1974

A phytochemical investigation of liverwort Frullania franciscana Howe

Timothy Ta-E Huang
Portland State University

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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, $R_f' = 25.8$, 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 $\delta$ 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 $\alpha$-cedrene and $\beta$-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 $\gamma$-lactone.
A PHYTOCHEMICAL INVESTIGATION OF LIVERWORT FRULLANIA franciscana HOWE

by

Timothy Ta-E Huang

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

CHEMISTRY

Portland State University
1974
TO THE OFFICE OF GRADUATE STUDIES AND RESEARCH:

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I wish to thank Dr. Alfred S. Levinson for his guidance during the course of this research, and for many personal kindesses.

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I wish to thank Dr. Doyle Daves and Mr. Bill Anderson of the Oregon Graduate Center for the gas chromatography-mass spectrum.

And, I wish to thank Mrs. Lillian Dixon for her help in the preparation of this manuscript.

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 thallophytes (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 Muller 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 Aneura pinguis (L) Dum., Riccardia sinnata (Hook) Trev., Conociphaleum 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 tamarisci 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. attenuata (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).

\[ \text{(I)} \]

\[ \text{(II)} \]

Frullanolide was reported in F. nisquallensis Sull. (37), F. dilatata (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).
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 \( \alpha \)-methylene-\( \gamma \)-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

* 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-
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 \( \alpha \)-methyl-\( \gamma \)-lactones in liverwort \textit{Frullania franciscana} Howe, the extract-
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 overall 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\(^{-1}\) (\(>\text{C=CH}\)), 1643 cm\(^{-1}\) (\(\text{C=C}\)), 888cm\(^{-1}\) \(^R\text{C=CH}_2\), or exo \(\text{C=CH}_2\); nuclear magnetic resonance: \(\delta 4.73 \text{ ppm}\), (exo double bond with the ring numbered six or larger, \(\text{C=CH}_2\)). 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 (ΔI/ΔT = 1.1 ± 0.25 for the entire group) and thus Kovats' indices can be reproduced to ± 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 identity 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 155° IAP-L 1530, 130° SE-30 1480.7, 132° Carbw-20M 1732.4, 165° Carbw-20M 1752.6, 205° Carbw-20M 1841.9, and 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 $R_{f}' = 25.7$ 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 $R_{f}' = 25.8$, 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. \( R_f' \) equals to \( R_f \times 100 \). (Refer to Table II.)

* The extraction was done by Dr. Levinson.
**TABLE I**

COLUMN CHROMATOGRAPHIC DATA
FOR SEVERAL FRACTIONS
OF COLUMN I**

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>Weight (g)</th>
<th>Solvent for Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A***</td>
<td>0.20</td>
<td>Benzene</td>
</tr>
<tr>
<td>3</td>
<td>0.05</td>
<td>Benzene</td>
</tr>
<tr>
<td>5</td>
<td>0.05</td>
<td>Benzene</td>
</tr>
<tr>
<td>7</td>
<td>0.05</td>
<td>4% Chloroform in Benzene</td>
</tr>
<tr>
<td>9</td>
<td>0.02</td>
<td>12% Chloroform in Benzene</td>
</tr>
<tr>
<td>11</td>
<td>0.01</td>
<td>20% Chloroform in Benzene</td>
</tr>
<tr>
<td>13</td>
<td>0.01</td>
<td>20% Chloroform in Benzene</td>
</tr>
</tbody>
</table>

** 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

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>No. of TLC</th>
<th>Solvent Used</th>
<th>No. of Spots</th>
<th>Rf'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>1</td>
<td>Benzene</td>
<td>3</td>
<td>67/22.4/13.2</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Benzene/Et Ac* 1/1</td>
<td>2</td>
<td>66.4/57.6</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Benzene/Acetone 1/1</td>
<td></td>
<td>Polarity of solvent was too high to give a good separation.</td>
</tr>
<tr>
<td>2</td>
<td>I. Cyclohexane/Et Ac</td>
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* Et Ac = ethyl Acetate
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** Considerable tailing noted.
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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 cm\(^{-1}\) 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.
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<tr>
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<td>25</td>
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<td>1710 &amp; 1738--carbonyl</td>
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<td>C</td>
<td>Benzene/Et Ac 9/1</td>
<td>75</td>
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<td>3300–3600 b,OH</td>
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<td></td>
<td></td>
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<td>1710,1738, &amp;</td>
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<td></td>
<td></td>
<td>1770--carbonyl</td>
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<tr>
<td>D</td>
<td>Benzene/Et Ac 8/2</td>
<td>50</td>
<td>34.8</td>
<td>3300–3600 b,OH</td>
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<td>--carbonyl</td>
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<td>3300–3600 b, OH</td>
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<td>1700–1780 s,b</td>
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<td></td>
<td>--carbonyl</td>
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<tr>
<td>L</td>
<td>Dioxane</td>
<td>80</td>
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Figure 1: COLUMN F-1-18

mg

50

45

40

35

30

25

20

15

10

5

0

A B C D E F G H I J K

34 ml Benzene

25 ml Benz./Et. Ac.

75 ml Benz./Et. Ac.

50 ml Benz./Et. Ac.

50 ml Benz./Et. Ac.

100 ml Benz./Et. Ac.

50 ml Benz./Et. Ac.

50 ml isopropyl Ether

50 ml CHCl3

50 ml abs. EtOH
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* Strong haloing and tailing observed.

** May have higher value due to the difficulty of fixing the real position of spot.
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Figure 2b : Column II (cont.)
Figure 2c: Column II (cont.)
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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

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* Haloing observed
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Figure 3: Thin Layer Chromatograms of Fractions II-1 Through II-32
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<th>Mass (mg)</th>
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<td>2.1</td>
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</tr>
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<tr>
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Figure 4: COLUMN F-11-44
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.
I344B (63.8 mg) → CHCl₃/Ether 8/2, then Cyclohexane/Et Ac 1/1

II44B (26.8 mg) → Cyclohexane/Ethyl Acetate 50/50 twice

CHCl₃/Ether 80/20 II44BIA (25.3 mg) → twice

II44BIA, 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
II44BVI, 3.9 mg, baseline

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

II44BIAα**, 6.4 mg, Rf' = 83.3
II44BIAβ**, 7.1 mg, Rf' = 73.4
II44BIAγ**, 2.6 mg, 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; II44BIAβ: blood-red; II44BIAγ: red-brown; II44BIAδ & ε: dark yellow
Under normal light: all green;
Analysis of sub-Fraction II44BIAγ, 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)

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<th>Butyl acetate</th>
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<tr>
<td>II-44C</td>
<td>53.9/27.3</td>
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</table>

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

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<td>haloing and tailing</td>
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Solvent used: Butyl acetate

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* May be higher
TABLE X
THIN LAYER CHROMATOGRAPHY OF COMBINATION FRACTIONS

Solvent: pet. ether/CHCl₃/Et Ac 4/4/2, conc. H₂SO₄ spray at room temperature, then 90 seconds, 120°C.

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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 in 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% CHCl₃/Benzene; Fraction 25: 60% CHCl₃/Benzene; Fraction 26: 70% CHCl₃/Benzene; Fraction 27-31: 80% CHCl₃/Benzene.
TABLE XI

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

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>Rf'</th>
<th>86.9</th>
<th>79.6</th>
<th>71.5</th>
<th>64.2</th>
<th>52.6</th>
<th>42.3</th>
<th>34.3</th>
<th>16.8</th>
<th>7.3</th>
<th>3.6</th>
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<td>31</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
TABLE XII

INFRARED ANALYSIS OF FRACTIONS
III-19 THROUGH 31

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

b=broad, m=medium, s=strong, w=weak, sd=shoulder
<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>Rf'</th>
<th>Mass of Extracted Material</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>93.3</td>
<td>5.5</td>
</tr>
<tr>
<td>B</td>
<td>88.7</td>
<td>?</td>
</tr>
<tr>
<td>C</td>
<td>81.3</td>
<td>6.4</td>
</tr>
<tr>
<td>D</td>
<td>69.3</td>
<td>11.7</td>
</tr>
<tr>
<td>E</td>
<td>60.0</td>
<td>5.5</td>
</tr>
<tr>
<td>F</td>
<td>56.7</td>
<td>3.9</td>
</tr>
<tr>
<td>G</td>
<td>53.3</td>
<td>5.7</td>
</tr>
<tr>
<td>H</td>
<td>49.3</td>
<td>9.9</td>
</tr>
<tr>
<td>I</td>
<td>32.0</td>
<td>14.1</td>
</tr>
<tr>
<td>J</td>
<td>21.3</td>
<td>4.4</td>
</tr>
<tr>
<td>K</td>
<td>12.0</td>
<td>3.5</td>
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<tr>
<td>L</td>
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<td>5.4</td>
</tr>
<tr>
<td>M</td>
<td>baseline</td>
<td>5.5</td>
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</tbody>
</table>
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 sub-
fractions 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 follow-
ing flow chart.

* sd = shoulder, b = broad, m = medium, w = weak, s = strong
** pet. ether: b.p. 30-60°C
Sample (80 g)
n-hexane extraction, 500ml x2 x 24 hrs
room temperature

Extract
Concentrated with Roto Vap.
Yellow-Green
0.9215 gm (1.15% from 80gm)

Infrared**
2D-TLC***
Sample was destroyed by accident

Residue
Ether Extraction,
500ml x2 x 40 hrs
room temperature

Extract
Residue
1.0013gm (1.25%
from 80gm)

Total % yield = 1.15% + 1.25% = 2.40%

Infrared**
2D-TLC***
Column IV

** 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

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Rf^1</th>
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<tbody>
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<td>A</td>
<td>92.6</td>
</tr>
<tr>
<td>B</td>
<td>86.8</td>
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<td>C</td>
<td>82.4</td>
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<td>D</td>
<td>73.5</td>
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<tr>
<td>E</td>
<td>61.0</td>
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<tr>
<td>F</td>
<td>57.4</td>
</tr>
<tr>
<td>G</td>
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<tr>
<td>H</td>
<td>46.7</td>
</tr>
<tr>
<td>I</td>
<td>46.7</td>
</tr>
<tr>
<td>J</td>
<td>29.2^g</td>
</tr>
<tr>
<td>K</td>
<td>7.4</td>
</tr>
<tr>
<td>L</td>
<td>7.4</td>
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<tr>
<td>M</td>
<td>No separation</td>
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</table>

^g: green
<table>
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<tr>
<th>Spot No.</th>
<th>Rf' in Solvent</th>
<th>Present in n-Hexane and/or Ether Extract</th>
<th>Color</th>
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<td></td>
<td>I</td>
<td>II</td>
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<td>A</td>
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<td>C</td>
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<td>D</td>
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<td>S</td>
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<tr>
<td>T</td>
<td>5.67</td>
<td>32.31</td>
<td>no</td>
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</table>
Figure 5: 2-D Thin Layer Chromatogram

13.0 cm
← n-Hexane
Extract

14.1 cm
← Benzene/Ethyl Ether
60/40

↑ Cyclohexane/Ethyl Acetate
80/20

Gray
A
B
C
Yellow

Brown
D

Green
G

Brown-Grey
E

Purple
I

Pink
J

K

Brown
P

Purple
M

Brown
N

F

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z


Figure 6: 2-D Thin Layer Chromatogram

13.0 cm²

Ethyl Ether

Extract

↑ 14.1 cm

cyclohexane:ethyl acetate 80:20

← Benzene/Ethyl Ether 60:40
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.

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<th>t_R, (min)</th>
<th>Peak Area %</th>
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</thead>
<tbody>
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</tr>
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<td>98.6</td>
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<td>Fr. No.</td>
<td>Solvent</td>
</tr>
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<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>1</td>
<td>n-hexane</td>
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<tr>
<td>2</td>
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<td>9</td>
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<td>10</td>
<td>ethanol</td>
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Figure 7: COLUMN IV
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<thead>
<tr>
<th>Fr. No.</th>
<th>Wave Number (cm(^{-1}))</th>
<th>Remarks</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>3080m, 1643s, 888s,</td>
<td>exo double bond</td>
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<tr>
<td>2</td>
<td>2930s, 2864s, 2848s, 1453m, 1441m, 1378m,</td>
<td>methyl, methylene</td>
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<tr>
<td>3</td>
<td>1729m, 1265m,</td>
<td>carbonyl (ester)</td>
</tr>
<tr>
<td>4</td>
<td>1735s, 1272-1285m,b,</td>
<td>carbonyl</td>
</tr>
<tr>
<td>5</td>
<td>poor spectrum</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1735m,</td>
<td>carbonyl</td>
</tr>
<tr>
<td>7</td>
<td>3005w, 1640w,</td>
<td>double bond</td>
</tr>
<tr>
<td></td>
<td>1736s,</td>
<td>carbonyl</td>
</tr>
<tr>
<td>8</td>
<td>3300-3600w,b,</td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td>3510w, 1762s,b, 1290s, 1244s,</td>
<td>γ-lactone</td>
</tr>
<tr>
<td></td>
<td>3060w, 1665m, 888m</td>
<td>double bond</td>
</tr>
<tr>
<td>9</td>
<td>3300-3600m,b,</td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td>3050w,</td>
<td>double bond</td>
</tr>
<tr>
<td></td>
<td>1700-1780b,s,</td>
<td>carbonyl</td>
</tr>
<tr>
<td>10</td>
<td>3300-3600w,b,</td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td>1700-1780b,s,</td>
<td>carbonyl</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>Fr. No.</th>
<th>Rf'</th>
<th>Similar to Spot of Table XV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1</td>
<td>67.5</td>
<td>85.3</td>
</tr>
<tr>
<td>7</td>
<td>59.3*</td>
<td>76.9*</td>
</tr>
<tr>
<td>8</td>
<td>58.0*</td>
<td>85.2*</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>$t_R$, (min)</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.33</td>
<td>2.35</td>
</tr>
<tr>
<td>2.35</td>
<td>0.32</td>
</tr>
<tr>
<td>3.63</td>
<td>97.33</td>
</tr>
</tbody>
</table>

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.

\[
\text{Area} = \frac{1}{2} AD \times BC
\]

The percentage of component $A = \frac{A}{\Sigma A_i} \times 100$
Figure 8a: 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

Operator: [Signature]
Date: 05/2/74

Column No.: Mat'l
Length: Disa.
10% Aperture: 0.25

Conc. - Coating: Support: Mesh
TEMP: Column: Isothermal: 18.6°C
Programmed: Initial: 100°C/min.
Rate: 10°C/min. Final: 200°C/min.
Inj. Temperature: 220°C
Detector Temperature: 300°C
CARRIER GAS: N2
Rate: 30 ml/min

Pressure: Inlet: Outlet:
DETECTOR: 210 V or Volts
Soak: Rate: 30 ml/min. Baseline: 0 v
F.I.D.: Rate: press Hydrogen: Air
SAMPLE: Fraction IV-1
Size: 0.15 ml
Solvent: X: Viscous: Concent.

98.6%
peak no. 3

1.4%
peak no. 1
Figure 9b  Gas Chromatogram of Fraction IV-1, Temperature

<table>
<thead>
<tr>
<th>Column</th>
<th>Mat'l</th>
<th>Length</th>
<th>Diam.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% SP 80 on Anderson AAS 100/110</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conc.: Coating Support Mesh

Col:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Programmed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Rate</td>
<td>Rate</td>
</tr>
</tbody>
</table>

Inj.:

- °C Detector

Carrier GAS:

- No. Rate ml/min.

Inlet

- Outlet

Detectors:

- FID

- O or Volts, Sens

- Rate ml/min. Rec Range mv

FID:

- Rate/press Hydrogen

Solvent:

- n-decane

<table>
<thead>
<tr>
<th>Solvent</th>
<th>n-decane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.</td>
<td>97.3%</td>
</tr>
<tr>
<td>Peak</td>
<td>No. 3</td>
</tr>
<tr>
<td></td>
<td>2.6%</td>
</tr>
<tr>
<td>Peak</td>
<td>No. 1</td>
</tr>
</tbody>
</table>
where A was the area of peak A, and Σ A_i was the sum of the areas of all the peaks.

Further Gas Chromatography of Fraction IV-I

Fraction IV-I from the nuclear magnetic resonance tube was coinjected 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.

\[
* I_x = I_{stdA} + \frac{\log t'_{R(X)} - \log t'_{std(A)}}{\log t'_{std(B)} - \log t'_{std(A)}} \times (I_{std(B)} - I_{std(A)})
\]

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>
<thead>
<tr>
<th>Component</th>
<th>Apiezon-L</th>
<th>SE-30</th>
<th>Carbowax-20M</th>
<th>DEGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-bourbonene</td>
<td>1418.3</td>
<td>1386</td>
<td>1547</td>
<td>1586.5</td>
</tr>
<tr>
<td>8-caryophyllene</td>
<td>1451.7</td>
<td>1417.5</td>
<td>1618.5</td>
<td>1655.5</td>
</tr>
<tr>
<td></td>
<td>(literature)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-caryophyllene</td>
<td>1451.3</td>
<td>1417.9</td>
<td>1622.6</td>
<td>1655.2</td>
</tr>
<tr>
<td></td>
<td>(calculation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-cedrene</td>
<td>1473.4</td>
<td>1414</td>
<td>1597.5</td>
<td>1640</td>
</tr>
<tr>
<td>peak no. 3</td>
<td>1530</td>
<td>1480.7</td>
<td>1732.4</td>
<td>1752.6</td>
</tr>
</tbody>
</table>
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

Operator Bill Anderson Date July 1974

Col No. Mat'l Length Diam.

Conc. - Coating Support Mesh

TEMP: Column: SE-30 Isothermal °C

Programmed Initial °C/min. Final °C/min.

Inj. °C Detector °C

CARRIER GAS Rate ml/min.

Pressures: Inlet Outlet

DETECTOR Flame Conduct or Volts, Sens.

Scav. Rate ml/min. Rec Range mv

F.I.D. Rates/press Hydrogen Air

SAMPLE Fraction IV-1 Size

Solvent CHCl₃ Concn. ~ 10 µg/ml

CHCl₃ n-Decane Fraction IV-1

175 °C

100 °C
Figure 11: Mass Spectrum of Fraction-IV-I
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: \( R_f' = 48.6 \) and \( R_f' = 25.7 \).

The band with \( R_f' = 25.7 \) 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:

- \( 3510 \text{ cm}^{-1} \text{ w, } 1762 \text{ cm}^{-1} \text{ s, } 1290 \text{ cm}^{-1} \text{ s, } 1244 \text{ cm}^{-1} \text{ s, } \gamma \text{-lactone; } 3060 \text{ cm}^{-1} \text{ w, } 1665 \text{ cm}^{-1} \text{ m, } 888 \text{ cm}^{-1} \text{ m, double bond.} \)

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.
BIBLIOGRAPHY


18. K.R. Markham, Phytochem., 11, 2047 (1972).


44. Hand-out from Dr. Alfred S. Levinson
### APPENDIX I

**TABLE OF COMPOUNDS ISOLATED FROM LIVERWORTS**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>From</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. N-alkanes and Ester Waxes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-alkanes</td>
<td><em>Calypogea meylanii</em> (Buch)</td>
<td>(5)</td>
</tr>
<tr>
<td><em>(C&lt;sub&gt;15&lt;/sub&gt;-C&lt;sub&gt;35&lt;/sub&gt;)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>esters</td>
<td><em>Jungermania aphaerocarpa</em> (Hook)</td>
<td></td>
</tr>
<tr>
<td><em>(C&lt;sub&gt;34&lt;/sub&gt;-C&lt;sub&gt;54&lt;/sub&gt;)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Calypogea trichomanis</em> (L.) Corda</td>
<td>(7)(8)</td>
</tr>
<tr>
<td></td>
<td><em>Calypogea trichomanis</em> (L.) Corda</td>
<td>(8)</td>
</tr>
<tr>
<td></td>
<td><em>Calypogea trichomanis</em> (L.) Corda</td>
<td>(9)</td>
</tr>
</tbody>
</table>

**B. Alkaloids**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>From</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-(3-methyl-2-bentenyl)-indole</td>
<td><em>Riccardia sinnata</em> (Hook). Trev</td>
<td>(6)</td>
</tr>
<tr>
<td>6-(3-methyl-2-bentenyl)-indole</td>
<td><em>Riccardia sinnata</em> (Hook). Trev</td>
<td></td>
</tr>
</tbody>
</table>

**C. Azulenes and Indenes**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>From</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4-dimethyl-azulene</td>
<td><em>Calypogea trichomanis</em> (L.) Corda</td>
<td>(7)(8)</td>
</tr>
<tr>
<td>4-methyl-1-methoxy-carbonyl-azulen</td>
<td><em>Calypogea trichomanis</em> (L.) Corda</td>
<td>(8)</td>
</tr>
<tr>
<td>3,7-dimethyl-5-methoxy-carbonyl-inden</td>
<td><em>Calypogea trichomanis</em> (L.) Corda</td>
<td>(9)</td>
</tr>
</tbody>
</table>
D. Alcohols and Acid

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Plant Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonacosan-10-ol</td>
<td>Bazzania Pompeana (Lac.) Mitt (10)</td>
</tr>
<tr>
<td>Lignoceric Acid (Tetracosanoic acid)</td>
<td>Conocephalum conicum (L.) Underw (11)</td>
</tr>
<tr>
<td>Triterpenic diols</td>
<td>Conocephalum conicum (L.) Underw</td>
</tr>
</tbody>
</table>

E. Flavonoids

<table>
<thead>
<tr>
<th>flavone</th>
<th>Plant Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponarin (I)</td>
<td>Madoteca platyphylla (12)</td>
</tr>
<tr>
<td>Saponaretin (II)</td>
<td>Pellia neesiana (Gettsche) Limpr. (14)</td>
</tr>
<tr>
<td>Pellepiphyllin</td>
<td>Marchantia foliacea (Mitt) and M. berteroana (L.) and (L.) (15)</td>
</tr>
<tr>
<td>7-O-B-D-glucuronides of Apigenin Chrysoeriol Tricin</td>
<td>Marchantia foliacea (Mitt) and M. berteroana (L.) and (L.) (15)</td>
</tr>
<tr>
<td>Apigenin-6,8-di-C-glucoside (Vincenin-2)</td>
<td>Hymmenophytum flabellatum (16)</td>
</tr>
<tr>
<td>Isovitexin</td>
<td>Rebonlia hemispherica (17)</td>
</tr>
<tr>
<td>Rhamnosyl-glucuronides of above flavones minor constituents</td>
<td>Monoclea forsteri (18)</td>
</tr>
<tr>
<td>6,8-di-C-glycosides of 5,7,4'-trihydroxyflavone</td>
<td>Porella platyphylla (13)</td>
</tr>
<tr>
<td>Acacetin 7-O-rhamnosyl-galacturonide 0-glycoside of an acadetin 8-C-glycoside</td>
<td>Porella platyphlla</td>
</tr>
<tr>
<td>8-methoxy-5,7,3',4'-tetrahydroxy-flavone (bound to [a] water soluble polysaccharide(s)</td>
<td></td>
</tr>
</tbody>
</table>
F. Steroids

\[ \text{β-sitosterol} \quad \text{Pingius (L.) Dum} \quad (11)(19) \]

\[ \text{Brassicasterol} \]

\[ \text{Campesterol} \]

\[ \text{Stigmasterol} \]

\[ \text{Cholesterol} \]

\[ \text{Cholest-5-en-3β-ol} \quad \text{Jungermanniacaeae (J. thermarum)} \quad (20) \]

\[ \text{24-methylcholest-5,22-dien-3β-ol} \quad \text{Scapaniaceae} \quad (20) \]

\[ \text{24-ethycholest-5,22-dien-3β-ol} \quad \text{Plagiochiclacea} \quad (20) \]

G. Mono-, di-, and Triterpenoids

\[ \text{Friedelin} \quad \text{Frullania tamarisci} \quad (21) \]

\[ \beta\text{-Carotene} \quad \text{Aneura Pinguis (L.) Dum} \quad (11) \]

\[ \text{ent-Kaurene} \quad \text{Solenostoma triste (Nees) K. Mull} \quad (22) \]
(-)-16β-Hydroxy-kauran

Anthelia julacea (L.) Dum
Anthelia juratzkana (Limpr.) Trev.

(-)-Manool

Jungermannia torticalyx

ent-Kaurene hydroxy-ketone

Solenostoma triste (Nees) K. Mull

ent-Kaurene unsaturated hydroxy-ketone

ent-kaurene diol

H. Sesquiterpenoids

Myliol

Mylia taylorii (Hook) Gray

Chiloscyphus polyanthus (L.) Corda

(23) (24) (25, 26) (27-29)
Isolongifolene

(-) longifolene

(-) Longiborneol

Bazzanene

Frullanolide

Costunolide

<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolongifolene</td>
<td><em>Scapania subalpina</em>, <em>Scapania uliginosa</em>, <em>Scapania undulata</em></td>
</tr>
<tr>
<td>(-) longifolene</td>
<td><em>Scapania undulata</em> (L.) Dum</td>
</tr>
<tr>
<td>(-) Longiborneol</td>
<td><em>Scapania parvitexta</em> (Steph.)</td>
</tr>
<tr>
<td>Bazzanene</td>
<td><em>Bazzania pompeana</em> (Lac.) Mitt</td>
</tr>
<tr>
<td>Frullanolide</td>
<td><em>Barbilophozia floerkei</em>, <em>Bazzania trilofata</em>, <em>Frullania tamarisci</em> (L.) Dum</td>
</tr>
<tr>
<td>Costunolide</td>
<td><em>Frullania tamarisci</em> (L.) Dum</td>
</tr>
</tbody>
</table>
\( \gamma \)-cyclocostunolide \( Frullania tamarisci \) (L.) Dum (35)

\( \alpha \)-cyclocostunolide \( Frullania tamarisci \) (L.) Dum

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

Bazzanenol

\( \delta \)-Cadinene \( Conocephalum Conicum \) (L.) Underw. (11)
β-Bourbonene

\[ \text{Scapania parvitexta (Steph.)} \] (11)

β-Ylangene

\[ \text{Scapania parvitexta (Steph.)} \] (1)

β-Cubebene

γ-Cuprenene

Calamenene

1. \text{Scapania parvitexta (Steph.)} (1)
2. \text{Barbilophozia species} (4)
3. \text{Bazzamia Trilobata} (37)

β-Chamigrene

\[ \text{Scapania parvitexta (Steph)} \] (1)
\[ \text{\textbf{Bazzania Pompeana (Lac.) Mitt}} \]

\[ \text{\textbf{α-Pompene}} \]

\[ \text{\textbf{β-Pompene}} \]

\[ \text{\textbf{Gymnomitrol}} \]

\[ \text{\textbf{Gymnomitrol acetate}} \]

\[ \text{\textbf{Scapania parvitexta (Steph.)}} \]

\[ \text{\textbf{Bazzania pompeana (L.) Mitt}} \] (1)

\[ \text{\textbf{Bazzania pompeana (L.) Mitt}} \] (39)

\[ \text{\textbf{Gymnomitron obtusum (Lindb) Pears}} \] (40)

\[ \text{\textbf{Gymnomitrol acetate}} \] (40)
Epoxygymnomitrol acetate

Gymnomitrane

α-Barbatene

Barbilophozia barbata (4)

Barbilophozia floerkei (34)

Bazzania tribobata

β-Barbatene

Barbilophozia barbata (34)

Bazzania tribobata

Chiloscyphus polyanthis (41)

(1) Corda

Scapania undulata (34)

Bazzania trilobata

Barbilophozia barbata (4)
Caryophyllene
(β-caryophyllene)

α-Cedrene

α-Selinene

Longipinenes

(+)α-himachalene

Barbilophozia barbata

Chiloscyphus polyanthus (L.) Corda

Scapania undulata

Plagiochila acanthophylla Gott.
Subsp. Japonica (Lac.) Inoue.

Scapania undulata

Scapania undulata

Scapania undulata

Unpublished by A. Matuso
APPENDIX II

Infrared Spectra of Fractions