

## CHEMICAL COMPOSITION OF THE CORTICAL ESSENTIAL OIL FROM *ABIES BALSAMEA*

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**Abstract**—Monoterpenoids and sesquiterpene hydrocarbons of *Abies balsamea* cortical oleoresin (Canada balsam) were analyzed by a combination of chromatographic and spectroscopic methods. Monoterpene hydrocarbons (21%) were composed of  $\beta$ -pinene,  $\alpha$ -pinene,  $\beta$ -phellandrene, limonene, 3-carene, myrcene and camphene (listed in order of decreasing percentages), and oxygenated monoterpenes (0.4%) contained 4,4-dimethyl-2-cyclohepten-1-one, linalool, bornyl acetate, methylthymol, citronellyl acetate,  $\alpha$ -terpineol, piperitone, citronellal, borneol, citronellol, two unknowns, and geraniol. From the sesquiterpene hydrocarbon fraction (1.1%) were isolated longifolene,  $\beta$ -bisabolene, longipinene, an unknown, sativene, cyclosativene, *cis*- $\alpha$ -bisabolene,  $\beta$ -himachalene,  $\alpha$ -himachalene,  $\beta$ -caryophyllene,  $\gamma$ -humulene, farnesene, longicyclene, an unknown, and  $\beta$ -selinene. Both himachalenes have been identified for the first time in Pinaceae outside of *Cedrus*, their co-occurrence with  $\gamma$ -humulene, longifolene, longipinene and longicyclene supports the biosynthetic mechanism by which all of these compounds arise through initial 1/11 cyclization of *trans*-*cis*-farnesylpyrophosphate.

### INTRODUCTION

IN AN earlier paper, we reported on the composition of essential oil present in *Abies balsamea* (L.) Mill. cortical oleoresin,<sup>1</sup> as well as on the geographic variability of its monoterpene hydrocarbon fraction.<sup>2</sup> In both cases, identification of individual compounds was by GLC which cannot be considered rigorous enough to reliably ascertain the identity of each component (with the possible exception of monoterpene hydrocarbons, a class containing smaller number of well-known compounds). In view of this, and because only two other *Abies* cortical oleoresins have been investigated by modern isolation methods (*Abies magnifica*<sup>3</sup> and *A. sibirica*<sup>4</sup>) we decided to subject the cortical oleoresin of *A. balsamea* to a critical chemical analysis.

Canada balsam represents one of the few *Abies* cortical oleoresins which is still commercially produced, being collected mainly in the eastern part of Canada. Although locally it is used medically as a vulnerary and as an internal remedy for coughs, its main field of utilization is as a cement for lenses and as mounting medium in microscopy. Due to its availability, it has formed the subject of several earlier chemical investigations resulting in isolation and identification of (–)- $\alpha$ -pinene, (–)- $\beta$ -pinene, (–)-limonene, (–)- $\beta$ -phellandrene, and bornyl acetate.<sup>5</sup>

<sup>1</sup> SMEDMAN, L. A., SNAJBERK, K., ZAVARIN, E. and MON, T. R. (1969) *Phytochemistry* **8**, 1471.

<sup>2</sup> ZAVARIN, E. and SNAJBERK, K. (1972) *Phytochemistry* **11**, 1407.

<sup>3</sup> SMEDMAN, L. A., ZAVARIN, E. and TFRANISHI, R. (1969) *Phytochemistry* **8**, 1457.

<sup>4</sup> CHIRKOVA, N. A. and PENTEGOVA, V. A. (1962) *Iz V Sibir Otd Akad Nauk SSSR, Ser Khim Nauk* 125.

<sup>5</sup> LOMBARD, R., ROTOVIC, B. and CRIQUI, A. (1958) *Peintures pigments vernis* **34**, 106; LOMBARD, R., ROTOVIC, B. and CRIQUI, A. (1956) *Compt Rend* **242**, 2033; DAVENPORT, J. B., SUTHERLAND, M. D. and WEST, T. F. (1951) *J Appl Chem* **1**, 527; SMITH, G. E. and WEST, T. F. (1937) *J Soc Chem Ind* **56**, 300T.

## RESULTS AND DISCUSSION

Monoterpene hydrocarbons amounted to 21.0% of the oleoresin and contained (–)- $\alpha$ -pinene (21.6%,  $[\alpha]_D^{25} -34.0^\circ$ ), camphene (trace), (–)- $\beta$ -pinene (39.3%,  $[\alpha]_D^{25} -11.3^\circ$ ), 3-carene (2.5%), myrcene (0.9%), (–)-limonene (14.5%,  $[\alpha]_D^{25} -104.0^\circ$ ), and (–)- $\beta$ -phellandrene (21.1%,  $[\alpha]_D^{25} -50.0^\circ$ ). Composition of the analyzed sample agreed well with its presumably eastern origin because of the presence of 3-carene, and lower limonene and higher  $\beta$ -phellandrene content.<sup>2</sup> The specific rotations, determined using >99% pure terpenoids, were in close agreement with earlier values.<sup>5</sup>

Oxygenated monoterpenoids amounted to only 0.4% of the total oleoresin. Of the compounds listed in Table 1 linalool, citronellal, piperitone, methylthymol, and 4,4-dimethyl-2-cyclohepten-1-one were isolated for the first time from *Abies* cortical oleoresin. The first three represent ubiquitous compounds. Methylthymol was previously isolated from the foliage oils of *Pinus nigra* and *P. muqo*,<sup>6</sup> and according to GLC, should also be common in *Abies* cortical oleoresins. 4,4-Dimethyl-2-cyclohepten-1-one is a new compound and its structure was determined earlier.<sup>11</sup> It probably derives biosynthetically from 3-carene through oxidation of the C<sub>10</sub> methyl to carbonyl followed by breaking of the C<sub>1</sub>–C<sub>6</sub> bond of the 3-membered ring and appropriate migrations of the double bonds.

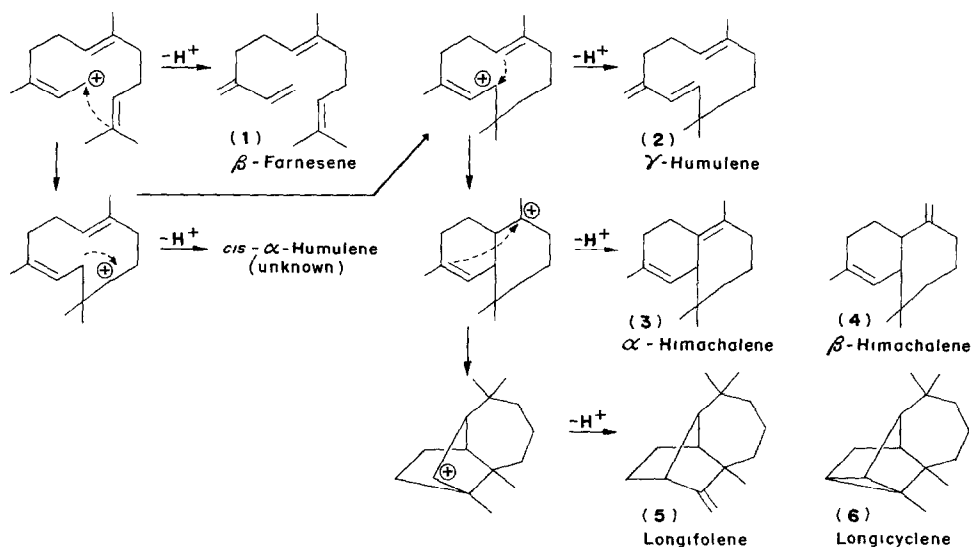
TABLE 1. OXYGENATED MONOTERPENOIDS ISOLATED

Class	Terpene	% of total	
Acyclics	Citronellol	37.0 { 2.7	
	Linalool		20.1
	Geraniol		0.6
	Citronellol acetate		9.0
	Citronellal		4.6
Monocyclics	$\gamma$ -Terpineol	44.6 { 6.0	
	Methylthymol		10.1
	4,4-Dimethyl-2-cyclohepten-1-one		23.4
	Piperitone		5.1
Bicyclics	Bornol	16.3 { 3.2	
	Bornyl acetate		13.1
Unknowns	CBX-258	2.1 { 1.0	
	CBX-305		1.1
Total oxygenated monoterpenoids in oleoresin: 0.4%			

Two unidentified oxygenated monoterpenoids were encountered, one of these, CBX-305 was present in too small quantity for further study. The other, CBX-258, appeared to be an aromatic compound (peaks at 232 and 277 nm in UV, strong bands 1575 and 1630 cm<sup>-1</sup> in IR, tropylium and cyclopentadienyl ions in MS) of MW 178, it had a conjugated carbonyl (band at 1675 cm<sup>-1</sup> in IR, loss of CO in MS), an isopropyl group (doublet at 1368 and 1383 in IR, loss of methyls in MS), and a methoxyl (bands at 1171, and 1240 cm<sup>-1</sup> in IR, loss of formaldehyde and appearance of presumably methoxyl-carrying fragments in MS). Although these data did not allow an unequivocal structural assignment, they agreed well with the formulation of CBX-258 as an oxidation product of methylthymol, namely, 3-methoxy-4-isopropyl-1-benzaldehyde.

<sup>6</sup> OGNYANOV, I. and TSANKOVA, E. (1966) *Riechst. Aromen. Koeperpfl.* **16**, 39.

Sesquiterpene hydrocarbons amounted to 1.1% of the oleoresin and their analysis agreed with that derived earlier on the basis of GLC retention times.<sup>1</sup>  $\beta$ -Farnesene (1), *cis*- $\alpha$ -bisabolene, and  $\alpha$ - and  $\beta$ -himachalenes (3 and 4) were isolated for the first time from *Abies* cortical oleoresins. The first is a ubiquitous compound, while the second has not been reported so far in Pinaceae, both himachalenes are reported for the first time outside of *Cedrus*.<sup>9</sup> Their co-occurrence in *Abies balsamea* cortical oleoresin, with  $\gamma$ -humulene (2), longifolene (5), longicyclene (6) and  $\alpha$ -longipinene has a biosynthetic significance as all six



SCHEME 1 POSTULATED BIOSYNTHESIS OF SOME SESQUITERPENOIDS PRESENT IN *Abies balsamea* CORTX

TABLE 2 SESQUITERPENE HYDROCARBONS ISOLATED

Class		Sesquiterpene	% in total	
Acyclics, 1.0%		$\beta$ -Farnesene	1.0	
Cyclics, from <i>trans</i> - <i>cis</i> -farnesyl pyrophosphate	Cyclization 1/6	$\beta$ -Bisabolene*	23.2 { 20.8	
		<i>cis</i> - $\alpha$ -Bisabolene		2.4
	Cyclization 1/10	Sativene	5.3 { 2.8	
		Cyclosativene		2.5
	Cyclization 1/11	$\gamma$ -Humulene	66.8 { 1.0	
		$\alpha$ -Himachalene		1.8
		$\beta$ -Himachalene		1.9
		Longifolene*		54.0
		Longicyclene		0.9
		Longipinene*		7.2
Total		95.3		
Cyclics, from <i>trans</i> - <i>trans</i> -farnesyl pyrophosphate	$\beta$ -Selinene	1.5 { 0.2		
	$\beta$ -Caryophyllene		1.3	
Unknowns	CBX-0.91†	3.9 { 0.4		
	CBX-1.60		3.5	
Total sesquiterpene hydrocarbons in oleoresin 1.1%				

\*Specific rotations  $[\alpha]_D^{25}$  for  $\beta$ -bisabolene  $-33.0^\circ$  for longifolene  $+18.2^\circ$  for longipinene,  $-19.3^\circ$

† See note added in proof (p. 183)

compounds were postulated as being formed from *trans-cis*-farnesylpyrophosphate through the initial 1/11 cyclization<sup>3</sup> (Scheme 1)

Two sesquiterpene hydrocarbons (Table 2) represent new compounds. One of these (CBX-091) had an identical IR spectrum to a sesquiterpene hydrocarbon isolated from the cortical oleoresin of *Pseudotsuga menziesii* (this will be discussed in a later publication). The IR spectrum of the other corresponded to a sesquiterpene isolated by Wenninger and Yates from *Opopanax* oil<sup>7</sup> and it is most likely  $\beta$ -*trans*-sesquicarene<sup>8</sup> (3-methyl-2-butenyl-3(10)-carene).

In addition to the compounds listed in Table 2, the occurrence in trace amounts of  $\alpha$ -humulene,  $\delta$ -elemene,  $\beta$ -copaene,  $\mu$ -cadinene,  $\gamma$ -muurolene and selina-3,7(11)-diene<sup>10</sup> has been confirmed. The first 5 compounds are ubiquitous, first three of which were isolated and identified in *Abies magnifica* oleoresin.<sup>3</sup> The assignment of the last structure, however, is much less certain.

### EXPERIMENTAL

Natural Canada balsam was purchased from the BDH Ltd. Repeated extraction of 1800 g of this material dissolved in 2800 ml Et<sub>2</sub>O with an aqueous solution of 35 g K<sub>2</sub>CO<sub>3</sub>, 42 g NaHCO<sub>3</sub> per l gave 976 g of mixed neutrals. From these fractions containing monoterpene hydrocarbons (365.1 g, 20.4%), oxygenated monoterpenoids (7.3 g, 0.4%), sesquiterpene hydrocarbons (20.8 g, 1.1%) and probably oxygenated sesquiterpenoids (0.6 g, 0.031%) were separated by a combination of fractional distillation at reduced pressure and chromatography on active alumina.

Separation of mono- and sesqui-terpene hydrocarbon fractions into individual compounds was performed by a combination of Ag-TLC on silica gel and GLC, for oxygenated monoterpenoids, a combination of preparative chromatography on active alumina and GLC was used. The course of liquid/solid chromatographic separation was monitored by TLC and by analytical GLC. To check the possibility that some of the compounds isolated might be isolation artifacts, the presence of each of these compounds in the original Canada balsam was checked by analytical GLC. Details of the chromatographic procedures used were reported earlier<sup>10,12</sup> except as follows. In argentative chromatography 20%, AgNO<sub>3</sub> on Silica gel 30/70 with light petrol/cyclohexane-C<sub>6</sub>H<sub>6</sub> mixtures was used. Length of GLC Carbowax was 18 m (130/150) and 9 m (125) for preparative and analytical work, respectively.<sup>11</sup> In analytical GLC 15% OV-17/0.1% Igepal (Chromosorb G 100/120/155, 9 m  $\times$  3 mm o.d., H<sub>2</sub>/N<sub>2</sub> flow rate 15 ml/min) and in isolation work involving bornyl acetate and methyl thymol the corresponding preparative column (165  $\times$  6 mm o.d., He flow 50 ml/min) were used. Identification of the compounds isolated was performed by IR using published or our own reference spectra as described before.<sup>12</sup>

#### Spectral data for the unknown CBX-2-58

*MS* Only peaks above mass 60, and of at least 20% intensity of the base peak as well as M-1 peak are listed. Intensity and tentative identifications of the fragments<sup>14</sup> are given in parentheses: 178 (42%, M<sup>+</sup>), 177 (7%, M-1), 163 (100%, M-Me), 145 (88%, M-Me-H<sub>2</sub>O), 135 (21%, methyl methoxytropylium), 130 (20%, M-CO-CH<sub>2</sub>O), 123 (25%), 122 (27%), 121 (33%, methoxytropylium), 119 (23%, dimethyltropylium), 109 (21%, methyl, methoxycyclopentadienyl), 107 (41%, hydroxytropylium), 105 (45%, methyltropylium), 95 (27%, methoxycyclopentadienyl), 93 (37%, dimethylcyclopentadienyl), 91 (55%, tropylium), 83 (40%), 82 (24%), 81 (25%, hydroxycyclopentadienyl), 79 (42%, methylcyclopentadienyl), 77 (47%, C<sub>8</sub>H<sub>8</sub>), 71 (23%), 69 (39%), 67 (28%), 65 (22%, cyclopentadienyl).

*IR* Peaks are listed in order of their decreasing intensity. Only well-defined peaks have been considered. When several peaks are reported together, this is indicated by parentheses; within such groups the peaks are listed again in order of decreasing intensity. The spectrum was run using CCl<sub>4</sub> solution and covers the

<sup>7</sup> WENNINGER, J. A. and YATES, R. L. (1969) *J. Assoc. Offic. Anal. Chem.* **52**, 1155.

<sup>8</sup> LAWRENCE, B. M. and WINNINGER, J. A. private communications.

<sup>9</sup> JOSEPH, T. C. and DEV, S. K. H. (1968) *Tetrahedron* **24**, 3809.

<sup>10</sup> BUTTNEY, R., LUNDIN, R. and LING, L. (1967) *Agric. Food Chem.* **15**, 58.

<sup>11</sup> BALOGH, B., WILSON, D. M., BURLINGAME, A. L., LEE, C. J., SNAJBEEK, K. and ZAVARIN, E. (1972) *Phytochemistry* **11**, 1481.

<sup>12</sup> ZAVARIN, E., SNAJBEEK, K., LEE, C. J., HENLEY, M. and MIROV, N. T. (1971) *Phytochemistry* **10**, 1857.

<sup>13</sup> ZAVARIN, E., SNAJBEEK, K., REICHERT, T. H. and TSIF, E. (1970) *Phytochemistry* **9**, 377.

<sup>14</sup> BUDZIKIEWICZ, H., DIETRICH, C. and WILLIAMS, D. H. (1967) *Mass Spectrometry of Organic Compounds*. Holden-Day, San Francisco.

500–4000  $\text{cm}^{-1}$  range excluding 830–720  $\text{cm}^{-1}$  area of  $\text{CCl}_4$  band 1575-aromatic, 1171-C–O, 1675-C=O, (1368, 1383, 1375-isopropyl doublet, methyl), 2960, 2925, 2870, 2820-C–H aliphatic, 3025-C–H aromatic), 972, 1240-C–O, 1630-aromatic, 897, (1420 to 1470-methylene multiplet), 865, 1130 1323, 1140-shoulder 875-shoulder, 1275, 1015, 680, 948, 550, 570

UV 232 nm,  $\log \epsilon = 3.17$ , 277 nm,  $\log \epsilon 2.69$  (EtOH)

Relative retention times for the unknowns isolated CBX-2.58 Carbowax 20 M,  $R_t -2.58$ , OV-17,  $R_t -1.71$ , CBS-3.05 Carbowax 20 M,  $R_t 3.05$  OV-17,  $R_t 1.94$  CBX-0.91 Carbowax 20 M,  $R_t 0.91$ , OV-17,  $R_t 1.52$ , CBX-1.60 Carbowax 20 M,  $R_t 1.60$ , OV-17,  $R_t 2.08$   $R_t$  given in relation to bornyl acetate for OV-17 and in relation to longifolene for Carbowax columns

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*Note added in proof* Unknown CBX-0.91 has now been identified as sibirene