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OXYGENATED MONOTERPENOIDS AND SESQUITERPENOID HYDROCARBONS OF THE CORTICAL TURPENTINE FROM DIFFERENT *ABIES* SPECIES*

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Abstract—Twelve samples of cortical oleoresin from most species and varieties of North American firs have been analyzed for monoterpenoids and sesquiterpenoid hydrocarbons by GLC using three different capillary columns. The analyses showed that large chemical differences exist between many species and varieties. Particularly important chemotaxonomically was the considerable difference in δ-elemene and longifolene content between *Abies magnifica* and *A. procera*, which are difficult to separate morphologically and also in farnesene, α-muurolene, α-cubebene, α-copaene and longifolene content between two varieties of *A. concolor*—var. *concolor* and var. *lowiana*.

INTRODUCTION

NINE distinct *Abies* species are generally recognized in North America outside Mexico: *Abies magnifica* A. Murr.; *A. procera* Rehd; *A. concolor* (Gord. & Glend.) Lindl.; *A. grandis* (Dougl.) Lindl.; *A. lasiocarpa* (Hook.) Nutt.; *A. balsamea* (L.) Mill.; *A. fraseri* (Pursh) Poir.; *A. amabilis* (Dougl.) Forbes and the rare *A. bracteata* D. Don. Of these, *A. concolor* and *A. lasiocarpa* occur in two distinct varieties each—*concolor* and *lowiana* (Gord.) Lemm., and *lasiocarpa* and *arizonica* (Merriam) Lemm., respectively. With exception of *A. amabilis* and *A. bracteata* all are parts of three larger species complexes with *A. magnifica* and *A. procera* in one, *A. concolor* and *A. grandis* in the other, and *A. lasiocarpa*, *A. balsamea* and *A. fraseri* in the third. Members of each of the three groups are spacially arranged in a chain. They replace each other geographically and tend to intergrade which partially accounts for the taxonomic difficulties encountered when dealing with this genus.

One of the characteristic properties of the *Abies* species is the development of blisters on the surface of young, smooth bark. These blisters are small cavities in the cortical tissue which are filled with an aromatic liquid chemically similar to gum oleoresin of pines. The cortical oleoresin of *Abies* is composed of a volatile fraction (turpentine) and nonvolatiles (rosin), the latter being a mixture of resin and fatty acids and their glycerides. The largest portion of the turpentine represents a mixture of monoterpenoid hydrocarbons consisting essentially of α-pinene, β-pinene, camphene, 3-carene, myrcene, limonene and β-phellandrene

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in various portions. This fraction has been extensively used in chemosystematic work in our laboratory.[1]

Little attention has been devoted to higher-boiling constituents of cortical turpentine which are composed mainly of oxygenated monoterpenoids and sesquiterpenoids, and which account for 4–25 per cent of the total volatiles. Among sesquiterpenoid hydrocarbons, (+)-longifolene, (−)-caryophyllene, (−)-ε-muurolene, (−)-α-humulene, (+)-γ-selinene and (+)-β-bisabolene have been identified in cortical turpentine of *A. sibirica* by Pentegova *et al.*,[2] and α-longipinene, longifolene and bisabolene in the turpentine of *A. balsamea* by Zavarin *et al.*[3] A "cadinene" has been also isolated from *A. lasiocarpa*.[4] Borneol and bornyl acetate were the oxygenated monoterpenoids most often encountered.[5] Linalool, camphor, α-terpineol and methyl thymol have been identified in *A. balsamea* turpentine.[3]

In our preceding paper[6] we reported on the composition of the higher-boiling constituents of cortical turpentine from *A. magnifica* in which nearly fifty constituents were identified chiefly by isolation and spectroscopic methods. In this paper we report on the GLC analyses of the cortical turpentines from other North American species. Because of the many higher-boiling constituents present in *Abies* cortical turpentines—over a hundred compared to less than a dozen of the monoterpenoid hydrocarbons—the identification reliability when using conventional packed columns is rather poor. For this reason high-resolution capillary column methodology was applied, with identifications based on retention volumes obtained using three types of immobile phases. Although not all compounds could be resolved even by these methods (e.g. α-ylangene did not separate from cyclosativene on either column), most identifications can be considered as trustworthy.

In most analyses prior to the injection in GLC instruments, volatiles were separated into monoterpenoid hydrocarbons, sesquiterpenoid hydrocarbons, acetates, and alcohols by fractional distillation and column chromatography. However, injection of the total balsam through an external injector, which retains acids and other high-boiling constituents, was used as a parallel method. Although separation was somewhat inferior in the latter case, the analyses required much less time (about an hour) and seemed to be particularly suitable for cases where processing of larger numbers of smaller oleoresin samples is required.

RESULTS AND DISCUSSION

Cortical turpentines from all previously mentioned species and varieties were investigated, with exception of *Abies lasiocarpa* var. *arizonica* which develops only very small cortical cavities imbedded in bark so that securing a larger amount of oleoresin becomes difficult. In addition, *A. balsamea* from the western (Saskatchewan–Manitoba) and eastern parts (Ontario to Newfoundland) of its range and *A. concolor* var. *concolor* from the Rocky Mountains and from southern California were investigated separately, because our previous studies showed larger chemical differences among monoterpenoid hydrocarbons.[3] The sesquiterpenoid hydrocarbon analyses are listed in Table 1. Analytical results for oxygenated monoterpenoids were combined with those of monoterpenoid hydrocarbons reported in

[1] E. ZAVARIN and K. SNAJBERK, *Phytochem.* **4**, 141 (1965).
[2] N. A. CHIRKOVA and V. A. PENTEGOVA, *Izv. Sibir. Otd. Akad. Nauk SSSR, Ser. Khim. Nauk* 125 (1962).
[3] E. ZAVARIN, unpublished.
[4] C. A. BICKFORD, S. C. CLARKE and E. C. JAHN, *Proc. 5th Pacific Sci. Congress, Canada*, 1933, Vol. V, p. 3941, Univ. of Toronto Press (1934).
[5] *Die Aetherischen Oele.* E. GILDEMEISTER and FR. HOFFMAN. Rev. by W. TREIBS and K. BOURNOT, Akademie Verlag, Berlin (1956).

our previous work[1] and are listed in Table 2 as percentages expressed on the basis of total monoterpenoid hydrocarbons set as 100. In both cases the compounds are arranged according to the number of rings, and taxonomically close species are grouped together.

Since the oleoresin in each case was secured from a large number of individual trees sampled in various localities, the analyses should give a reliable picture for the average differences expected. Still, because of the lack of data on the individual and population

TABLE 1. SESQUITERPENOID HYDROCARBONS OF *Abies* CORTICAL OLEORESINS

Compound	Species or lower taxa*											
	magnifica	*procera*	*fraseri*	*balsamea* (West)	*balsamea* (East)	*lasiocarpa*	var. *lowiana*	var. *concolor* (southern Calif.)	var. *concolor* (Rocky Mts.)	*grandis*	*amabilis*	*bracteata*
Acyclic												
Farnesene	×	1	2	×	—	2	—	3	4	×	×	×
Monocyclic												
β-Elemene	5	9	1	×	—	×	5	14	6	8	2	×
δ-Elemene	9	40	×	×	×	×	3	×	×	×	—	—
α-Humulene	2	2	×	1	×	×	2	1	3	1	3	12
γ-Humulene	3	1	7	4	5	3	1	—	—	1	—	—
β-Bisabolene	2	5	18	22	19	35	12	10	10	1	30	1
Bicyclic												
α-Guaiene	1	1	—	×	—	×	×	—	2	1	×	1
Guaiazulene	×	×	—	—	—	—	—	—	—	—	—	—
α-Selinene	2	3	×	×	—	1	14	10	10	11	9	—
β-Selinene	2	4	1	×	—	×	15	—	6	4	9	—
Selina-4(14),7(11)-diene	×	×	1	×	—	1	—	—	×	—	—	—
Selina-3,7(11)-diene	×	×	1	1	1	7	3	—	×	—	6	—
β-Caryophyllene	10	7	2	5	6	×	8	11	11	8	6	59
β-Santalene	1	2	×	—	—	×	1	—	×	×	—	—
β-Bergamotene	×	5	—	—	—	×	8	2	3	1	×	—
γ-Cadinene	×	1	1	×	1	10	1	8	3	3	4	2
δ-Cadinene	×	1	1	×	—	1	2	5	6	8	1	×
α-Muurolene	×	×	×	—	2	8	1	3	9	5	3	11
γ-Muurolene	×	1	×	×	—	×	2	×	×	2	×	2
Tricyclic and Tetracyclic												
α-Cubebene	—	×	—	—	—	×	2	21	9	24	×	—
α-Copaene	×	×	1	—	—	×	1	3	8	18	1	—
β-Copaene	×	3	1	×	×	×	2	1	3	×	17	—
Sativene	2	×	4	4	4	1	—	—	×	×	×	1
Cyclosativene] α-Ylangene]	1	×	1	3	3	1	4	—	×	×	1	1
β-Ylangene	×	2	—	×	—	—	—	×	×	—	—	—
α-Longipinene	6	1	1	8	9	3	×	3	×	×	×	3
Longifolene	48	6	49	48	50	24	5	2	—	—	1	4
Longicyclene	2	×	3	1	×	×	3	—	—	—	×	—

* Vertical lines separate groups of closely related species; "×" denotes trace amounts.

TABLE 2. MONOTERPENOIDS OF *Abies* CORTICAL OLEORESINS*

	magnifica	*procera*	*fraseri*	*balsamea* (East)	*balsamea* (West)	*lasiocarpa*	var. *lowiana*	var. *concolor* (South)	var. *concolor* (Rocky Mt.)	*grandis*	*amabilis*	*bracteata*
Acyclics												
Myrcene	2·0	2·0	0·5	1·0	2·0	0·5	0·5	0·5	8·0	0·5	2·0	3·0
Geraniol	0·3	0·4	—	×	0·4	0·1	×	2·0	0·4	0·1	0·9	0·05
Geranyl acetate	×	0·5	0·2	×	0·4	0·8	2·2	—	0·8	1·7	1·7	0·05
Nerol	—	1·5	×	—	—	×	×	0·2	—	×	—	×
Neryl acetate	×	0·5	×	—	—	×	×	×	0·1	—	—	×
Citronellol	4·0	11·7	×	—	0·3	0·1	1·4	2·0	1·5	0·3	0·9	0·1
Citronellyl acetate	4·0	1·5	—	×	×	0·8	2·7	0·3	4·1	1·7	—	×
Total	10·3	18·1	0·7	1·0	3·1	2·3	6·8	5·0	14·9	4·3	5·5	3·2
Monocyclics												
Limonene	3·0	28·0	2·0	13·0	44·0	65·0	2·0	2·0	2·0	5·0	1·0	4·5
α-Phellandrene	—	2·0	—	—	—	—	—	×	—	—	1·0	5·0
β-Phellandrene	55·0	20·0	4·0	21·0	5·0	13·0	35·0	26·0	16·0	15·0	46·0	4·0
Terpinolene	×	1·0	0·5	—	—	—	—	0·5	×	0·5	2·0	—
α-Terpineol	×	2·0	—	—	—	0·3	0·3	0·6	0·4	—	×	0·2
Methyl thymol	0·3	×	1·0	×	0·1	0·2	0·4	0·4	0·4	0·1	3·0	0·2
Total	58·3	53·0	7·5	34·0	49·1	78·5	37·7	29·5	18·8	20·6	53·0	13·9
Bi- and Tricyclics												
Santene	—	—	—	—	—	—	—	—	—	×	—	—
Tricyclene	—	—	—	—	—	—	—	—	1·0	5·0	—	—
Camphene	0·5	1·0	0·5	×	—	×	×	×	12·0	46·0	—	9·5
Borneol	1·1	0·7	0·4	×	0·6	0·2	0·3	1·0	1·7	0·3	0·9	0·2
Bornyl acetate	1·0	13·0	0·2	0·1	1·5	1·0	1·5	9·3	23·0	11·0	1·6	0·1
Sabinene	—	0·5	—	—	—	—	—	—	—	—	×	×
α-Pinene	13·0	24·0	60·0	23·0	9·0	6·0	9·0	10·0	23·0	13·0	12·0	61·0
β-Pinene	24·0	11·0	20·0	36·0	41·0	6·0	53·0	42·0	28·0	15·0	16·0	13·0
3-Carene	1·0	10·0	13·0	5·0	—	10·0	0·5	20·0	11·0	1·0	20·0	×
Total	40·6	60·2	94·1	64·1	52·1	23·2	64·3	82·3	99·7	91·3	50·5	83·8
Grand Total	109·2	131·3	102·3	99·1	104·3	104·0	108·8	116·8	133·4	116·2	109·0	100·9

* Percentage total monoterpenoid hydrocarbons basis, thus excess over 100 per cent represents amount of oxygenated materials; "×" denotes trace amounts.

variability of the species in question, the data must be considered as preliminary observations only, and further work supplementing this statistical aspect is thus indicated.

Biosynthetic speculations on the natural formation of terpenoids have been reviewed on a number of occasions. In our discussion we follow essentially the treatment of monoterpenoids by Richards and Hendrickson,[7] and of sesquiterpenoids by Parker *et al.*[8] The monoterpenoids are generally considered to form through the geranyl- or neryl-pyrophosphate precursor. Dephosphorylation in combination with oxydation or reduction of these precursors leads to acyclic compounds. Cyclization of neryl-pyrophosphate gives the 1-p-menthene-8-carbonium ion which can react in several ways leading to most common cyclic monoterpenoids. Two of these ways stand aside as they lead to two sets of compounds commonly found together. One goes through a 4/8 hydride shift to 1-p-menthene-4-carbonium ion and then, by proton loss, to sabinene, α-thujene and terpinenes. The other route goes through the second cyclization to 2-bornane carbonium ion, and leads to tricyclene, santene, camphene, camphor, borneol, and its acetate.

The hypothetical precursors of sesquiterpenoids are *trans–trans-* and *trans–cis* farnesyl pyrophosphates. The first, through initial 1/10 cyclization followed by transformations of the carbonium ion formed, leads to elemenes, selinenes, α-guaiene and guaiazulene, while 1/11 cyclization gives caryophyllene and α-humulene. The second pyrophosphate can cyclize in four ways, 1/6, 1/7, 1/10 and 1/11. The 1/6 cyclization represents the gateway to bisabolenes, santalenes and bergamotenes, while 1/10 cyclization leads to cadinenes, muurolenes, copaenes, ylangenes, sativenes, and cubebenes, although it is possible to arrive at the latter structures also assuming 1/6 cyclization.[9] Longifolene, longicyclene and α-longipinene, possibly together with γ-humulene[6] form through the initial 1/11 cyclization of *trans–cis* farnesyl pyrophosphate.

As can be seen from Table 2 in oleoresins investigated the acyclic monoterpenoids, although not inconsiderable, still occupy only a secondary position as far as their amount is concerned. Of the cyclic monoterpenoids, the 1-*p*-menthane-4-carbonium ion path leading to sabinene, terpinenes and related compounds is poorly represented. With several important exceptions, this is also true for the bornane-2-carbonium ion path leading to camphene, borneol and related materials. The bulk of the monoterpenoids seems to be formed by other routes through the same 1-*p*-menthane-8-carbonium ion, with α- and β-pinenes, limonene and β-phellandrene being most important products. Among constituents not reported in the previous paper, methyl thymol has been detected in all species investigated. It has been previously separated and identified by infra-red spectroscopy in the *A. balsamea* turpentine.[3]

The *Abies* sesquiterpenoid hydrocarbons (Table 1) are synthesized through both *trans–trans* and *trans–cis* farnesyl pyrophosphate cyclizations, although the *trans–cis* path usually accounts for the larger part of the material (on the average about 2:1). No representative of the 1/7 cyclization of the *trans–cis* farnesyl pyrophosphate has been detected so far, and acyclic sesquiterpenoid hydrocarbons are poorly represented.

The *A. magnifica group* of firs, including *A. procera*, is distinguished from other firs, with the exception of the *A. concolor* group and *A. amabilis*, by the higher amounts of sesquiterpenoids formed through *trans–trans* farnesyl pyrophosphate 1/10 cyclization, chiefly

[6] L. SMEDMAN, E. ZAVARIN and R. TERANISHI, *Phytochem.* **8**, 1457 (1969).
[7] J. H. RICHARDS and J. B. HENDRICKSON, *The Biosynthesis of Steroids, Terpenes and Acetogenins*, W. A. Benjamin, New York (1964).
[8] W. PARKER, J. S. ROBERTS and R. RAMAGE, Sesquiterpene Biosynthesis, *Quarterly Reviews* **21**, 331, The Chemical Society (London) (1967).
[9] L. WESTFELT, *Svensk Kem. Tidsk.* **79**, 441 (1967).

δ-elemene. With the exception of the *A. balsamea* group, the lesser participation of *trans–cis* farnesyl pyrophosphate 1/10 cyclization path (cadinenes, muurolenes and related compounds) is also characteristic. As far as monoterpenoids are concerned the oxygenated, acyclic materials—mainly citronellol and its acetate—seem higher than usual.

The presence of larger amounts of limonene in *A. procera* has been so far the only chemical characteristic distinguishing the two species.[3] To this can be now added the occurrence of δ-elemene in significantly higher amounts in *A. procera*, and of longifolene and α-longipinene in *A. magnifica*. Among monoterpenoids the presence of thirteen times larger amounts of bornyl acetate in *A. procera* could also be significant.

Characteristic for the *A. balsamea* group of firs, including also *A. lasiocarpa* and *A. fraseri*, seems to be the low percentage of sesquiterpenoid hydrocarbons formed through *trans–trans* farnesyl pyrophosphate 1/10 cyclization (elemenes and selinenes), the large amount of material synthesized through *trans–cis* farnesyl pyrophosphate 1/11 cyclization (longifolene, α-longipinene and γ-humulene) as well as the presence of β-bisabolene in higher than usual amounts. Within monoterpenoids, oxygenated materials are relatively low; neither acyclics nor camphene and sabinene type compounds are well represented.

In our work with monoterpenoid hydrocarbons a difference was noted previously between the *A. balsamea* turpentines from eastern (Ontario and Newfoundland) and western (Saskatchewan and Manitoba) parts of its range.[3] Not much difference was noted, however, with sesquiterpenoid hydrocarbons this time. On the other hand acyclic monoterpenoids—hydrocarbons and oxygenated—as well as oxygenated materials derived through bornane-2-carbonium ion (borneol and bornyl acetate) were found to be significantly higher in the west.

Not much difference was noted between *A. fraseri* and *A. balsamea*. *A. lasiocarpa* differed from other members of this group by having greater amounts of selina-3,7(11)-diene, β-bisabolene, γ-cadinene and α-muurolene and lesser amounts of β-caryophyllene, sativene, and longifolene. Within oxygenated monocyclics, acyclic acetates (citronellyl- and geranyl-) were considerably higher in this fir.

The *A. concolor* group is characterized by the higher content of the *trans–cis* and *trans–trans* farnesyl pyrophosphate 1/10 cyclization compounds, separating it from the *A. balsamea* group and *A. bracteata*, as well as by the low content of the *trans–cis* farnesyl pyrophosphate 1/11 cyclization materials (longifolene and related structures). Among the acyclic monoterpenoids, geraniol, citronellol and their acetates occurred sporadically in higher than usual amounts. More striking, however, was the appearance of larger quantities of materials formed through the bornane-2-carbonium ion path: camphene, bornyl acetate, and related monoterpenoids.

The two varieties of white fir—*A. concolor* var. *concolor* (found in the Rocky Mountains), and *A. concolor* var. *lowiana* (found in the Sierra Nevada)—were investigated separately. The taxonomic status of the two is still controversial—while some botanists, chiefly European, regard them as different species, many American botanists do not accord them even varietal status. In our previous publication[1] we mentioned the much larger amount of camphene in var. *concolor* as being the chemical difference separating the two varieties. To this should be added now also bornyl acetate, synthesized through the same 2-bornane-carbonium ion path.* Some differences in acyclic oxygenated monoterpenoids could be also significant.

* It has been found in parallel studies that the ratio of camphene to bornyl acetate remains the same irrespective of their per cent occurrence in total turpentine. This suggests the lack of genetic control over the rates of formation of these two compounds from their common precursor, bornane-2-carbonium ion, and requires that the above two-compound-difference be considered as the one-compound-difference in taxonomic evaluations.

Also among sesquiterpenoid hydrocarbons, var. *lowiana* differed from the other firs of this group by the somewhat larger amounts of *trans–trans* farnesyl pyrophosphate 1/10 cyclization materials (selinenes and δ-elemene) as well as of β-bergamotene. Muurolenes and related compounds formed by *trans–cis* 1/10 cyclization were appreciably less than were found in other firs of this group, while *trans–cis* farnesyl pyrophosphate 1/11 cyclization (longifolene and others) was more common. These differences could supply further evidence for advocates of the varietal or specific status of the Rocky Mountain white fir.

Our previous investigations indicated[3] that populations of *A. concolor* in southern California (Los Angeles and its nearby mountain ranges) differed from the Rocky Mountain populations of the same fir by the absence of camphene in the turpentine. Additional differences found this time included acyclic terpenoids, a higher percentage of α-cubebene, and a lower of β-selinene, in the south; overall the southern California fir seemed closer to var. *concolor* than to var. *lowiana.*

In *A. grandis* the occurrence of β-bisabolene in small amounts and of α-cubebene and α-copaene in particularly large amounts seems characteristic, in addition to a large percentage of bornane-2-carbonium ion compounds in which the presence of camphene has been stressed before.[1] Overall, chemically *A. grandis* seemed to be closer to var. *concolor* than to var. *lowiana.*

Besides the species belonging to the three groups above, cortical turpentine from *A. amabilis* and *A. bracteata* was examined. As far as oxygenated monoterpenoids are concerned *A. amabilis* had an exceptionally high percentage of methylthymol. Among sesquiterpenoid hydrocarbons it was rather low in *trans–cis* farnesylpyrophosphate 1/11 cyclization materials (longifolene and rel.), while it was very high on β-bisabolene and β-copaene; it seemed to show affinity with the *A. concolor* group as well as with *A. lasiocarpa.*

A. bracteata had a viscous oleoresin almost devoid of oxygenated monoterpenoids. Its particular characteristic was the presence of exceptionally large amounts of *trans–trans* farnesyl pyrophosphate 1/11 cyclization compounds (71 per cent as compared to 3 to 15 per cent for other firs), i.e. β-caryophyllene and α-humulene, at the expense of most others; α-muurolene seemed to be also unusually high. This is in good agreement with the taxonomic position of this fir, which is rather distantly related to other members of its genus.

EXPERIMENTAL

Sampling

A composite sample of 20–45 g of oleoresin was collected in at least eight distinct locations involving more than fifty individual trees in order to provide good averaging. A small portion of each sample was retained for the direct oleoresin injection experiments and the rest processed.

Separation

The oleoresins were dissolved in Et_2O and extracted with cold 0·1 N NaOH. The ethereal extracts were washed with weak aqueous acid, followed by H_2O, while maintaining the pH above 7. After drying over Na_2SO_4, Et_2O was distilled and the residue fractionated. The fraction distilling up to 70° at 5 mm pressure was composed essentially of monoterpenoid hydrocarbons, while oxygenated monoterpenoids, sesquiterpenoid hydrocarbons and small amounts of oxygenated sesquiterpenoids were recovered between 70–190°. Table 3 gives the weight of the distillation residue together with the acids removed by KOH extraction in per cent.

Sesquiterpenoid hydrocarbons, monoterpenoid acetates and alcohols were further separated by column chromatography on deactivated Al_2O_3 (basic), using a 1:40 ratio, and a column 1 in. in dia. The successive elutions included 300 ml of light petroleum (b.p. 30–60°), 150 ml light petroleum/benzene, 1:1, 100 ml benzene,

TABLE 3. COMPOSITION OF VOLATILE MATERIALS FROM *Abies* CORTICAL OLEORESINS*

	Distillation residue	Monoterpenoid hydrocarbons	Sesquiterpenoid hydrocarbons	Acetates	Alcohols
A. magnifica	48·0	22·0	6·2	2·4	21·3
A. procera	56·0	21·1	3·7	3·7	15·0
A. fraseri	58·5	32·0	1·2	1·2	7·1
A. balsamea	59·5	24·7	2·0	1·2	12·2
A. lasiocarpa	62·0	26·6	0·7	1·1	9·7
A. concolor var. *lowiana*	54·0	22·6	1·6	2·2	19·4
A. concolor var. *concolor*	54·5	26·2	2·5	2·5	12·7
A. grandis	36·0	35·3	4·5	12·4	11·8
A. amabilis	42·5	35·1	3·0	1·5	17·1

* Expressed in per cent of total oleoresin.

100 ml of benzene/ether, 1:1, and 300 ml of ether. Chromatographic separations were followed by TLC on Al_2O_3 with light petroleum as solvent; the similar fractions were combined, and after evaporation of the solvent and distillation the yield of each of the three fractions established (Table 3).

Gas Chromatographic Procedures

For GLC of the total oleoresin a Hamilton injector, No. 86800, was used. The injector tube (i.d. 0·1 in. and 3 in. length) was packed to 1/3 with Chromosorb G coated with 4 per cent of SF (96)50, and the injector was heated to 190°. The needle of the block was inserted into the injector of the gas chromatograph, and the carrier gas (helium) was switched on to pass through the block. After 15 sec the sample (0·05–0·10 μl) was injected and the carrier gas allowed to pass through for another 30 sec, whereupon it was switched back to the normal passageway and the external injector block removed. The column used was 1000 ft by 0·03 in. OV 101 operated at 150°, 50 lb/in². However, to resolve the peaks in the monoterpenoid hydrocarbon region, programming at 10° per min from 120° to 195° was found to be necessary.

For GLC of Sesquiterpenoid Hydrocarbons three analytical columns, (*a*) 500 ft × 0·02 in., SF 96(50) (Fig. 1), (*b*) 500 ft × 0·02 in., Apiezon L and (*c*) 500 ft × 0·02 in., Carbowax 20 M, were used, and most of the major peaks were identified on the basis of three sets of retention data.

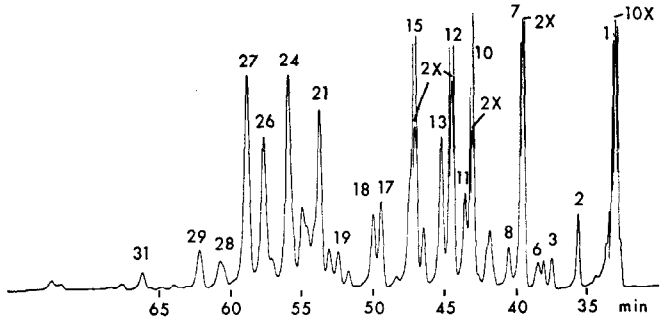

FIG. 1. GAS/LIQUID CHROMATOGRAM OF *A. procera* CORTICAL TURPENTINE FRACTION CONTAINING SESQUITERPENOID HYDROCARBONS, COLUMN A (SF96(50)) AT 125°C.

(1) δ-elemene, (2) α-longipinene, (3) cyclosativene overlapping, (4) α-ylangene, (5) α-copaene, (6) longicyclene, (7) β-elemene, (8) sativene, (9) unknown SH X_1, (10) longifolene, (11) β-ylangene, (12) caryophyllene, (13) β-copaene, (14) α-guaiene, (15) β-bergamotene, (16) farnesene, (17) β-santalene, (18) α-humulene, (19) unknown SH X_2, (20) γ-muurolene, (21) unknown SH X_3, (23) γ-humulene, (24) β-selinene, (25) α-muurolene, (26) α-selinene, (27) β-bisabolene, (28) γ-cadinene, (29) δ-cadinene, (30) selina-4(14),7(11)-diene, (31) selina-3,7(11)-diene.

The percentage compositions were calculated from the peak areas, but the accuracy achieved is only approximate. Additional work, including determination of flame-head response correction factors particularly for oxygenated materials, is necessary to achieve an accuracy necessary for quantitative operations on a wider, statistical basis. The percentage figures for the oxygenated monoterpenoids and sesquiterpenoid hydrocarbons were based on peaks spanning the region from linalool to selina-3,7(11)-diene.

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