

**Volatile Wood Oils of the Brazilian *Pinus Caribaea* Var.  
*Hondurensis* and Spanish *Pinus Pinaster* Var. *Mediterranea***

**María Concepción García Vallejo**

*CIFOR-INIA, Apartado 8111, 28080 Madrid - Spain*

**Evandro A. Nascimento and Sérgio A.L. Morais**

*Departamento de Química, Universidade Federal de Uberlândia,  
38400 Uberlândia - MG - Brazil*

Received: June 7, 1994; October 6, 1994

Na produção de furfural a partir de madeira por meio de corrente de vapor, a alta temperatura e pressão, são arrastados também componentes voláteis. Para entender o comportamento destes componentes durante o processo industrial, madeira moída de *Pinus caribaea* var. *hondurensis* e de *Pinus pinaster* var. *mediterranea* foram submetidas a uma destilação por arraste de vapor em um aparelho de Clevenger e os óleos essenciais resultantes foram analisados por cromatografia gasosa acoplada à espectrometria de massas. Ambas as espécies apresentaram uma grande quantidade de componentes voláteis, os quais se distribuíram nas frações monoterpênica, sesquiterpênica e, surpreendentemente, diterpênica. 77 constituintes foram identificados.

The extraction of furfural from wood at high temperature and pressure also furnishes volatile components which are steam distilled during the process. In order to understand the behavior of these components during industrial production, milled wood of both *Pinus caribaea* var. *hondurensis* and *Pinus pinaster* var. *mediterranea* were submitted to steam distillation in a Clevenger apparatus, and the resulting essential oils were analyzed by gas chromatography - mass spectrometry. The wood oils of both species presented a great variety of components distributed in the monoterpene, sesquiterpene, and surprisingly, diterpene fractions. 77 constituents were identified.

**Keywords:** *essential oils, Pinus caribaea, Pinus pinaster, terpene, monoterpene, sesquiterpene, diterpene*

## Introduction

The extraction of furfural from wood by acid catalysis is well known. The theory of the process is reviewed in detail by Browning<sup>1</sup>. A process without the addition of a mineral acid catalyst was introduced in the last decade<sup>2</sup>. In *Coniferae* woods, the furfural concentrate contains volatile oils, in addition to acetic acid, methanol, acetone, and other minor soluble constituents. Thus, it is of interest to study the volatile oil composition in the wood to compare with that produced by the industrial process.

Two *Pinus* species were treated: *P. caribaea* var. *hondurensis* (from Brazil) and *P. pinaster* var. *mediterranea* (from Spain), which were further hydrolyzed at 190 °C and 0.1 MPa, for 4 h with steam, to produce furfural. This

process is currently being introduced at the Companhia Triângulo de Participações (COTRIPAR), Uberlândia - MG. The residual lignocellulosic material will be used to produce active carbon.

The essential oils of pine oleoresins obtained by steam distillation (turpentines) and the non-volatile residue (gum rosins) have been studied for many years. Mirov's work<sup>3</sup> is the classic review of turpentines, and recently Zinkel and Han<sup>4</sup> have published a good review on gum rosins. In general, turpentine constitutes 20-25% of the oleoresin, and contains two main fractions of monoterpenes and sesquiterpenes.

The great development achieved by gas chromatography and mass spectrometry techniques in the 1960's has led to an intensive characterization of the constituents of vege-

table materials. Currently, turpentines and gum rosins (upon derivation) are preferentially analyzed by these techniques<sup>5,6,7,8,9</sup>.

The essential oils of pine needles have also been intensively studied, and a review on this subject was recently published<sup>10</sup>. For some species, more than fifty components were identified<sup>11</sup>. The composition of the needle oils is essentially the same as that of the turpentines.

At present, the importance of these studies is extended to the chemotaxonomic characterization of vegetable species<sup>10,12</sup>. For instance, two varieties of *Pinus sylvestris* were identified in Europe by the presence of the monoterpene  $\Delta^3$ -carene. This compound is only present in the trees of the North and not in those from the Mediterranean regions<sup>13</sup>.

A detailed study of the essential oils of the *Pinus caribaea* var. *hondurensis* could not be found in the literature. There was a report in the 1960's<sup>14</sup> and another in the early 1980's<sup>15</sup>, but the number of constituents identified was relatively low,  $\alpha$ - and  $\beta$ -pinenes and  $\beta$ -phellandrene being the most expressive of them.

The characterization of the essential oils of *Pinus pinaster* is more advanced than that of *Pinus caribaea*. A large number of components, including five diterpene hydrocarbons, were detected in the essential oils of the needles of these species<sup>16-19</sup>.

Finally, the literature reports only one work on the essential oils obtained from wood chips from *Pinus balfouriana*<sup>20</sup>, in which a large number of monoterpenes and sesquiterpenes, but no diterpenes, were detected.

## Materials and Methods

### *Pinus caribaea* var. *hondurensis*

It came from the forestation of COTRIPAR in Sacramento - MG. A fifteen year old trunk was left to air dry for six months, and then debarked and chipped. The chips were ground to pass through a 0.1 mm screen.

### *Pinus pinaster* var. *mediterranea*

It was cultivated in the region of Cuenca - Spain. A thirty-four year old trunk was processed in the same way as the *P. caribaea*.

### Steam distillation

The ground wood was steam distilled for 4 h in a Clevenger apparatus, which enables an accurate quantification of the distilled oil at any time during the process in a very simple way<sup>18</sup>.

### Gas chromatography - mass spectrometry

A HP 5890 gas chromatograph was used, equipped with a HP 5971 selective mass detector, a SE 30 capillary column of 12 m, i.d. of 0.25 mm, energy of 70 eV and temperature program: 70 °C (5 min) - 235 °C (3 °C/min);

injector and detector temperatures: 250 °C; and carrier gas: nitrogen (12 mL/min). The calculations were based on total ion chromatograms (TIC).

For compound identification references 21-23 were used. The mass spectrometer data bank (nbs54k) was also very important for the elucidation of many structures.

Fortunately, the massive utilization and good uniformity of the capillary columns, and the high sensibility of the modern equipment permitted accurate reproducibility of retention times. Thus, this parameter can be used as a valuable tool for the identification of the compounds<sup>24</sup>.

Finally, in a few cases, a standard compound was employed to confirm its presence in the essential oil, and compounds at low concentrations (< 0.3%) were not considered.

## Results and Discussion

Figures 1 and 2 show the chromatograms of the essential oils of *Pinus caribaea* var. *hondurensis* and *Pinus pinaster* var. *mediterranea*, respectively. Table 1 shows the identified and non-identified compounds with their percentages based on Figs. 1 and 2.

Both wood oils present significant concentrations of diterpenes. This fact is not characteristic of turpentines, needles and wood oils. Further, the diterpene fraction is the most abundant at all in the tropical *P. caribaea*. Normally, the exhaustive distillation of oleoresins furnishes terpenoids whose molecular weight is not above those of sesquiterpenes<sup>25</sup>. As the main components of oleoresins are

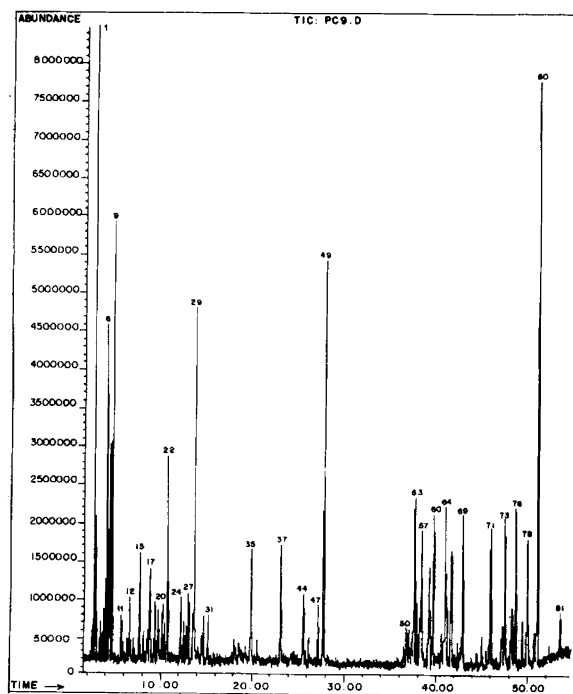


Figure 1. Chromatogram of the essential oil of *Pinus caribaea* var. *hondurensis*.

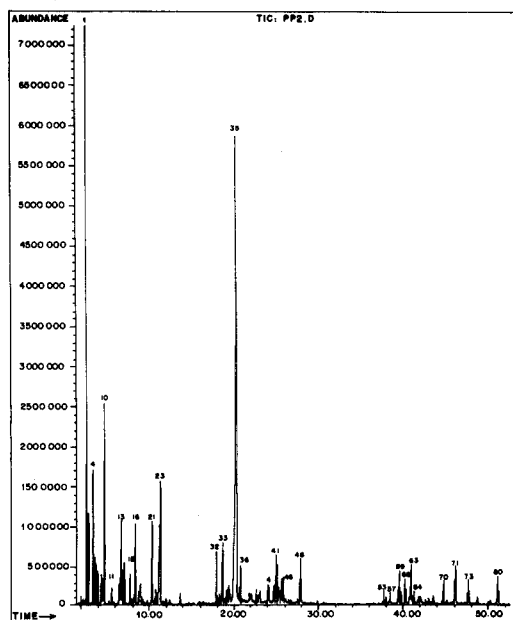


Figure 2. Chromatogram of the essential oil of *Pinus pinaster* var. *mediterranea*.

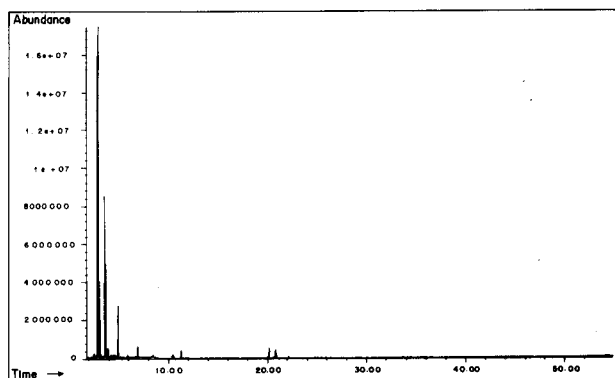


Figure 3. Chromatogram of the *Pinus pinaster* sulfate-turpentine.

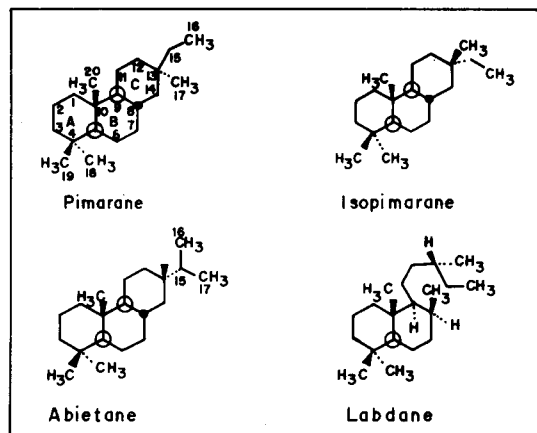


Figure 4. Basic structures of diterpene hydrocarbons.

the diterpene resin acids, it is outstanding that the wood oils are richer in diterpenes than turpentines.

In comparison with needle oils, the diterpenes fraction obtained from wood contains more components and in a greater amount. Taking into account that the furfural production is carried out at 190 °C and 0.7 MPa of pressure, the quantity of volatile oil will be more expressive than that obtained in this study: 0.5% yield (based on dried wood).

Pine sulfate-turpentine is a by-product of the pulping of this wood. During the digestion of the chips the oil is distilled and collected. Figure 3 shows the chromatogram of a *Pinus pinaster* sulfate-turpentine. The diterpenes fraction is not present. The basic medium can hydrolyze the resin esters, but we can not account for the absence of the other diterpenes which have structures related to those shown in Fig. 4. The norditerpene hydrocarbons have the same structure, except that they carry only one methyl group instead of two at the carbon 4 of the ring.

Comparing the essential oil composition of the two pine species, the difference between them is outstanding. Both pines contain about the same amount of monoterpenes; *P. pinaster* is richer in sesquiterpenes than *P. caribaea*, while the latter is richer in diterpenes.

These general remarks are already sufficient to distinguish both types of pine. However, there are other groups of components which are characteristic of each species. For example, the compounds of  $M^+$  176 and 178 are not present in *P. pinaster*, which also lacks various sesquiterpene hydrocarbons. Norditerpene hydrocarbons are present in *P. pinaster* at low levels, as are methyl resin acid esters.

Longifolene and  $\alpha$ -pinene are responsible for almost half of the *P. pinaster* wood oil, although no component reaches 10% in *P. caribaea*.

Due to their relatively large amount, compounds 29 and 49 of the *P. caribaea* oil could be isolated by preparative gas chromatography (SE 30 packed column, 4.0 m and 2.5 mm i. d.). Figures 5 and 6 show the IR and mass spectra of both compounds. Compound 29 is phellandral contaminated with a small amount of an unknown compound, and compound 49 could be propanoic acid, 2-methyl-1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester ( $M = 286$  and  $243 = M - (CH_3)_2CH$ ). Both the IR and the mass spectra confirm this structure suggested in the literature. However, we are not aware of this identification because there was no sample to be used as standard.

To our knowledge, compound 49 has been not detected in pine oils so far. It is, however, present in poplarbud exudate used by bees to make propolis<sup>26</sup>. It was also detected in the volatiles of PVC film to which it is added as a plasticizer<sup>27</sup>.

Looking for a possible variety among *Pinus caribaea* var. *hondurensis* trees, we selected a group whose appearance was very different from those in the previous study. Figure 7 shows the gas chromatogram of its essential oil.

**Table 1.** Composition of the essential oils of *Pinus caribaea* var. *hondurensis* and *Pinus pinaster* var. *mediterranea* (%).

Peak	Component	<i>P. caribaea</i>	<i>P. pinaster</i>	Peak	Component	<i>P. caribaea</i>	<i>P. pinaster</i>
1	$\alpha$ -pinene	3.64	11.72	48	sesquit. alcohol, M <sup>+</sup> 222	–	1.87
2	camphene	0.99	1.46	49	ester of 2-methylpropanoic acid <sup>2</sup>	5.70	–
3	sabinene	0.68	0.65	50	phtalic acid ester <sup>3</sup> , M <sup>+</sup> 278	0.70	–
4	$\beta$ -pinene	0.33	2.16	51	norditerpene hydroc., M <sup>+</sup> 258	0.47	–
5	myrcene	0.54	1.03	52	" 256	0.57	–
6	$\alpha$ -phellandrene + 1,3,8-p-mentatriene	3.17	0.70	53	norpimara-8(14) - 15-diene	2.93	–
7	$\alpha$ -terpinene	1.94	0.62	54	norditerpene hydroc., M <sup>+</sup> 256	0.98	–
8	p-cymene	2.25	0.59	55	" 256	0.45	–
9	limonene	4.95	t <sup>1</sup>	56	" 256	0.51	–
10	$\beta$ -phellandrene	t	5.68	57	norisopimara-8(14), 5-diene	2.72	–
11	$\Delta^3$ -carene	0.47	0.36	58	norabieta-8(14), 12-diene	1.77	–
12	2-p-tolylpropene	0.74	0.75	59	pimara-8(14), 15-diene	0.78	1.54
13	terpinolene	t	2.30	60	norabieta-8, 13-diene	2.84	–
14	2-nonen-1-ol	t	1.11	61	isopimara-7, 15-diene	–	0.85
15	myrtenal	1.48	0.84	62	norabieta-7, 13-diene	0.72	–
16	fenchol	–	2.34	63	abieta-7, 13-diene	–	1.63
17	umbellulone	2.14	–	64	norabieta-8, 11,13-triene	2.64	–
18	isopinocampone	0.86	–	65	norabieta-8(14),11,13(15)-triene	1.67	–
19	naphthalene	0.72	–	66	abieta-8(14), 13(15)-diene	0.73	–
20	myrtenol	0.66	–	67	norabieta-8(14), 13(15)-diene	1.63	–
21	pinocampone	0.52	3.01	68	norabieta-7, 13,15-triene + dit. hydroc., M <sup>+</sup> 270	1.76	–
22	estragol	3.12	–	69	abieta-8, 11,13-triene	2.61	–
23	verbenone	–	5.13	70	norpimara-8(14), 15-dien-3-one	–	0.91
24	p-isopropylbenzaldehyde	1.04	–	71	pimara-8, 14(15)-dienal	2.06	1.53
25	methyltymol	0.56	–	72	abieta-8(14),11,13(15)-triene + isopimara-7,15-dienal	0.91	–
26	not identified, M <sup>+</sup> 148	0.51	–	73	abieta-7,13-dienal + abieta-6,8,11,13-tetraene	1.97	0.86
27	" 178	0.84	–	74	abieta-7,13,15-triene	0.69	–
28	" 176	0.63	–	75	methyl-8(14),15-pimara dienoate	0.82	–
29	phellandral + contaminant	5.43	–	76	abieta-8,11,13-trienal	2.52	–
30	" 178	0.59	–	77	abieta-8(14),11,13(15)-trienal	0.68	–
31	" 178	0.91	–	78	methyl-7,15-isopimara-dienoate	2.28	–
32	sesquit. hydroc. M <sup>+</sup> 204	–	1.83	79	estolid	t	t
33	" 204	–	2.22	80	methyl-8,11,13-abieta-trienoate	9.70	2.10
34	$\alpha$ -cubebene	–	0.70	81	methyl-6,8,11,13-abieta-tetraenoate	0.65	–
35	longifolene	1.61	34.63		monoterpene hydrocarbons	19.70	28.02
36	$\beta$ -caryophyllene	–	1.26		oxygenated monoterpenes	16.53	13.14
37	sesquiterpene ketone	1.89	–		compounds of M <sup>+</sup> 176 and 178	3.46	–
38	$\beta$ -hymachalene	–	0.25		sesquiterpene hydrocarbons	3.50	45.67
39	$\alpha$ -muurolene	–	0.25		norditerpene hydrocarbons	23.60	2.14
40	calamenene	–	0.76		diterpene hydrocarbons	6.24	4.45
41	$\gamma$ - + $\delta$ -cadinene	–	1.70		oxygenated diterpenes	6.70	1.96
42	cadina-1,4-diene	–	0.51		methylesters of resin acids	13.45	1.02
43	calacorene	–	0.51				
44	not identified, M <sup>+</sup> 198	1.12	–				
45	dihydrovalencene	–	1.05				
46	sesquit. alcohol, M <sup>+</sup> 220	–	0.86				
47	not identified, M <sup>+</sup> 198	1.01	–				

1: t = traces; 2: see text; 3: contaminant

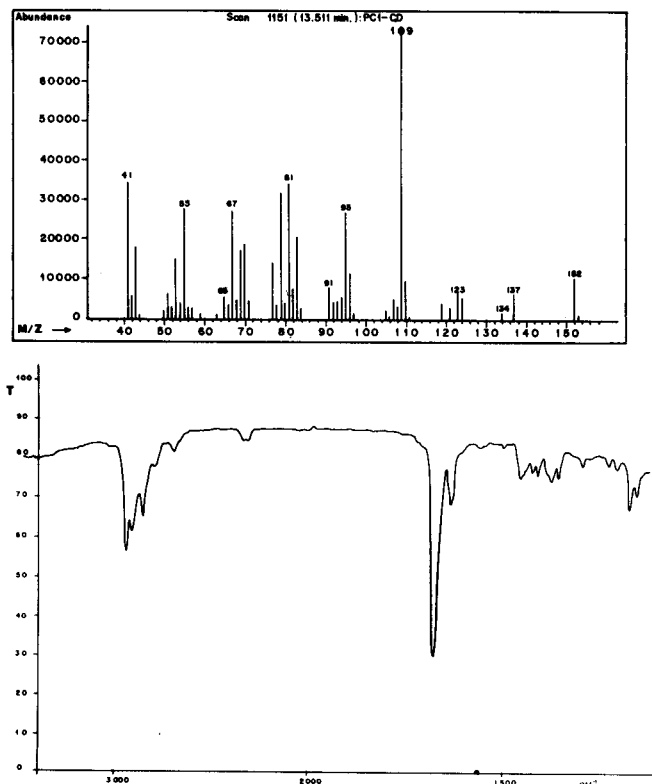


Figure 5. IR and mass spectra of compound 29.

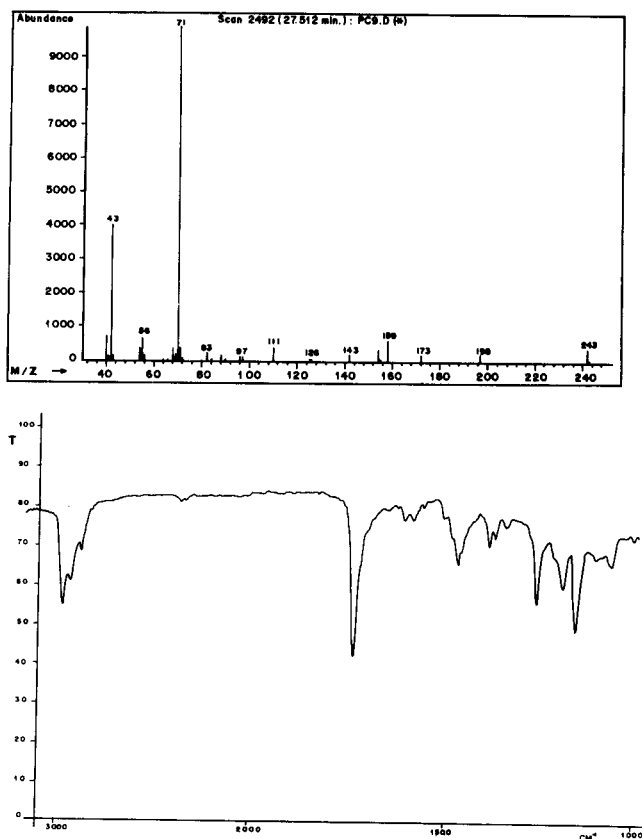


Figure 6. IR and mass spectra of compound 49.

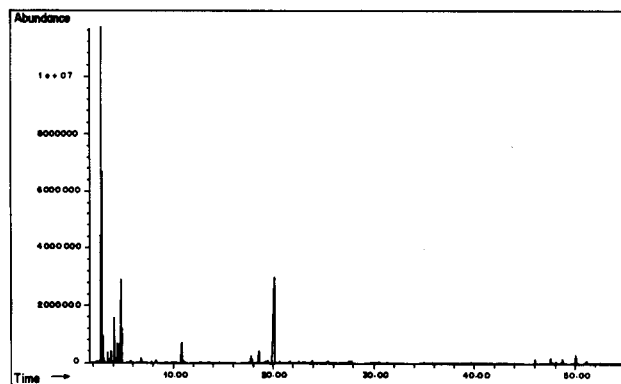


Figure 7. Chromatogram of the essential oil of another representative of *P. caribaea* var. *hondurensis*.

Comparing this chromatogram with that shown in Fig. 1, one can see that this new essential oil is very different from those studied so far. This fact confirms the morphological difference observed in both groups of trees and suggests new studies to be conducted on well selected species representatives<sup>10</sup> to clarify this distinction.

### Acknowledgments

We thank Prof. Francisco Isabel (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) for the kind supply of *Pinus pinaster* sulfate-turpentine, and also CNPq for financial support.

### References

1. B.L. Browning, *Methods in Wood Chemistry* (Interscience Publishers, New York, 1967), p. 615.
2. Patent Pol. appln. 336,962 (June 15, 1982), *Process for Utilizing Aqueous Solution of Acetic Acid in Hydrolyzing Vegetable Raw Materials*.
3. N.T. Mirov, *Composition of Gum Turpentine of Pines*, Technical Bull. No. 1239, Forest Service - U.S. Department of Agriculture (1961).
4. D.F. Zinkel and J.S. Han, *Naval Stores Review* **96** (2), 14 (1986).
5. J.S. Han and Byung-Ho Hwang, *J. Korean Wood Sci. Technol.* **16** (2), 62 (1988).
6. S. Zhanqian, L. Xing, and L. Zhiqin, *Naval Stores Review* **103** (2), 6 (1993).
7. J.A. Pardos and G. Weissmann, *Holzforchung* **44**, 143 (1990).
8. J.S. Han and D.F. Zinkel, *Naval Stores Review* **100** (1), 11 (1991).
9. W. Lange and T.S. Janezic, *Holzforchung* **47**, 207 (1993).
10. O. Ekundayo, *Flavor and Fragrance J.* **3**, 1 (1988).
11. R.P. Adams and G.F. Edmunds Jr., *Flavor and Fragrance J.* **4**, 19 (1989).
12. D.F. Zinkel, *Pine Resin Acids as Chemotaxonomic and Genetic Indicators*, TAPPI Conf. Papers, For. Biol. Wood Chem. Conf., Madison, Wis. (1977), p. 53.

13. W. Lange und G. Weissmann, *Holz als Roh- und Werkstoff* **46**, 157 (1988).
14. J. Drew and G.D. Pylant Jr., *Tappi* **49** (10), 430 (1966).
15. K. Löyttyniemi, R. Löyttyniemi, R. Hiltunen and Räisänen, *Silva Fennica* **16**, 351 (1982).
16. G. Pauly, M. Gleizes, and C. Bernard-Dagan, *Phytochemistry* **12**, 1395 (1973).
17. M.M. Carmo e S. Frazão, *The Essential Oil of Portuguese Pine Needles, Progress in Essential Oil Research*, (Walter de Gruyter & Co., New York, 1986), p. 169.
18. M. Domínguez G., D. García M., and M.C. García V., *The Essential Oil of Needles from Spanish Pinus Pinaster Aiton*, Proc. 10th Int. Congress of Essential Oils, Fragrances and Flavors, Washington, D.C., 1986 (Elsevier Science Publishers B.V., Amsterdam, 1988).
19. M. Domínguez G., M.C. García V., D. García M. and J. Sanz P., *Diterpene Hydrocarbons in the Essential Oils of Pinus Pinaster Aiton*, Proc. Int. Symposium Biol. Chem. and Biochem. of Plant Terpenoids, Murcia, Spain, 1989.
20. K.S. Snajberk, E. Zavarin, and D. Bailey, *Biochem. System. Ecol.* **7**, 269 (1979).
21. S.R. Heller and G.W.A. Milne, *EPA/NIH Mass Spectral Data Base* (US Government Printing Office, Washington, 1983).
22. M.G. Moshonas and E.D. Lund, *The Flavor Industry* **1** (6), 375 (1970).
23. S.K. Ramaswami, P. Briscese, R.J. Gariullo and T. von Geldern, *Flavors and Fragrances: a World Perspective*, Proc. 10th Int. Congress of Essential Oils, Fragrances and Flavors, 951, Washington, DC, 1986.
24. Analytical Methods Committee, Application of Gas-Liquid Chromatography to the Analysis of Essential Oils, *Analyst* **109** (1984).
25. W. Lange and G. Weissmann, *Holz als Roh- und Werkstoff* **46**, 157 (1988).
26. W. Greenaway, J. May, T. Scaysbrook and F.R. Whatley, *Z., Naturforsch.* **46c**, 111 (1991).
27. H. Kim and S.G. Gilbert, *J. Food Sci.* **54** (3), 770 (1989).