Phytochemistry, 1965, Vol. 4, pp. 141 to 148. Pergamon Press Ltd. Printed in England

CHEMOTAXONOMY OF THE GENUS *ABIES*—I.
SURVEY OF THE TERPENES PRESENT IN THE *ABIES* BALSAMS

EUGENE ZAVARIN* and KAREL SNAJBERK†

University of California, Forest Products Laboratory, Richmond, California

(*Received* 2 *May* 1964)

Abstract—Terpenes of the balsams of seventeen *Abies* and three *Pseudotsuga* species and varieties were analyzed by gas–liquid chromatography. The differences in composition found indicate that this method can be used for distinguishing between some closely related species, although substantiation will have to be made through further population studies.

INTRODUCTION

THE genus *Abies* (firs) comprises about fifty species distributed in North and Central America, Asia, Europe, and Northern Africa. In spite of their common occurrence many aspects of their taxonomy seem greatly confused, with the morphological variability of species and lack of clear-cut distinguishing characteristics often resulting in controversies regarding the definition of taxa and the assignment of a particular population to the correct taxon. The likelihood of interspecific hybridization and introgression in some instances further complicates the situation.[1]

The present chemotaxonomic investigation was started in the hope of developing a method which will allow sharper delineation of the fir species, throw light on their relationships and which will be useful later in the studies of artificial and natural hybrids. Although fir wood is low in extractives and does not offer much promise for chemotaxonomic investigations,[2] young fir bark is generally covered with blisters up to a few milliliters in volume. The blisters are filled with balsam, an aromatic, honey-like liquid, composed largely of terpenes, sesquiterpenes, resin acids, fatty acids (in free form and as glycerides), and neutral nonvolatiles—thus it is somewhat similar to pine oleoresin. Since terpenoids of pine oleoresins have been successfully used to solve a number of taxonomic problems,[3] it was decided first to try the volatile portion of the fir balsam.

Surprisingly, in spite of the availability and technical and pharmaceutical importance of the *Abies* balsams, comparatively little work has been done on the chemical composition of their terpene constituents, and what has been done is usually of an unsystematic and fragmentary nature. The individual terpenes identified at one time or another included santene, α-pinene, camphene, β-pinene, Δ^3-carene, α-phellandrene (?), limonene, β-phellandrene, and terpinolene. Essentially only the composition of the terpenoid compounds

* Associate Forest Products Chemist.
† Assistant Specialist.

[1] E. L. PARKER, *Forest Sci.* 9, 207 (1963).
[2] G. L. CARLSBERG and E. F. KURTH, *T.A.P.P.I.* 43, 982 (1960).
[3] N. T. MIROV, *Composition of Gum Turpentine of Pines*, Tech. Bull. No. 1239, U.S. Dept. of Agri. (1961).

from balsams of *A. alba* Mill.,[4] *A. lasiocarpa* (Hook.) Nutt.[5] *A. balsamea* (L.) Mill.,[6] *A. mayriana* Miyabe and Kudo,[7] *A. sachalinensis* Mast. (mixture of the two was used), *A. sibirica* Ledeb.,[8] *A. amabilis* (Dougl.) Forbes, and *A. grandis* (Dougl.) Lindl.[9] has been determined. The preparative methods used did not permit complete analysis in some cases, however. Also, with the last two species the steam distillates of the barks, rather than volatile portions of the blister balsams were used. The difference, however, should not be large, as exemplified by the analyses of both materials from *A. sibirica*.[8] Apparently gas chromatographic technique was employed only once, for the analysis of the balsam of *A. balsamea*;[6a] the authors used only a silicone column and the resolution was relatively poor. Probably because this stationary phase is not well suited for separating β-phellandrene from limonene, the latter was not detected; the claimed identification of a small amount of α-phellandrene also should be regarded with suspicion because Δ^3-carene could have appeared in about the same position.[10]

This paper presents a report on the terpene composition of balsams from seventeen *Abies* species and three varieties, and of balsams from two *Pseudotsuga* species and one variety run for comparison.

The first inspection of the balsams immediately revealed differences in color, viscosity and odor. For example, the balsam of *A. magnifica* was greenish to greenish-yellow and relatively thick, whereas the balsam of *A. concolor* var. *lowiana* was yellow and definitely thinner. The balsam of *A. magnifica* had a mild pine odor; that of *A. concolor* var. *lowiana* a sharp, somewhat unpleasant smell, distinctly different from *A. concolor* var. *concolor* balsam. The odor of *A. grandis* balsam was also characteristic and different from the first two, whereas the balsam of *Pseudotsuga menziesii* had a fruit-like odor. The odors of the other balsams fell roughly between, with distinctive interspecific differences present in many cases.*

The balsams were analyzed by gas–liquid chromatographic methods for the terpenes, and the results are presented in Table 1. As can be seen, α-pinene, β-pinene, camphene, limonene, and β-phellandrene were found in the balsams of all species, in varying quantities. Δ^3-Carene seems to be somewhat less common, however; at times it can be one of the main constituents of a volatile oil. Myrcene is about as common as Δ^3-carene, being always a secondary constituent. Santene, tricyclene, sabinene, α-phellandrene, and terpinolene seem to be present only in a few balsams and usually in small quantities.

In many cases the differences in composition were found to be taxonomically significant. Thus within the group of the closely related *A. lasiocarpa* var. *arizonica*, *A. lasiocarpa* var.

* At times the difference in odor is so striking it becomes the most practical and surest distinguishing characteristic for preliminary identification of species, e.g., distinguishing between *A. magnifica* and *A. concolor* var. *lowiana* in areas of intergrowth.

[4] H. WIENHAUS and K. MUCKE, *Ber.* **75**, 1830 (1942).
[5] C. A. BICKFORD, S. C. CLARKE and E. C. JAHN, *Proc. Pacif. Sci. Cong.* V, 3941 (1934).
[6a] H. J. PETROWITZ, F. NERDEL and G. OHLOFF, *Riechstoffe u. Aromen* **12**, 1 (1962).
[b] R. LOMBARD, B. ROTOVIC and A. CRIQUI, *Peintures, pigments, vernis.* **34**, 106 (1958).
[c] R. LOMBARD, B. ROTOVIC and A. CRIQUI, *Compt. rend.* **242**, 2033 (1956).
[d] J. B. DAVENPORT, M. D. SUTHERLAND and T. F. WEST, *J. Appl. Chem.* **1**, 527 (1951).
[e] G. E. SMITH and T. F. WEST, *J. Soc. Chem. Ind.* **56**, 300T (1937).
[7] T. SHIBAMOTO, K. MINAMI and M. MIYAZAKI, *Bull. Tokyo University, Forests* **38**, 95 (1950).
[8] A. P. PENTEGOV, V. A. PENTEGOVA and M. A. CHIRKOVA, *Trudy Khim. Metallurg. Inst. Sibirsk. Otdel Akad. Nauk SSSR* **13**:5 (1959). (*Refer. Zhur. Khim.* **4**, 492 (1960)).
[9] M. S. TRUPP and L. FISCHER, *J. Amer. Pharmaceut. Assoc.* **28**, 433 (1939).
[10] M. H. KLOUWEN and R. TER HEIDE, *J. Chromatog.* **3**, 297 (1962).

TABLE 1. ANALYSIS OF TERPENE HYDROCARBONS FROM BALSAMS OF VARIOUS *Abies* AND *Pseudotsuga* SPECIES

Species	Origin of species	Total terpene hydrocarbons (%)											
		Santene	Tricyclene	α-Pinene	Camphene	β-Pinene	Δ³-Carene	Sabinene	α-Phellandrene	Myrcene	Limonene	β-Phellandrene	Terpinolene
A. concolor var. *concolor*	N.A.	—	1·0	20·5	14·5	38·0	15·0	—	tr.	tr.	5·0	5·5	0·5
A. concolor var. *lowiana*	N.A.	—	—	8·0	0·5	69·0	0·5	—	tr.	tr.	1·0	21·0	tr.
A. grandis	N.A.	0·5	1·5	13·0	34·5	18·5	0·5	—	—	0·5	5·0	26·0	—
A. magnifica var. *magnifica*	N.A.	—	—	10·5	4·5	25·0	1·5	—	—	2·0	2·5	54·0	—
A. magnifica var. *shastensis*	N.A.	—	—	29·5	0·5	19·0	0·5	—	tr.	2·0	7·5	40·5	0·5
A. procera	N.A.	—	—	32·0	2·0	12·5	1·5	0·5	2·0	2·5	28·5	18·0	0·5
A. amabilis	N.A.	—	—	15·5	0·5	16·5	38·0	—	1·0	1·0	1·0	26·5	tr.
A. lasiocarpa var. *arizonica*	N.A.	—	—	15·5	0·5	28·0	tr.	tr.	—	0·5	18·5	36·0	0·5
A. lasiocarpa var. *lasiocarpa*	N.A.	—	—	9·0	tr.	28·5	6·5	—	—	1·0	11·5	43·0	0·5
A. balsamea	N.A.	—	—	17·0	tr.	31·0	4·0	—	—	0·5	28·5	19·0	tr.
A. fraseri	N.A.	—	—	59·0	0·5	21·5	12·5	tr.	—	0·5	4·0	1·0	1·0
A. bracteata	N.A.	—	—	61·0	0·5	13·0	tr.	tr.	4·5	3·0	4·5	4·5	—
A. alba	Europe	—	—	39·0	tr.	3·0	4·5	—	—	tr.	53·5	tr.	—
*A. sibirica**	Europe & Asia	2·0	—	37·0	30·0	10·5	5·5	—	—	—	—	12·0	3·0
A. pinsapo	Europe	—	—	55·0	tr.	35·5	tr.	—	—	tr.	5·0	4·5	—
A. forrestii	China	—	—	41·5	3·0	3·5	13·0	tr.	—	7·0	24·0	8·0	tr.
A. sachalinensis	Japan	—	tr.	12·0	3·0	21·0	—	—	—	—	40·0	24·0	—
A. veitchii	Japan	—	4·0	29·5	52·0	4·5	tr.	—	—	—	5·5	4·5	—
A. mariesii	Japan	tr.	—	71·5	1·5	15·0	tr.	—	—	—	1·0	11·0	—
A. koreana	Japan	—	tr.	40·0	19·0	9·0	0·5	—	—	tr.	30·0	0·5	1·0
P. menziesii var. *menziesii*	N.A.	—	—	31·0	0·5	36·0	10·0	9·5†	—	1·0	5·0	1·5	5·5
P. menziesii var. *glauca*	N.A.	—	—	76·0	1·5	6·5	1·0	—	—	1·0	10·5	2·5	1·0
P. macrocarpa	N.A.	—	—	63·0	0·5	10·0	7·0	tr.	—	3·5	16·0	tr.	tr.

* This analysis was taken from literature.[8] Percentages of terpenes were recalculated, assuming sum of all terpene hydrocarbons being equal to 100 per cent and rounding figures to nearest 0·5 per cent.

† In this case the figure was showing very strong fluctuation from individual to individual.

lasiocarpa, *A. balsamea* and *A. fraseri*[11] the content of Δ^3-carene seems to separate the two varieties of *A. lasiocarpa* and the content of limonene and β-phellandrene *A. balsamea* from *A. fraseri*. The separation of *A. lasiocarpa* var. *lasiocarpa* from *A. balsamea* seems to be less clear-cut however. Not much difference has been found in the analyses of the balsams from the two varieties of *A. magnifica*; there are some indications, however, that the limonene content might separate *A. magnifica* (both varieties) from *A. procera*, although more analyses are needed. The content of camphene and Δ^3-carene seems to differentiate clearly *A. grandis* from either of the two *A. concolor* varieties. Significant differences were found between the closely related Japanese firs, although statistical substantiation is needed here too.

TABLE 2. ANALYSIS OF *Abies grandis* BALSAMS OBTAINED FROM INDIVIDUAL TREES NEAR STEWART'S POINT, CALIFORNIA

	Total terpene hydrocarbons (%)								
Sample No.	Santene	Tricyclene	α-Pinene	Camphene	β-Pinene	Δ^3-Carene	Myrcene	Limonene	β-Phellandrene
5	0·5	1·5	8·7	31·6	20·0	2·2	—	6·8	24·7
6	0·5	0·9	12·0	54·7	6 9	0·5	—	6·3	16·5
8	0·2	0·8	7·3	40·5	18·7	0·1	-	7·2	25·2
11	0·2	0·8	10·2	16·7	27·4	0·1	0 5	3·4	40·7
12	0·2	1·4	7·8	19·4	37·3	—	0·4	2·8	30·7
13	0·2	1·0	15·6	26·1	18·3	0·5	0·1	2·8	35·4
14	0·6	3·1	28·7	41·2	7·0	0·3	0·7	7·0	11·4
15	0·5	2·0	12·6	45·8	14·0	0·7	0,2	4·9	20·3
Mean	0·4	1·4	13·0	34·5	18·7	0 6	0·4	5·2	25·8
Mean deviation	0·15	0·56	4·66	11·0	5·98	0·40	0·18	1·67	7·52

The largest surprise, however, was the gap found between *A. concolor* var. *concolor* and *A. concolor* var. *lowiana*, which are generally considered as two different species by European botanists but in America by and large are treated as conspecific.[12] The final decision should be made by critically considering both botanical and chemical evidence. Should further chemotaxonomic work on different populations substantiate the large difference found in content of Δ^3-carene and camphene, the chemical evidence would definitely suggest at least varietal status for these two groups.

Tables 2–4 exemplify the variation of the terpene composition from tree to tree within the same population as found with three of the species investigated. The degree of fluctuation was about the same with the rest of the species analyzed. As can be seen, the general character of the composition was retained throughout.

[11] B. BOIVIN, *Naturaliste Can.* **86**, 219 (1959).

[12a] W. DALLIMORE and A. B. JACKSON, *Handbook of Coniferae*, pp. 96 and 108, E. Arnold, London (1931).

[b] G. B. SUDWORTH, *Forest Trees of the Pacific Slope*, p. 116, Washington Government Printing Office (1908).

[c] E. L. LITTLE, JR., *Check list of Native and Naturalized Trees of the United States* (including Alaska), Washington Government Printing Office (1953).

TABLE 3. ANALYSIS OF *Abies lasiocarpa* var. *lasiocarpa* BALSAMS OBTAINED FROM INDIVIDUAL TREES AT PARTRIDGE CREEK, B.C. CANADA

	Total terpene hydrocarbons (%)								
Sample No.	α-Pinene	Camphene	β-Pinene	Δ^3-Carene	α-Phellandrene	Myrcene	Limonene	β-Phellandrene	Terpinolene
105	9·9	0·3	23·4	10·7	tr.	0·5	0·9	52·3	1·0
106	12·4	1·9	34·0	0·4	—	0·4	3·2	47·7	tr.
107	7·8	0·1	28·0	2·2	tr.	0·6	1·7	59·6	tr.
108	6·9	0·3	23·4	7·6	0·1	1·2	2·2	57·7	0·6
109	7·0	0·6	25·6	3·2	0·2	0·4	1·4	60·5	1·1
110	8·2	0·8	24·3	9·4	—	0·5	2·2	53·7	0·9
111	9·3	0·5	35·6	2·1	tr.	1·0	1·3	49·7	0·5
Mean	8·8	0·6	28·0	5·1	0·2	0·7	1·8	54·2	0·6
Mean deviation	1·50	0·39	3·98	3·57	0·05	0·27	0·59	4·12	0·33

The variation of the composition along the stem of an individual tree is exemplified by the analyses of *P. menziesii* var. *menziesii* balsams, obtained at the various heights (Table 5). The variation seems much less than that between balsams obtained from different trees.

In the present work identification of the individual terpenes was made by relative retention data on columns of different polarity, only. Although this method has been used in the terpene field with fair success particularly when separation of *all* known terpenes is taken in consideration, it is not a rigorous means of identification. For this reason the present

TABLE 4. ANALYSIS OF *Abies amabilis* BALSAMS OBTAINED FROM INDIVIDUAL TREES NEAR KITIMAT, B.C., CANADA

	Total terpene hydrocarbons (%)								
Sample No.	α-Pinene	Camphene	β-Pinene	Δ^3-Carene	α-Phellandrene	Myrcene	Limonene	β-Phellandrene	Terpinolene
174	27·4	0·1	9·5	39·7	3·2	0·2	1·4	18·7	tr.
175	18·2	0·7	18·2	31·5	1·6	0·7	0·8	28·0	tr.
176	14·2	1·3	15·3	40·5	0·4	1·0	1·1	26·2	tr.
177	9·5	0·6	7·3	62·5	0·5	1·2	0·4	17·8	tr.
178	8·2	0·2	21·7	44·3	1·3	1·3	1·5	21·5	tr.
179	17·6	1·0	22·1	26·5	0·9	1·0	0·4	30·5	tr.
180	12·6	0·8	23·0	19·2	0·7	0·6	1·1	42·0	tr.
Mean	15·4	0·7	16·7	37·7	1·2	0·9	1·0	26·4	tr.
Mean deviation	4·92	0·31	5·17	10·30	0·69	0·30	0·36	6·10	—

work is being continued through preparative analysis of the balsams to substantiate the gas–liquid chromatographic identification and also to isolate the constituents, probably sesquiterpenes and oxygenated terpenes, responsible for the differences in odor. In addition, population studies are being carried out to put the interspecific and intervarietal differences found on a sounder basis statistically.

TABLE 5. ANALYSIS OF BALSAMS COLLECTED AT DIFFERENT HEIGHTS OF A *Pseudotsuga menziesii* var. *menziesii* SINGLE TREE

	Total terpene hydrocarbons (%)									
Height (ft)	α-Pinene	Camphene	β-Pinene	Δ³-Carene	Sabinene	Myrcene	Dipentene	β-Phellandrene	Unknown	Terpinolene
0–3	40·0	0·8	14·8	30·2	3·0	2·3	3·2	1·6	tr.	4·1
3–6	41·7	0·5	14·2	32·9	1·8	2·0	2·8	0·8	0·1	3·2
6–9	40·2	0·5	16·8	29·7	1·7	2·5	3·5	0·8	0·1	2·4

EXPERIMENTAL

Collection

Balsam was collected by perforating the upper part of a blister with a needle and applying pressure just under the perforation with the lip of the collecting vessel; the balsam flows directly into the vial. For collection, screw-cap vials of ½–1 dram were found most appropriate. To suppress air oxidation a vial was filled as full as possible, a few crystals of pyrogallol were added, the remaining air replaced with nitrogen, and the samples refrigerated. Although in some instances a single tree was tapped, every effort was made to analyze several trees of the same species. Where trees sampled were in the field, herbarium specimens were prepared for the record. Table 6 summarizes the sources of the materials investigated. In case of species indigenous to United States the nomenclature of the "Check List"[12c] was followed. In addition balsams were obtained from the following species, growing in Wind River Arboretum, Oregon (one balsam sample in each case): *A. grandis* (Dougl.) Lindl.; *A. fraseri* (Pursh) Poir.; *A. magnifica* Murr. var. *magnifica*; *A. alba* Miller; *A. magnifica* var. *shastensis* Lemm.; *A. pinsapo* Boiss.; *A. procera* Rehd.; *A. sachalinensis* Mast.; *A. amabilis* (Dougl.) Forbes; *A. veitchii* Lindl.; *A. lasiocarpa* (Hook) Nutt. var. *lasiocarpa*; *A. mariesii* Mast.; *A. koreana* Wilson.

The blistering habit varied considerably from species to species and also from locality to locality. Thus *Pseudotsuga menziesii* from a number of localities visited on the western slope of the Sierra Nevada did not have blisters, whereas the bark of the same species from the Pacific Coast, north of Fort Ross, California, was covered by a fair number of blisters. *Abies magnifica* was nearly always covered with blisters, while *A. concolor* var. *lowiana* exhibited quite a variable degree of blistering. The blisters of *A. forrestii* were very small and difficult to detect due to the bark character of the species. With the *A. lasiocarpa* var. *arizonica* (Corkbark fir) the nature of the bark precludes blistering; in this case the balsam is contained in small cavities (usually 0·01–0·05 ml in volume) in the bark portion nearer to the cambium.

The balsam was obtained by collecting bark and locating the cavities by cutting the bark with a razor blade.

TABLE 6. SOURCES OF MATERIALS INVESTIGATED

Species	Provenance	Number of trees sampled	Identified by
A. concolor (Gord. & Glend.) Lindl.	½ mile below Ladybug Summit, Pinaleno Mts., Arizona	10	W. B. Critchfield
var. *concolor*	Institute for Forest Genetics, Placerville, Calif.**	2	W. B. Critchfield
var. *lowiana* (Gord.) Lemm.	Greenspring Area, East of Medford, Jackson Cty., Oregon	4	E. L. Parker
	Wagner Creek, near Canyonville, Douglas Cty., Oregon	1	E. L. Parker
	Strawberry Flat, near Hiway 50, Eldorado Cty., Calif.*	1	Authors
	Markleeville Hot Springs, Alpine Cty., Calif.*	8	Authors
A. grandis (Dougl.) Lindl.	Near Stewart's Point, Sonoma Cty., Calif.	8	Authors
	Clear Lake, Yakima Cty., Calif.	1	J. W. Duffield
	Institute for Forest Genetics, Placerville, Calif.**	1	W. B. Critchfield
A. magnifica Murr. var. *magnifica*	Near Wright's Lake, Eldorado Cty., Calif.*	20–30	Authors
var. *shastensis* Lemm.	S–SE slope of Mt. Shasta, Siskiyou Cty., Calif.	5	E. L. Parker
A. procera Rehd.	Mt. Hood, Clackamas Cty., Oregon	6	E. L. Parker
A. amabilis (Dougl.) Forbes	Near Kitimat, B.C., Canada	7	Authors
A. lasiocarpa (Hook) Nutt. var. *lasiocarpa*	Near Partridge Creek, Alaska Hiway, B.C., Canada	7	Authors
	Near Bear Lake Campground, Hiway 97, B.C., Canada	6	Authors
	Near Steamboat, Mt. Alaska Hiway, B.C., Canada	5	Authors
var. *arizonica* (Merriam) Lemm.	Graham Mt., Graham Cty., Arizona, 12 miles N. of Ladybug Summit, Pinaleno	8	W. B. Critchfield
A. balsamea (L.) Mill.	At Paul Smiths, near Saranac Lake, Franklin Cty., New York	7	J. W. Duffield
A. fraseri (Pursh) Poir	Balsam Gap, Blue Ridge Parkway, N. Carolina*	25	Ch. F. Spears
A. bracteata D. Don	Institute for Forest Genetics, Placerville, Calif.* **	3	W. B. Critchfield
A. forrestii Craig	University of California Botanical Garden, Berkeley, Calif.**	1	Botanical Garden records
P. Menziesii (Mirb.) Franco var. *menziesii*	Near Stewart's Point, Sonoma Cty., Calif.	6	Authors
var. *glauca* (Beissn) Franco	Near Missoula, Montana	4	J. R. Habeck
P. macrocarpa (Vasey) Mayr.	Tilden Park, Botanical Garden, Berkeley, Calif.**	1	Botanical Garden records

* The balsams from the locations marked with an asterisk were analyzed as a mixture. With the unmarked locations the balsam of each individual tree was analyzed separately and the mean values were computed.

** Double asterisk indicates an arboretum.

Analysis

The analysis was performed by vapor-phase chromatography. A Wilkens Aerograph Hi-Fi 600-C Instrument equipped with hydrogen flame detector, in combination with a Minneapolis Honeywell Brown Recorder equipped with automatic integrator, was employed.

The most expedient procedure appeared to be the direct injection of the balsam. In cases where balsam was too thick to be drawn into a Hamilton syringe, it was diluted with a small amount of ethyl ether. The amount of balsam injected was usually very small amounting roughly to 0·1λ. The interference of higher boiling constituents, mostly oxygenated terpenes (broad peaks overlapping the narrow, normal, terpene peaks), was noticeable only with the Ucon and Silicone columns and this only after about eight runs. Very little interference was noted with β,β-oxydipropionitrile column, although it was found advisable to purge all of the columns after use by allowing nitrogen to continue flowing overnight at the same temperature.

Three $\frac{1}{8}$ in. × 5 ft columns, Silicone 550—20 per cent on acid-washed 60/80 chromosorb, Ucon polar 20 per cent on firebrick 60/80[13] and β,β-oxydipropionitrile 10 per cent on acid-washed chromosorb 60/80 were used.[10] The last column (nitrogen flow rate of 12–20 ml/min 64° column and 75–80° injector temperature) gave by far the best separation of the components. Identification of the individual terpenes was made by comparing the relative retention volume values with those of the authentic samples run on the same columns and under the same conditions. Although nitrogen was used as carrier gas in our case the values obtained were about the same as reported by Klouwen and Heide for hydrogen.[10]

The per cent composition of the individual terpene components was computed directly from the integration results with no response correction, as the variation of the terpene composition between individual trees exceeds by far the error committed.[14]

Acknowledgements—We wish to express our appreciation to Dr. Nicholas T. Mirov, Harvard University, for suggesting the problem as well as for many discussions. We wish also to thank Prof. John W. Duffield, University of North Carolina, Dr. William B. Critchfield, Mr. Roy R. Silen, Dr. Charles F. Spears, U.S. Department of Agriculture, Forest Service, Mr. Eugene L. Parker, Medford, Oregon, Prof. James R. Habeck, Montana State University, Missoula, Montana, and Dr. Donald A. Fraser, Department of Forestry, Ontario, Canada, for procuring certain balsam samples.

[13] N. T. MIROV, E. ZAVARIN and J. G. BICHO. *J. Pharm. Sci.* **51**, 1131 (1962).

[14a] G. PERKINS, JR., R. E. LARAMY and L. D. LIVELY. *Analyt. Chem.* **35**, 360 (1963).

[b] A. Y. ANDREATCH and R. FEINLAND. *Analyt. Chem.* **32**, 1021 (1960).

[c] L. S. ETTRE and F. J. KABOT. *J. Chromatog.* **11**, 114 (1963).