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AN ABSTRACT OF THE THESIS OF
BURL C. CARTER for the degree of MASTER OF SCIENCE
in CHEMISTRY presented on May 7, 1968

Title: BACTERIAL DEGRADATION of METHYL DEHYDROABIETATE

ABSTRACT APPROVED:

Thesis Advisor

ABSTRACT

Bacteria (Arthrobacter sp.), isolated from lodgepole pine (Pinus contortus), are capable of utilizing methyl dehydroabietate as the sole source of carbon. Degradation products of methyl dehydroabietate have been obtained by manually extracting the cell-free supernatant solution of a liquid growth medium with methylene chloride. Gas chromatography (G. L. C.) was used for separation of the neutral products. The acid products were converted to their methyl esters by diazomethane and then separated by gas chromatography. Structures have been proposed for two G. L. C. fractions (V, II) on the basis of infrared (I. R.) and mass spectral (M. S.) data.

The molecular weight of V is 328, corresponding to the introduction of one oxygen atom into methyl dehydroabietate, (M. W. 314) and loss of two protons. Infrared peaks at 5.73μ and 5.77μ , and the absence of a large P-1 mass spectral peak (P-1 is due to loss of the aldehydic hydrogen) indicate that the material is a keto-ester. A comparison of the fragmentation pattern of V and the fragmentation pattern of methyl dehydroabietate indicates that the ketone carbonyl group is in ring A of methyl dehydroabietate and is β to the ester carbonyl group.

The molecular weight of II is 260. Present in its infrared spectrum are peaks associated with a non-conjugated methyl ester (5.73μ C=O; 8.30μ , 8.39μ , 8.54μ , C-O) and the aromatic ring

(6.21 μ , 6.67 μ , and 6.89 μ) with isopropyl substitution(7.30 μ , 7.37 μ). Significant mass spectral peaks at (1) 233 (P-27), (2) 201 (P-59), (3) 200 (P-60), (4) 159 (P-101) and (5) 129 (P-131) are consistent with structure II.

V

II

BACTERIAL DEGRADATION OF METHYL DEHYDROABIETATE

by

Burl C. Carter

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BACTERIAL DEGRADATION OF METHYL DEHYDROABIETATE

by

Burl C. Carter

A Thesis submitted to the Graduate Committee
for partial fulfillment of the requirements for
the degree of Master of Science.

APPROVED:

Thesis Advisor

Department Head

Dean, Graduate Studies

Date thesis presented: May 7, 1968.

Typed by: Mrs. Delia D. Green

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INTRODUCTION

There has been an interest in resin acids for many years.

One of the results of this long interest is a reasonably good knowledge of the processes involved in chemical oxidation of resin acids.

Another area of interest, and one in which there is little knowledge, is the area of microbial oxidation of resin acids. This research is part of a research program to elucidate the processes involved in bacterial oxidation of methyl dehydroabietate. Methyl dehydroabietate was chosen as a substrate because it contains both aromatic and saturated ring systems, and existing purification methods are efficient and effective.

HISTORY

Chemical Oxidation

Oxidation of dehydroabietic acid to a 7-keto derivative has been accomplished by molecular oxygen passed through an alkaline solution.¹ The same keto-acid was prepared by alkaline permanganate oxidation.² The 7-keto derivative of methyl dehydroabietate was obtained by oxidation with molecular oxygen. Oxidation at the 18 position to the corresponding hydroperoxide was also detected.³ Chromic acid oxidation of methyl dinitrodehydroabietate resulted in a keto-acid (A) in equilibrium with its lactol form (B). The same reaction was also carried out on the unsubstituted ester.⁴

Podocarpa-8, 11, 13, trien-15-oic acid-- , 13-isopropyl; methyl ester of _____.
(methyl dehydroabietate)

Dehydroabietic acid has been converted indirectly to 1-ketonordehydroabietane. Treatment with lead tetraacetate gave the intermediate (C) which upon oxidation yielded the 1-keto compound (D).

(C)

(D)

Oxidation following condensation of (D) with ethyl formate yielded the diacid (E).⁵

(E)

Microbial Oxidation

In view of structural similarity, one might expect that microbial oxidation of methyl dehydroabietate would be similar to that of phenanthrene. Phenanthrene is end-ring oxidized to a dihydroxy derivative (F). Further oxidation (enzymatic) of F resulted in ring cleavage. Non-enzymatic oxidation of F yielded the diketone G.⁶

(F)

(G)

Androst-5-en-7-one (H) has been oxidized by microbes to the 12-hydroxy derivative (J) and to the 3-hydroxy derivative (K).⁷

(H)

(J)

Recently, Biellmann and co-workers have shown that the 3-keto compound (L) is obtained upon bacterial oxidation of dehydroabietic acid.⁸

(K)

(L)

DISCUSSION

Compound III. R. Data:

An intense peak at 5.73μ indicates the presence of at least one non-conjugated carbonyl group. The presence of aromatic 6.21μ , 6.67μ , and 6.89μ peaks and a gem-dimethyl doublet (7.30μ , 7.37μ) indicate that ring C of methyl dehydroabietate has been left intact. The prominent peaks at 8.30μ , 8.39μ , and 8.54μ are undoubtedly those peaks associated with the C-O bond of a methyl ester.* The peak of moderate intensity at 7.0μ is probably due to C-H bending vibrations of a cyclic hydrocarbon.

M. S. Data:

The molecular weight of II, determined by mass spectrometry, is 260. The known presence of an isopropylphenyl group (mass 118 for 3 substituents on the ring, or mass 119 for 2 substituents on the ring), and a carbomethoxyl group (mass 59) accounts for either 177 or 178 of the 260 molecular weight.

The remaining mass (83 for 2 substituents on the aromatic ring, or 82 for 3 substituents on the aromatic ring) corresponds to C_6H_{11} (mass 83) or C_6H_{10} (mass 82). These carbon-hydrogen ratios require either unsaturation or cyclization. Evidence supporting cyclization, i. e. retention of the B ring of methyl dehydroabietate,

* The acid of II was converted to a methyl ester by diazomethane.

is found in the mass spectrometry fragmentation patterns of II. The base peak (most intense peak) of II, corresponding to the most stable ion or ion fragment, is at 159. Dimethyl substituted 1, 2, 3, 4-tetrahydronaphthalene* (with one abstracted H) is a reasonable choice, especially in view of the stability requirement. Moreover, there are fragments corresponding to the loss of H and one methyl (mass 143, 16.7% relative intensity), two methyls (mass 129, 28.5% relative intensity), and two methyls and H (mass 128, 21.8% relative intensity) from the dimethyl substituted 1, 2, 3, 4-tetrahydronaphthalene fragment. In view of this, four isomers can be drawn.**

*1, 2, 3, 4-tetrahydronaphthalene skeleton is obtained by removal of ring A of methyl dehydroabietate.

**A fifth isomer, II E, is considered unlikely since no reasonable fragmentation can be drawn for the P-27 peak; also, due to the fact that a P-29 (231) peak (loss of C_2H_5) is absent in fragmentation of II.

The fragmentation patterns of II require a structure that can satisfy the following conditions: a structure that can lose mass 27, mass 41, mass 55, and mass 59 with apparent rearrangement such that H migration gives rise to a P-60 fragment.

Bond cleavage seems to occur predominately between the most substituted carbons.⁹

Cleavage of the C₍₅₎-C₍₆₎ and C₍₇₎-C₍₈₎ bonds followed by rearrangement (where hydrogen migrates from the 28 mass fragment) would account for the P-27 peak for the four previously mentioned isomers.

*Podocarpane numbers are used here and on all subsequent structures.

The most probable rearrangement to occur is one similar to the McLafferty rearrangement of esters whereby a hydrogen atom on the carbon γ to the carbonyl group migrates to the oxygen of the carbonyl group.¹⁰ Only II_B , II_C and II_D can assume the proper steric position to accommodate such a rearrangement. Cleavage of the $\text{C}_{(9)}-\text{C}_{(10)}$ and $\text{C}_{(5)}-\text{C}_{(10)}$ bonds of II_B with the same type of rearrangement would account for the P-27 fragment. II_D could also lose 27 a. m. u. by $\text{C}_{(5)}-\text{C}_{(10)}$ and $\text{C}_{(5)}-\text{C}_{(6)}$ bond cleavage with the same rearrangement.

Moreover, only II_D , by very similar fragmentation, can lose 41, and 55 a. m. u.

Loss of 59 and 60 a. m. u. follows much the same scheme. P-59 corresponds to the loss of carbomethoxyl and P-60 corresponds to the loss of carbomethoxyl plus proton where the hydrogen on the ester carbonyl oxygen comes from the carbon γ to the carbonyl group. See table M. S. - II.

Fraction IIII. R.

Fraction III is a mixture of at least two compounds (see gas chromatograph). Although no positive identification could be made, some interesting points are suggested by the infrared spectrum. Two peaks are present in the region of carbonyl absorption. One peak of high intensity is at 5.77μ and the second peak of lower intensity is at 6.03μ . A carbonyl absorbing near 6.03μ could be either a carbonyl adjacent to the aromatic ring, or a carbonyl of an ester chelated by the enol form of a keto-ester.¹¹ A weak O-H peak at 2.9μ supports the latter.

Compound IV

The infrared spectrum of IV is very similar to that of methyl dehydroabietate. The $6.03\ \mu$ peak was suspected to be due to an unresolved minor component. The G. L. C. retention times of IV and methyl dehydroabietate were the same, thus affording identification of the major component of IV. The presence of peaks at 260 a. m. u. (7% intensity) and 233 a. m. u. (4.5% intensity) in the mass spectrometry fragmentation pattern of IV (peaks lacking in the fragmentation pattern of methyl dehydroabietate) confirmed the suspicion that a minor component was present in IV.

Since IV was obtained on acid extraction, methyl dehydroabietate must have been hydrolyzed during the growth period. A control was run in order to determine whether hydrolysis was due to chemical action or due to bacterial action. A 1.0 gram sample of methyl dehydroabietate was subjected to the same condition found during growth except that the media was not inoculated with the bacteria. Upon acid extraction, a very small amount (less than 1 mg) of material was obtained. An infrared spectrum showed the material to be methyl dehydroabietate with a very small amount of impurity. It was concluded that hydrolysis of the ester was due to bacterial action rather than chemical action.

Fraction VG. L. C.

The presence of one peak suggests a reasonably pure fraction, although mass spectral data indicate a small amount of impurities.*

I. R.

Two intense peaks in the infrared spectrum at 5.73μ and 5.77μ indicate the presence of at least two non-conjugated carbonyl groups. Also present are the peaks associated with the aromatic ring (6.21μ , 6.67μ and 6.89μ) and the isopropyl group (7.30μ and 7.37μ) indicating that ring C and the isopropyl group of methyl dehydroabietate have been left intact.

M. S.

The molecular weight of V was determined by mass spectrometry to be 328 a. m. u. This corresponds to the introduction of one oxygen atom into methyl dehydroabietate (m. w. 314) and loss of two protons. Since infrared data indicates non-benzilic oxidation, the following sites are possible:

(A) Ketones: $C_{(1)}$, $C_{(2)}$, $C_{(3)}$, $C_{(6)}$; (B) Aldehydes: $C_{(16)}$, $C_{(17)}$.

Absence of an intense mass spectral peak at 327 (P-1) indicates that introduction of oxygen led to formation of a ketone rather than an aldehyde. (Aldehydes have large P-1 mass spectral

*Peaks 314, 312, 297, 296 requiring very unusual fragmentation of the parent (P 328) are considered to be due to impurities.

peaks due to cleavage of the aldehydic proton).¹²

A comparison of the mass spectrum of V with that of methyl dehydroabietate reveals that both have peaks at 128, 129, and 141

a. m. u. corresponding to the following:

This would suggest that oxidation occurred in ring A of methyl dehydroabietate, and that oxidation occurred at either C₍₂₎ or C₍₃₎ since the fragments (128, 129, 141) are a result of ring A cleavage.

A ketonic carbonyl at C₍₁₎ would result in ring B cleavage upon electron impact.¹³ Therefore, the ketone carbonyl is either at C₍₂₎, or C₍₃₎.

Microbial oxidation of dehydroabietic acid results in oxidation at the 3-position.¹⁴ The structure of V is proposed to be:

EXPERIMENTAL *

Bacterial Isolation:**

Bacteria were isolated by the enrichment technique using bark and /or dry needles of lodgepole pine as the inoculum source.

A mineral salt medium consisting of:

(1)	KH_2PO_4	1.7	grams/liter
(2)	K_2HPO_4	4.4	grams/liter
(3)	NH_4Cl	2	grams/liter
(4)	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.04	grams/liter
(5)	$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.05	grams/liter
(6)	$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.05	grams/liter
(7)	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.01	grams/liter
(8)	NaMoO_4	0.01	grams/liter
(9)	Methyl abietate***	1.0	grams/liter

was used for the enrichment procedure and for subsequent growth to obtain degradation products.

*Bacteria were aerated with a New Brunswick Gyrotory shaker model G-25. Removal of cells was accomplished with a Servall model RC-2 centrifuge. Melting points (uncorrected) were taken on a Büchi melting point apparatus. An Aerograph autoprep model A-700 was used for separation of extracted portions. Liquid nitrogen was used as a coolant during collection. Infrared spectra were taken on a Perkin-Elmer model 137 B Infracord spectrophotometer and on a Beckman IR 12. Mass spectral data was obtained on an Atlas model CH-4 mass spectrometer operating at 70 eV voltage, 10 μ A current and an inlet temperature of 180° C.

**Work of Dr. M. Taylor, Dept. of Biology, P. S. C.

***Matheson, Coleman and Bell (Practical). Methyl abietate was used as the nutrient only during bacterial isolation.

Isolation of bacteria was by streaking the liquid enrichment cultures onto plates containing the above medium with 2% agar. After testing each strain of bacteria under various conditions, optimum yields of degradation products were obtained from cultures of an uncharacterized species of Arthrobacter cultivated at room temperature (24-26° C).

Stock cultures were maintained on nutrient agar (Difco), stored at 4° C, and were transferred every two months.

Purification of methyl abietate by chromatography (gas chromatography, column chromatography) proved to be impractical, therefore, the nutrient was changed to methyl dehydroabietate.

Purification of Methyl Dehydroabietate:

N-wood resin* (230.03 g) was disproportionated by heating and stirring under nitrogen atmosphere with 0.1% (0.2375 g) of 10% palladium on carbon at 260° C for 1½ hours.¹⁵ After cooling, 575 ml. of 95% ethanol was added. The solution was filtered using a fritted disc glass filter with a 3/16 inch Celite filter aid. The solution was heated to 70-80° C and 45 ml. of monoethanolamine was added, followed by 500 ml. of water at 80° C. The reaction mixture was extracted 3 times using 150-200 ml. aliquots of cyclohexane. Care was taken to keep the solution above 60° C during extraction. After extraction, the solution was refrigerated and the crystals were

*Hercules Powder Co.

collected, slurried with 500 ml. of 50% ethanol and collected again. The product was recrystallized twice from 95% ethanol; the volume of the mother liquor was reduced and a second crop of crystals was collected, and recrystallized twice from 95% ethanol. The combined first and second crop crystals were recrystallized a third time from 95% ethanol. The crystals (ethanolamine salt of dehydroabietic acid) were dissolved in 310 ml. of 95% ethanol by stirring while heating. To the solution, 50 ml. of hydrochloric acid (54% by volume) was added in order to give a pH of 3. Crystals of dehydroabietic acid were collected following cooling in a refrigerator. The volume of the mother liquor was reduced and more crystals were collected. The combined crystals were recrystallized twice from 1000 ml. of 75% ethanol. A total of 56.07 g (48.7% theoretical yield) of pure (m. p. 170.5-171.5) dehydroabietic acid was obtained.

Dehydroabietic acid (12.7570 g) was allowed to react with 15 ml. of purified thionyl chloride over a steam bath for one hour.¹⁶ By-products and unreacted thionyl chloride were swept from the acid chloride by a nitrogen jet. Disappearance of a 5.94μ peak and appearance of a 5.62μ peak in the infrared spectrum of the reaction mixture indicated that the reaction was complete.

To the acid chloride was added 25 ml. of distilled methanol, followed by 15 ml. of distilled methylene chloride to obtain miscibility. The reaction mixture was placed in a water bath (55°C) for

2 hours and then let stand overnight. Unreacted methanol and methylene chloride were swept from the ester by a nitrogen jet. The solution, obtained from addition of 150 ml. of methylene chloride to the oily product, was washed with dilute aqueous base, dried with sodium sulfate, and evaporated to dryness. The oily product was dissolved in 50 ml. of 95% ethanol, and Norite was added to the solution. The solution was filtered with a fritted disc glass filter with a 1/4 inch Celite filter aid, and the filtrate was refrigerated. Needle-like crystals were collected; a second crop of crystals was collected from the mother liquor. Both crops were recrystallized from 95% ethanol, and air dried for two days. A yield of 6.913 grams (54.2% theoretical) of methyl dehydroabietate (m.p. 61.5-62.5° C) was obtained. G. L. C. data showed but one peak.

Growth of Bacteria on Methyl Dehydroabietate:

Two-liter flasks containing 500 ml. of sterile salt medium and 0.4 g of purified methyl dehydroabietate, inoculated with bacteria from nutrient agar plates, were vigorously aerated at 24-26° C for 15-20 days. Cells were removed by centrifugation at 10,000 x g for 15 minutes at 4° C. The supernatants were combined and any solid material remaining in the yellow-pink solution was removed by vacuum filtration through Whatman #1 filter paper.

Extraction of Degradation Products:

Method 1. A ten-liter jug containing 4 liters of yellow-pink solution was placed over a magnetic mixer. The solution was made alkaline with 5 Normal sodium hydroxide and extracted 3 times with 125 ml. aliquots of methylene chloride by allowing the magnetic mixer to operate overnight for each aliquot. The methylene chloride was dried with sodium sulfate, filtered with a fritted disc glass filter, and the neutral degradation products were obtained after removal of methylene chloride by reduced pressure. The acidic products were obtained in a similar operation after acidification with hydrochloric acid.

Method 2. The yellow-pink solution (2 liters) was made alkaline with 10 ml. 5 Normal sodium hydroxide, and manually extracted 4 times with 100 ml. aliquots of methylene chloride. The solvent was dried and filtered as in method 1. Neutral degradation products were obtained upon removal of methylene chloride by careful distillation.* Acidic products were obtained in the same way following addition of 20 ml. of 5 Molar hydrochloric acid. From a total of 4 liters containing 2.8 grams of methyl dehydroabietate, extracted as in method 2, 18.0 mg of neutral and 67.8 mg of acidic degradation products were obtained.

*Distillation was performed with a 90 cm column packed with glass helices.

From 4 liters of solution, extracted as in method 1, 15.0 mg of acidic degradation products was obtained. The percent yield obtained on extracting as in method 2 was greater than the percent obtained on extracting as in method 1. Method 2 was preferred since the method was much quicker and more efficient. The results on all of the extractions (method 2) gave the same results.

Methylation of Acidic Degradation Products:

The acidic products were converted to methyl esters by diazomethane.¹⁷ A 500 ml. flask and two 8 inch test tubes were connected by glass tubing, arranged such that nitrogen entering the container bubbled through the contents of that container. A nitrogen tank was connected to the 500 ml. flask which had been filled with 200 ml. of ethyl ether. Connected to the flask was an 8 inch test tube containing 7 ml. of ethyl ether, 7 ml. of 2-(2'-ethoxy-ethoxy)-ethanol, and 10 ml. of a solution of 6 grams of potassium hydroxide in 10 ml. water. The second 8 inch test tube, connected to the first test tube, contained 175.7 mg of acidic degradation products dissolved in 20-30 ml. of 10% methanol in ethyl ether. A trap of acetic acid in ethyl ether was connected to the second 8 inch tube, and was employed for the destruction of excess diazomethane. After all leaks in the system were eliminated, 253.0 mg of N-methyl-N-nitroso-p-toluene sulfonamide dissolved in a minimum of ethyl ether was added to the tube containing potassium hydroxide. The nitrogen was adjusted to a very slow rate, and the reaction was run for 4 hours.

Separation by Gas Chromotography:

The methyl esters of the acidic degradation products were separated into five fractions by an Aerograph model A-700 operating at the following conditions: *

column:	20% SE 30, 10 ft x 3/8 in. 60/80 mesh. (Chrom. W.)
column temperature:	290° C
collector temperature:	293° C
detector temperature:	315° C
injector temperature:	297° C
filament current:	175 mA
carrier gas:	Helium
gas flow rate:	200 ml/min.
attenuation:	16
sample size: **	100 μ liters
collecting coolant:	liquid nitrogen

The following quantities of the fractions II, III, IV, V were obtained:

II	15.8 mg
III	7.0 mg
IV	3.3 mg
V	3.6 mg

*The neutral degradation product consisted mostly of unused methyl dehydroabietate, with a presently inseparable minor component.

**Total number of 100 μ liter sample injections to collect 175.7 mg of sample was 4.

Infrared spectra were taken as a liquid film on KBr plates with a Perkin-Elmer model 137B spectrophotometer, and with a Beckman 12 spectrophotometer for precise wave numbers.

Mass spectral data (see Appendix I) were obtained on an Atlas CH-4 Mass Spectrometer located at Oregon State University in the Food Technology Department.

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APPENDIX I - SPECTRA

GAS CHROMATOGRAPHY

	% Area	Fraction
1.	_____	Solvent
2.	trace *	_____
3.	trace *	_____
4.	41.7	II
5.	0.7 *	_____
6.	1.6 *	_____
7.	10.1	III
8.	12.6	
9.	1.8 *	
10.	0.4 *	_____
11.	10.1	IV
12.	19.5	V

* Not collected.

Methyl Dehydroabietate/film, KBr

Fraction II. 1,2-dimethyl, 7-isopropyl, 1-carboxylic acid, 1, 2, 3, 4-tetrahydro-naphthalene; methyl ester of...../ film, KBr.

Fraction III. Mixture, unidentified/film, KBr

Fraction IV. Mixture, methyl dehydroabietate and unidentified component./film,KBr

Fraction V. Podocarpa-8, 11, 13-trien-15-oic acid, 13-isopropyl, 3-one: methyl
 ester of..... / film, KBr.

Fraction N. (Neutral). Methyl dehydroabietate and unidentified compound./film,KBr

MASS SPECTRUM

Methyl Dehydroabietate

Peak	Relative Intensity	Peak	Relative Intensity
27	3	59	3
28	13	60	*
29	4	61	*
30	*	63	*
31	2	64	*
32	1	65	1
33	*	66	*
39	2	67	3
41	11	68	*
42	1	69	3
43	15	70	*
44	1	71	1
45	1	73	1
46	*	76	*
51	*	77	3
52	*	78	1
53	2	79	2
54	*	81	3
55	6	82	*
56	*	83	1
57	*	84	1

*designates less than 1%.

Methyl Dehydroabietate (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
85	2	114	*
89	1	115	6
90	*	116	1
91	6	117	6
92	2	118	1
93	2	119	1
94	*	120	*
95	1	121	4
96	*	122	*
97	*	123	1
98	*	127	*
99	*	128	6
101	2	129	7
102	*	130	1
103	*	131	6
104	*	132	1
105	3	133	3
106	*	134	*
107	1	135	*
108	*	139	*
109	1	140	*
110	*	141	9
112	3	142	4
113	*	143	6

*designates less than 1%

Methyl Dehydroabietate (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
144	1	168	2
145	2	169	4
146	2	170	1
147	2	171	3
148	*	172	*
149	*	173	7
150	*	174	1
151	*	175	*
152	2	176	*
153	3	177	*
154	2	178	1
155	6	179	1
156	2	180	*
157	4	181	4
158	1	182	1
159	4	183	3
160	1	184	1
161	*	185	4
162	*	186	2
163	1	187	*
164	*	195	2
165	2	196	2
166	1	197	8
167	2	198	2

*designates less than 1%

Methyl Dehydroabietate (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
199	3	267	1
200	*	268	*
207	*	270	*
209	1	299	16
210	*	300	4
211	2	314	13
212	1	315	3
213	*		
214	*		
223	*		
224	*		
225	2		
226	*		
237	1		
238	1		
239	100		
240	21		
241	2		
243	*		
253	*		
254	*		
255	3		
256	*		

*designates less than 1%

MASS SPECTRUM

Fraction II

Peak	Relative Intensity	Peak	Relative Intensity
27	10.5	51	7.8
28	21.6	52	1.2
29	9.5	53	5.7
30	1.9	54	0.8
31	4.6	55	7.0
32	3.1	56	1.8
33	0.9	57	4.0
35	0.9	57 $\frac{1}{2}$	1.2
36	0.8	58	1.2
37	0.4	59	15.8
38	0.4	60	0.9
39	7.0	61	0.4
40	0.9	62	1.1
41	21.6	63	3.6
42	2.5	64	3.1
43	51.5	64 $\frac{1}{2}$	0.8
44	5.0	65	6.7
45	4.3	65 $\frac{1}{2}$	0.4
47	2.7	66	1.2
48	1.5	67	2.3
49	13.3	68	0.5
50	1.9	69	2.9

Fraction II (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
70	1.3	89	2.6
70½	1.2	90	0.9
71	4.3	91	26.6
71½	0.8	92	2.7
72	3.2	92½	0.4
72½	0.8	93	2.7
73	1.2	94	1.1
74	2.4	95	1.5
75	2.3	96	0.8
76	3.0	97	1.2
77	10.0	98	0.8
77½	0.9	99	0.8
78	6.3	99½	0.4
79	10.8	100	1.6
79½	1.9	101	2.8
80	0.8	102	3.1
81	1.8	103	4.6
82	0.9	104	2.7
83	1.3	105	9.3
84	7.8	106	1.5
85	1.3	107	1.5
86	6.3	108	0.8
87	2.3	108½	1.5
88	1.9	109	9.3

Fraction II (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
109½	2.7	139	1.3
110	0.4	140	1.2
111	0.6	141	8.9
113	1.2	142	7.8
114	1.2	143	16.7
115	24.8	144	5.4
116	11.4	145	12.4
116½	1.5	146	2.7
117	64.0	147	6.4
118	8.4	148	1.7
119	4.3	149	8.8
120	0.8	150	1.2
121	1.5	151	0.8
122	0.8	152	1.9
123	1.5	153	2.3
125	1.2	154	2.5
126	1.9	155	3.1
127	7.8	156	2.7
128	21.7	157	18.6
129	28.5	158	6.7
130	10.1	159	100.0
131	46.7	160	20.1
132	7.8	161	4.0
133	3.9	162	0.8

Fraction II (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
163	13.9	190	1.9
164	2.3	191	5.4
165	1.5	192	1.5
167	2.5	195	1.5
168	0.9	197	1.2
169	1.9	198	0.8
170	1.0	199	2.7
171	3.9	200	43.3
172	12.2	201	38.7
173	25.1	202	6.2
174	6.2	203	3.9
175	1.4	204	1.5
176	1.4	205	2.3
178	0.4	206	0.4
179	0.4	207	0.4
180	0.4	208	0.4
181	0.4	209	0.4
183	1.5	210	0.6
184	1.2	211	0.4
185	8.4	212	0.4
186	3.9	213	1.5
187	43.3	214	0.6
188	8.5	215	0.6
189	7.0	216	3.5

Fraction II (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
217	12.0	274	1.5
218	3.4	277	1.9
219	5.0	278	0.4
220	1.1	292	4.3
221	0.4	293	0.8
224	0.5	299	1.1
226	0.5	300	0.3
228	0.6	314	1.0
229	5.8		
230	1.5		
232	15.2		
233	27.9		
234	4.6		
235	0.4		
239	7.2		
240	1.7		
242	1.7		
243	0.5		
244	0.5		
245	7.4		
246	1.4		
260	68.1		
261	12.4		
262	1.9		

MASS SPECTRUM

Fraction IV

Peak	Relative Intensity	Peak	Relative Intensity
27	12.5	56	5.5
28	72.5	57	9.5
29	13.5	59	12.0
31	3.5	63	5.0
32	9.0	65	8.5
35	3.0	67	13.5
36	3.0	68	4.0
38	3.0	69	15.0
39	10.0	70	3.0
40	3.0	71	5.5
41	42.5	73	5.5
42	4.5	74	5.5
43	57.5	76	5.5
44	15.5	77	14.0
45	3.0	78	6.5
47	9.0	79	10.0
48	5.0	80	4.5
49	45.0	81	18.0
50	3.0	82	5.5
51	17.0	83	10.0
53	10.0	84	30.0
55	32.0	85	8.0

Fraction IV (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
86	20.0	115	20.0
88	7.5	116	10.0
89	10.0	117	17.5
90	3.0	118	5.0
91	22.5	119	8.0
92	7.5	120	4.5
93	8.5	121	17.5
94	3.0	122	5.5
95	13.0	123	8.5
96	4.0	125	3.0
99	7.5	127	8.0
100	5.0	128	19.0
101	4.0	129	25.5
102	3.0	130	8.0
103	5.0	131	17.5
104	3.0	132	3.0
105	16.0	133	10.5
106	4.0	134	6.0
107	11.0	135	5.0
108	3.5	136	2.5
109	12.5	137	2.5
111	6.5	139	3.0
112	8.0	141	25.0
113	2.5	142	17.5

Fraction IV (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
143	19.0	167	13.5
144	5.0	168	7.5
145	8.0	169	15.0
146	8.0	170	6.5
147	8.0	171	12.5
148	3.5	172	5.0
149	7.0	173	21.5
150	3.5	174	7.5
151	3.5	175	6.0
152	6.5	177	3.0
153	10.0	178	7.5
154	7.5	179	10.0
155	20.0	180	5.0
156	7.5	181	18.0
157	14.0	182	6.5
158	5.0	183	12.5
159	17.5	184	5.5
160	7.5	185	14.0
161	5.0	186	7.5
162	4.5	187	10.5
163	10.0	188	2.5
164	3.0	189	7.5
165	12.0	190	5.0
166	7.5	191	8.0

Fraction IV (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
192	8.0	219	7.5
193	8.0	223	10.0
194	2.5	224	3.0
195	12.5	225	7.5
196	6.0	226	3.0
197	27.5	233	4.5
198	7.5	234	4.5
199	10.0	235	3.5
200	7.5	237	20.0
201	7.5	238	5.0
202	5.0	239	100.0
203	3.0	240	45.0
204	3.0	241	10.0
205	3.0	251	8.5
206	3.0	252	5.0
207	5.5	253	8.5
208	3.0	254	5.0
209	7.0	255	10.0
210	4.5	256	5.0
211	11.0	260	7.0
212	5.0	261	2.5
213	5.0	267	6.0
214	3.0	268	3.5
215	5.0	269	2.5

Fraction IV (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
296	2.5		
297	2.5		
298	2.5		
299	37.5		
300	10.0		
312	7.5		
313	2.5		
314	25.0		
315	7.5		
316	3.5		

MASS SPECTRUM

Fraction V

Peak	Relative Intensity	Peak	Relative Intensity
27	25	63	8
28	225 (?)	64	6
29	25	65	8
30	6	67	17
31	25	69	19
32	49	71	10
39	21	73	10
41	61	74	10
42	6	75	6
43	107	76	6
44	47	77	18
45	6	78	11
47	14	79	21
48	6	81	11
49	78	82	6
50	6	83	17
51	33	84	50
53	11	85	10
55	35	86	22
56	8	87	6
57	10	88	8
59	21	89	12

Fraction V (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
91	35	129	38
92	8	130	15
93	14	131	46
95	15	132	7
96	7	133	15
97	19	135	10
101	14	139	14
103	12	140	7
104	6	141	35
105	28	142	21
107	15	143	38
109	18	144	7
110	8	145	15
111	6	146	8
112	7	147	11
115	32	149	24
116	14	151	6
117	33	152	15
118	15	153	19
119	18	154	12
121	10	155	33
123	6	156	14
127	18	157	28
128	38	158	11

Fraction V (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
159	70	185	24
160	14	186	12
161	11	187	47
163	17	188	8
165	25	189	12
166	12	191	8
167	53	192	8
168	17	193	31
169	21	194	8
170	11	195	22
171	21	196	6
172	11	197	32
173	31	198	8
174	6	199	15
175	6	200	36
176	6	201	26
177	8	202	7
178	14	203	8
179	21	204	8
180	12	205	8
181	21	207	14
182	11	208	7
183	31	209	28
184	11	210	24

Fraction V (Continued)

Peak	Relative Intensity	Peak	Relative Intensity
211	19	253	29
212	11	254	8
213	11	255	8
217	10	260	36
219	11	261	7
221	7	268	8
222	7	269	6
223	8	281	14
224	6	296	29
225	33	297	19
226	10	300	8
228	11	310	6
232	12	312	6
233	14	313	8
234	8	314	14
235	10	328	28
237	33	329	6
238	15		
239	100		
240	32		
241	12		
245	6		
251	18		
252	8		

APPENDIX II - CHARTS

Proposed M. S. Fragmentation of Methyl Dehydroabietate

Proposed M. S. Fragmentation of Fraction II

(Table M. S. II)

Proposed M. S. Fragmentation of Fraction V