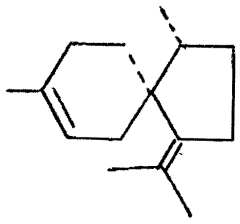


Barbilophozia barbata (34)
B. floerkei
B. lycopodioides
B. attenuata

Chiloscyphus polyanthis (L) Corda (41)

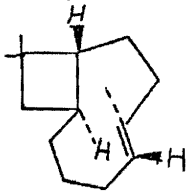
Scapania undulata
Bazzania trilobata (34)

α-Alaskene

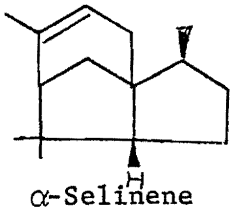


Barbilophozia barbata (4)

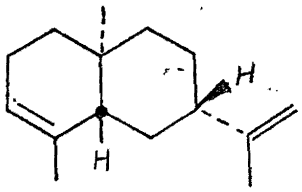
Caryophyllene (β-caryophyllene) — Barbilophozia barbata — (4)



α-Cedrene — Barbilophozia barbata — (4)



α-Selinene — Chiloscyphus polyanthus (L.) Corda — (41)

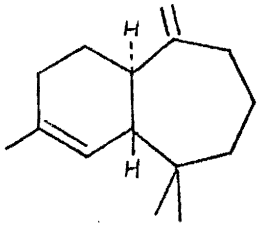


Longipinenes — Scapania undulata — (4,32)

(+)-Maalioxide — Plagiochila acanthophylla Gott. Subsp. Japonica (Lac.) Inoue. — Unpublished by A.Matuso

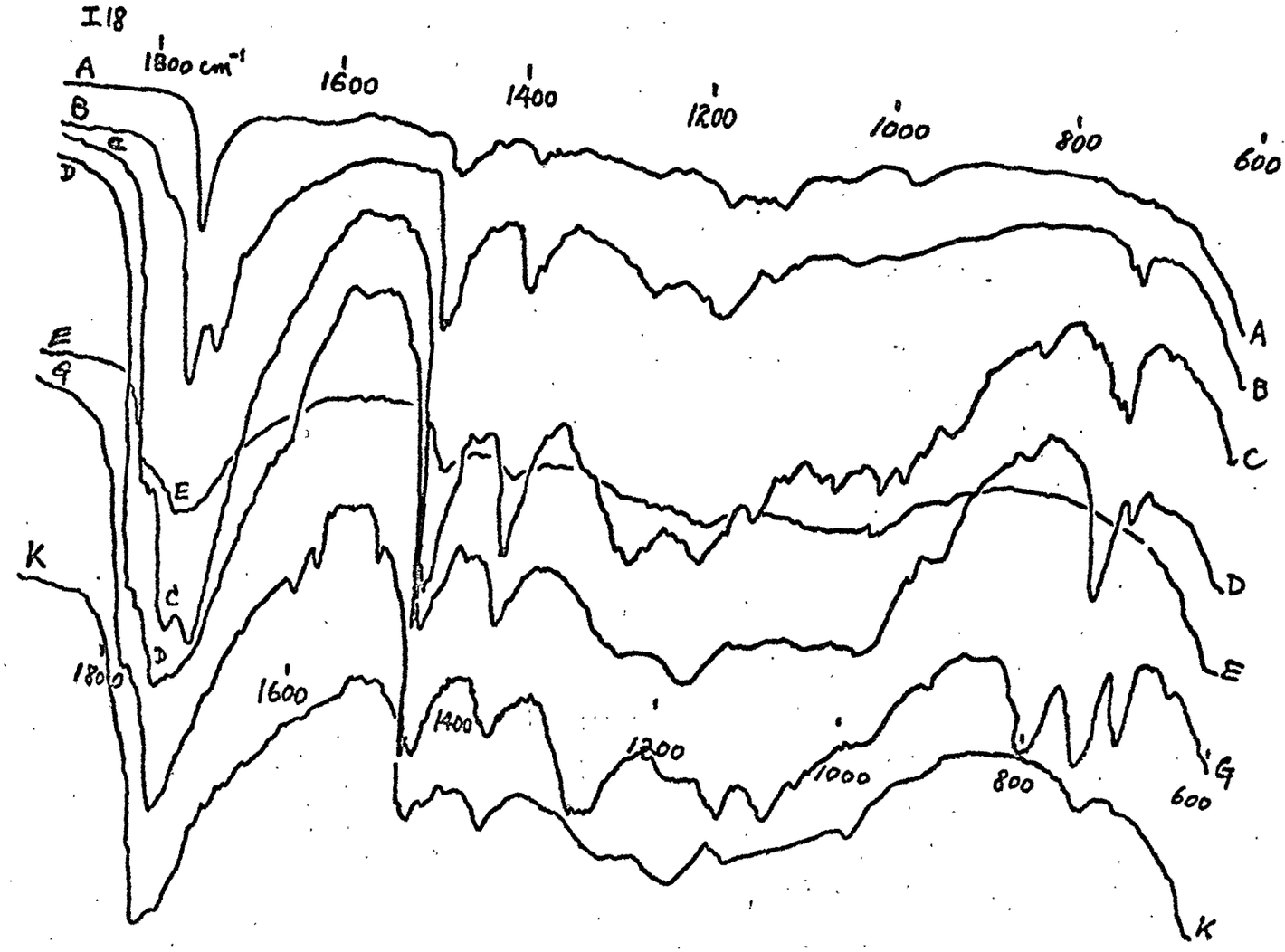
δ -Cuparenol — Scapania undulata — (42)

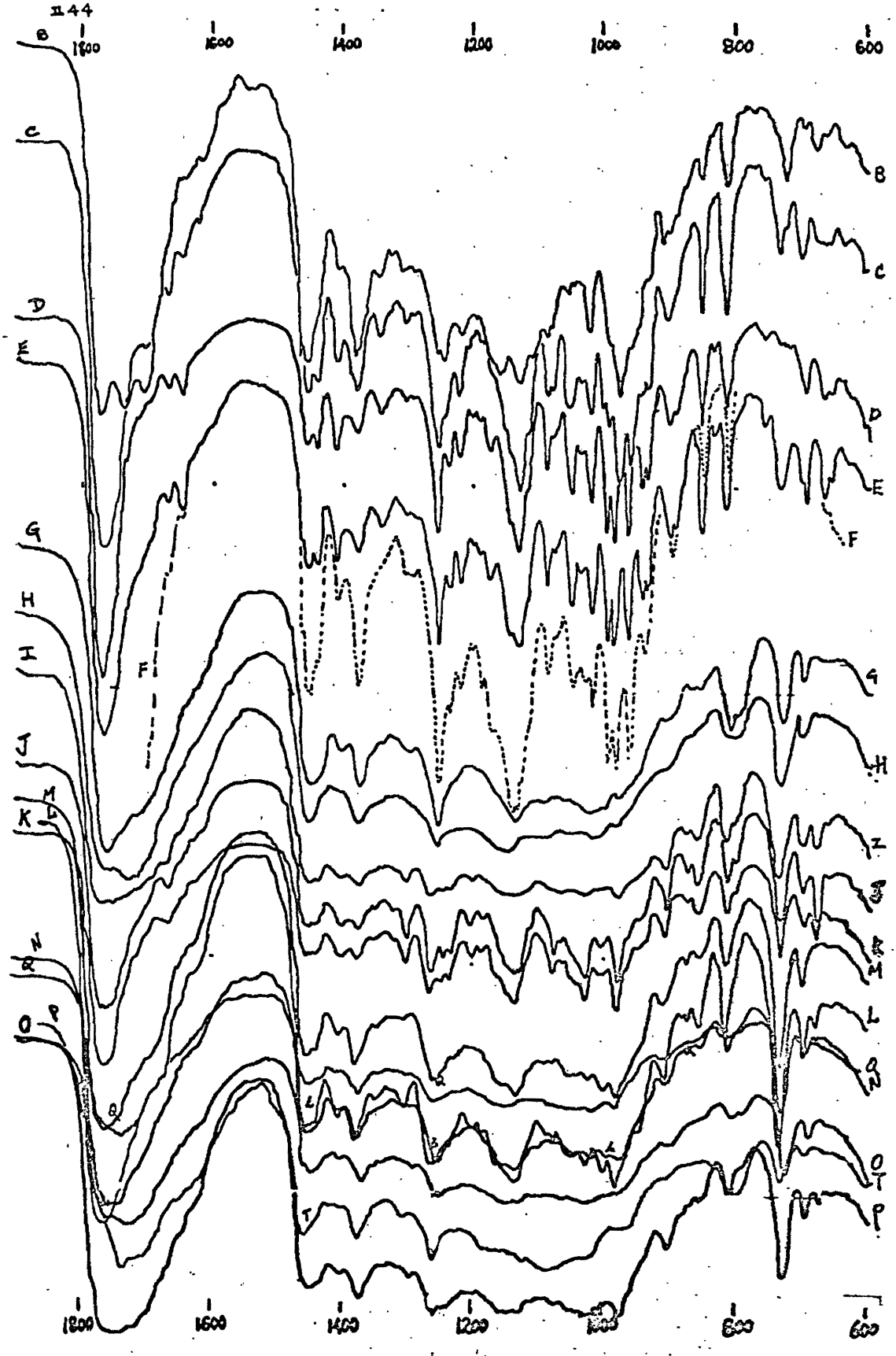
(+)α-himachalene — Scapania undulata — (32)

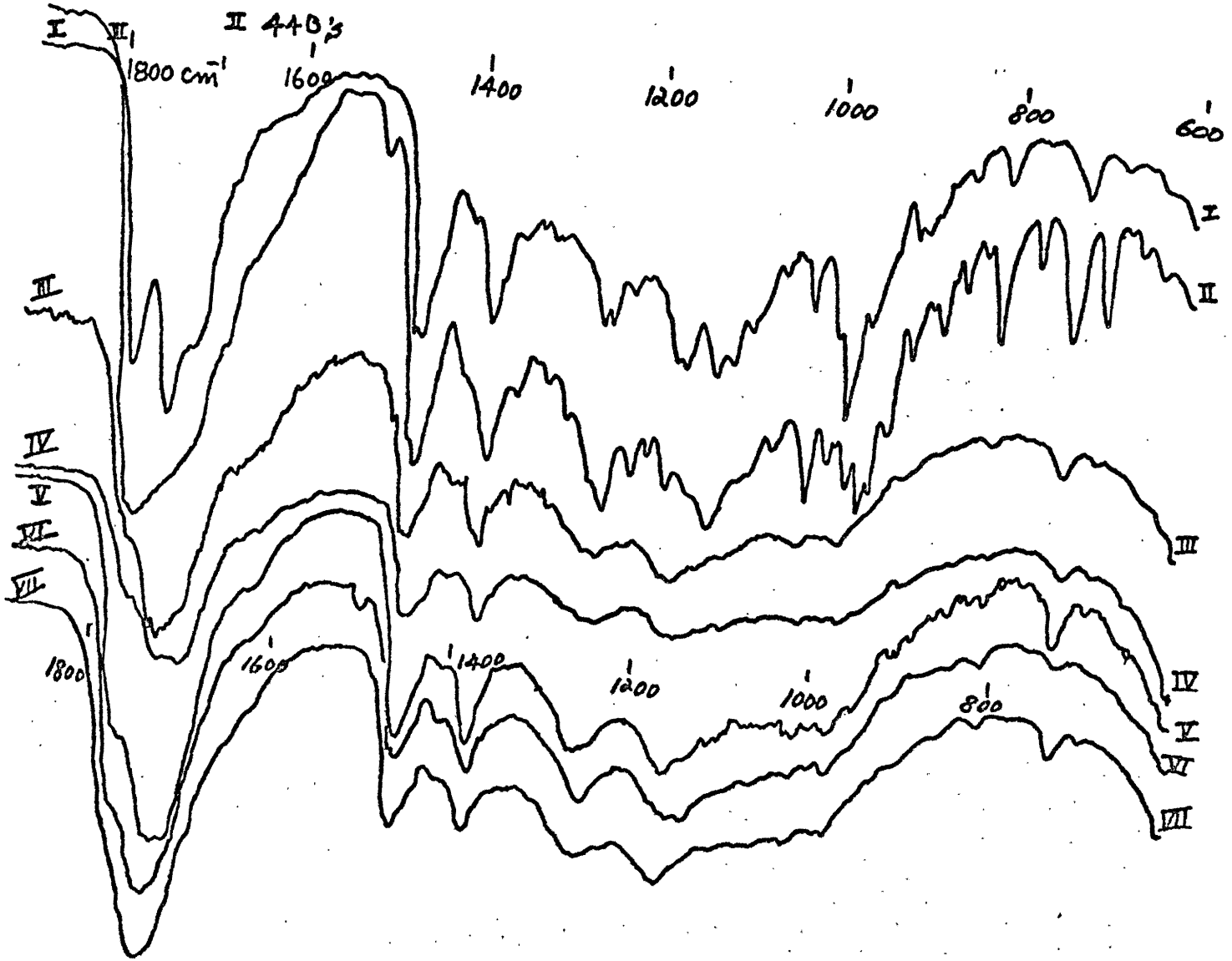


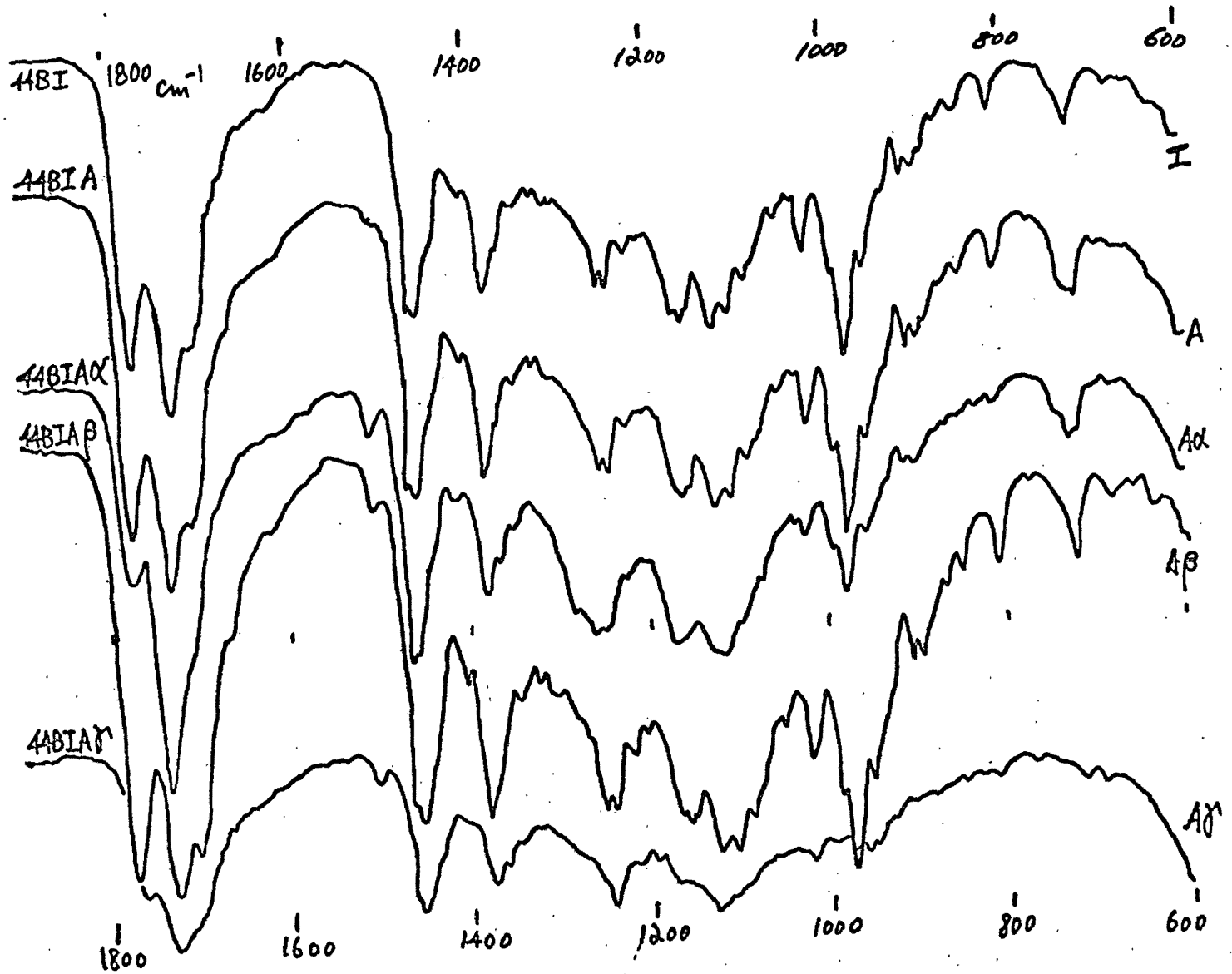
APPENDIX II

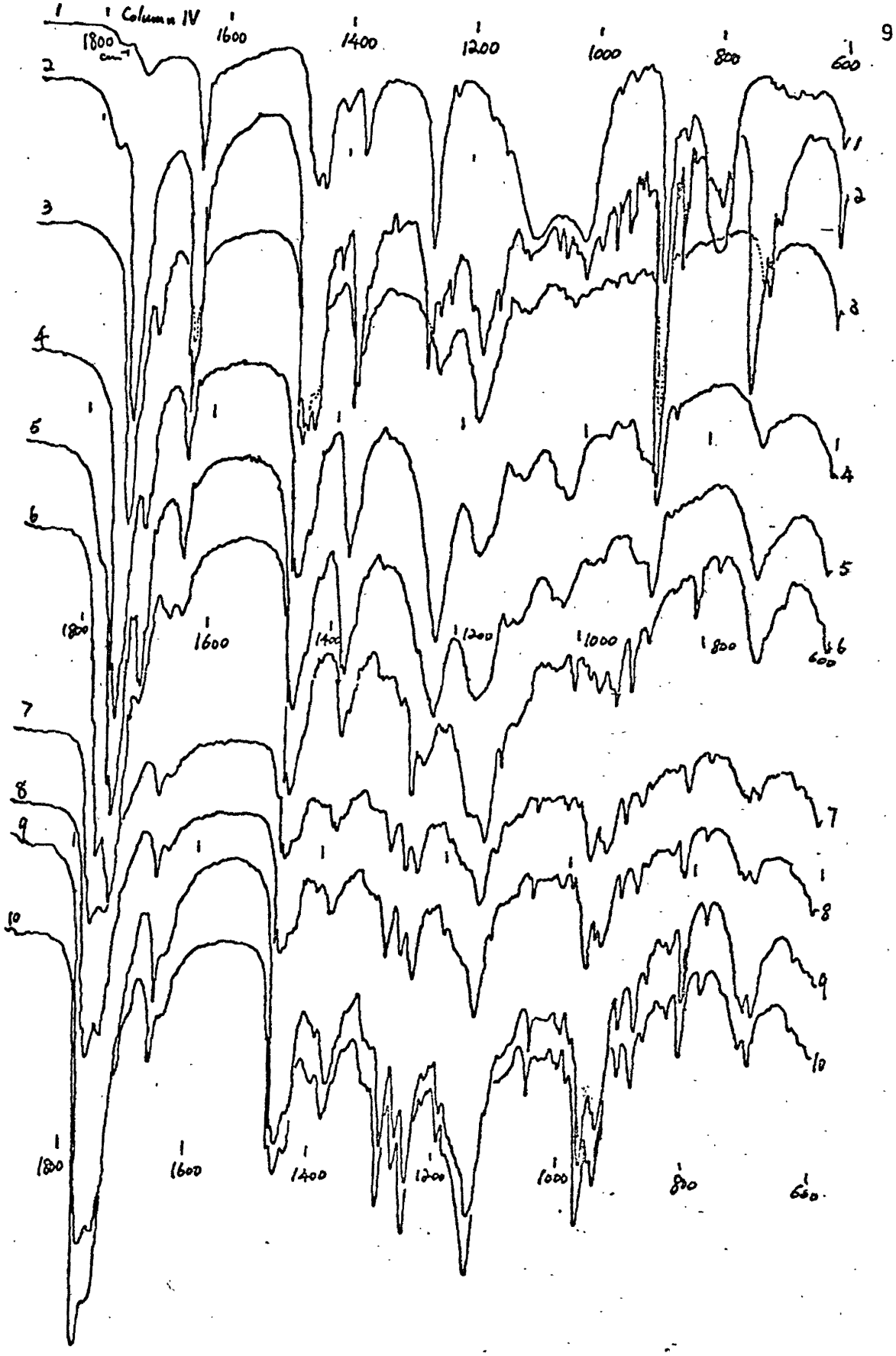
Infrared Spectra of Fractions


I18
A
1800 cm⁻¹
1600
1400
1200
1000
800
600
B
C
D
E
G
K
A
B
C
D
E
G
K
1800
1600
1400
1200
1000
800
600


II 44
B
C
D
E
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
1800
1600
1400
1200
1000
800
600


I
II
III
IV
V
VI
VII
1800 cm⁻¹
1600
1400
1200
1000
800
600


44BI
1800 cm-1
1600
1400
1200
1000
800
600
I
44BIA
A
44BIAα
Aα
44BIAβ
Aβ
44BIAγ
Aγ
1800
1600
1400
1200
1000
800
600


1
Column IV
1800
1600
1400
1200
1000
800
600
cm⁻¹
2
3
4
5
6
7
8
9
10
VI
2
3
4
5
6
7
8
9
10