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The Composition of Wood Extracts from Spanish *Pinus pinaster* and Brazilian *Pinus caribaea*

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Madeira sem casca de duas espécies de *Pinus, pinaster var. mediterranea,* espanhola, e *caribaea var. hondurensis,* brasileira, foi extraída com diclorometano em um soxhlet e os extratos foram analisados por CG-EM, após prévia separação dos ácidos resínicos e graxos e da fração insaponificável. A quantidade de insaponificáveis de ambas as espécies é parecida (*P. caribaea*, 9,2%; *P. pinaster* 7,9%), mas a *P. pinaster* apresenta maior conteúdo em ácidos resínicos que *P. caribaea* (86,3 e 76,4%, respectivamente); a *P. caribaea*, por outro lado, possui maior quantidade de ácidos graxos que a *P. pinaster* (11,4 e 3,6%, respectivamente). Com respeito à composição de cada fração, existem grandes diferenças entre elas, tanto no número de constituintes quanto na concentração de cada um deles.

Dichloromethane extracts from the debarked wood of *Pinus caribaea var. hondurensis* from Brazil and of *Pinus pinaster var. mediterranea* from Spain were analyzed by GC-MS, after prior separation of the resinous and fatty acid, and unsaponifiable fractions. Both pine species present practically the same unsaponifiables content (*P. caribaea:* 9.2% and *P. pinaster:* 7.9%), but the latter is richer in resinous acids than the former (86.3 and 76.4%, respectively). On the other hand, *P. caribaea* presents a higher content of fatty acids than *P. pinaster* (11.4 and 3.6%, respectively). Comparing the composition of the corresponding fractions of both *Pinus* species, one can see great differences in the constituents as well as their concentration.

Keywords: Pinus caribaea, Pinus pinaster, *Pinus extracts, terpenes, sesquiterpenes, diterpenes, triterpenes, sterols*

Introduction

Continuing our studies on minor wood components of the *Pinus* species, *caribea var. hondurensis* from Brazil and *pinaster var. mediterranea* from Spain[1], we present in this work the constituents of the dichloromethane extracts obtained from ground debarked wood in a soxhlet apparatus.

Usually such studies focus on *Pinus* gum resins, due to the interests of the naval stores industry. The essential oils have of needles also been investigated, though less intensively for their importance to the flavor and fragrance industries. However, in recent years, the need to increase throughput in the pulp and paper industries and increasing environmental concerns have forced detailed studies on

wood extracts. Low extract-content wood species are preferred because their pulp is of better quality and the pitch problems are reduced. In addition, fewer chemicals are required in the pulping and bleaching processes.

Pinus gum resins contain resinous acids as the major constituents (75-80%), as well as mono-, sesqui-, and diterpenes (20-25%)[7]. In addition to these components, *Pinus* wood extracts contain fatty acids, triglycerides, waxes, steryl esters, sterols, triterpene alcohols, and fatty alcohols.

The literature did not reveal any studies on wood extracts of *P. caribaea,* but the composition of petroleum ether extracts of *P. pinaster var. atlantica* has already been reported[8]: 1.86% dried wood 52,7%; resinous acids (pimaric, sandaracopimaric, isopimaric, abietic, and dehydroabietic); 10% fatty acids (palmitic, oleic, and linoleic) and 21,4% unsaponifiable constituents. Other constituents were not mentioned.

Another study carried out on the same subspecies[9] provided different results: 1.12% extracts, 61.92% resinous acids, 5.93% fatty acids; and 32.15% unsaponifiables constituents.

Wood extracts of other *Pinus* species have frequently been reported in the literature[5,10-16], references 11-15 being very comprehensive. A large number of resinous and fatty acids and unsaponifiable constituents have been identified.

The present work intends to reproduce the recent results obtained for pine wood extracts of *P. caribaea var. hondurensis* and *P. pinaster var. mediterranea.*

Materials and Methods

Pinus caribaea var. hondurensis

The wood was cut during the rainy season at the reforestation of COTRIPAR - Sacramento - MG. A fifteen year old trunk was left to air dry for six months, and was then debarked and chipped. The chips were ground to pass through a 0.1 mm screen.

Pinus pinaster var. mediterranea

This wood was cultivated in the Cuenca region of Spain. A thirty four year old trunk was processed in the same way as *Pinus caribaea.*

Extracts

The extraction was carried out in a soxhlet with dichloromethane to avoid low molecular-weight carbohydrates, salts, and water soluble substances which are extracted by ethanol-benzene. Dichloromethane also allows low temperature extraction. The procedure was based on Tappi standard T 204 om-88. The dichloromethane solution was then filtered and the solvent evaporated at 30 °C in a rotavapor. The extracts were further dried in a vacuum oven.

Saponification and fractionation

Two gram of extracts were dissolved in 200 mL of an ethanolic (90%) 0.5 N KOH solution and refluxed for 1 h over a water bath. An equal amount of water was then added, and the unsaponifiable were constituents extracted with 50 mL of petroleum ether (3 times). Free acids were liberated with 1.5 N HCl and extracted with 50 mL of petroleum ether (3 times).

Fatty acids were selectively separated from resinous acids with a methyl sulfuric acid solution (ASTM D-1585-63, 100 g of concentrated sulfuric acid in 400 g of methanol). Two gram of extracts were dissolved in 20 mL of sulfuric acid solution and refluxed for 1/2 h over a water bath. Then the solution was cooled and a diluted KOH solution was added until pH $\cong$ 14. The fatty acid methyl esters were extracted with 25 mL of petroleum ether (3 times). The resinous acids were liberated with 1.5 N HCl and extracted with 25 mL of diethyl ether (3 times).

Gas chromatography - mass spectrometry

Unsaponifiable constituents and fatty esters were diluted in diethyl ether and injected diretly in to a gas chromatograph; resinous acids were methylated with diazomethane before the injection. A gas chromatograph HP 5890 was used equipped with a selective mass detector HP 5971, SE 30 capillary column of 12 m, i. d. of 0.25 mm, electronic energy of 70 eV and the following temperature program: 70 °C (5 min) - 300 °C (3 °C/min); injector and detector temperatures of 250 and 325 °C, respectively, and carrier gas: helium (0.5 mL min). The quantifications were based on the total ion chromatograms (TIC), and the components at concentrations lower than 0.3 % were considered.

For compound identification a extensive bibliography was employed. The literature began with the classic works on mass spectroscopy of diterpenoids[17-24], which were exhaustively applied by Lange and Weissmann in their studies of gum resins[7,25-31], and also by Ekman[32-34] and Hafizoglu[15,35-38] for wood extracts; and finally in the book by Adams[39]. The mass spectrometer library (nbs54k) was also very useful.

Results and Discussion

The content of *Pinus* species extracts normally lies between 1 and 4% dried wood. The results for *P. caribaea* and *P. pinaster* for various solvents are shown in Table 1. As can be seen, the polarity of the solvent has influence on the content of the extracts. So, the less polar solvents diethyl-ether and dichloromethane extract fewer substances than acetone and ethanol. The latter is less polar than acetone, but as a protonic solvent it can form more hydrogen bonds, and therefore extracts a greater number of compounds.

Similar studies carried out on *P. pinaster* using methyl benzene, diethyl ether, dimethyl benzene, ethanol, acetone,

Table 1. Wood extracts contents of *P. caribaea var. hondurensis* and *P. pinaster var. mediterranea**.

Solvent	*P. caribaea*	*P. pinaster*
dichloromethane	2.50	2.75
ether	2.69	2.22
acetone	3.07	3.04
ethanol	3.51	3.82

Table 2. Composition of dichloromethane extracts of *P. caribaea var. hondurensis* and *P. pinaster var. mediterranea* (%).

Fraction	*P. caribaea*	*P. pinaster*
unsaponifiables	9.2	7.9
resinous acids	76.4	86.3
fatty acids*	11.4	3.6
total	97.0	97.8

* as methyl esters.

and trichloromethane showed that the quantity of material extracted from wood chips slightly decreased in the order indicated[16].

Table 2 presents the composition of the extracts of both species of *Pinus* under consideration, obtained by saponification and fractionation of the material extracted with dichloromethane. We employed this procedure because the Zinkel Technique[40], which uses ion exchange chromatography, is tedious and very time consuming, in spite of the more precise results it provides. The present technique can also furnish good results if well connected to a GC-MS as this work intends to show.

The amount of unsaponifiable constituents is practically the same for both *Pinus* species, but the fatty acid content differs greatly: *P. pinaster* presents a very low content of these components.

The gas chromatogram of the resinous ester fraction is presented in Fig. 1, and Table 3 shows the corresponding quantification. The feature observed in Fig. 1 is similar to that reported by Zinkel and Han using a DB-1 capillary column[41].

The high content of dehydroabietic acid apparent in both species may be due to the oxidation of palustric and levopimaric acids (Fig. 2) during the long period of air exposure after cutting. Compound 1 presents M^+ 314 and a fragmentation pathway similar to pimaric acid, but has not been reported yet. Compound 10 has been identified in *Pinus luchuensis*[28].

Figure 3 and the corresponding Table 4 present the distribution of fatty acids in *P. caribaea* and *P. pinaster*. Almost 75% of the total for both pines is composed represented of palmitic, linoleic, oleic, and stearic acids. These findings are in good agreement with those reported for

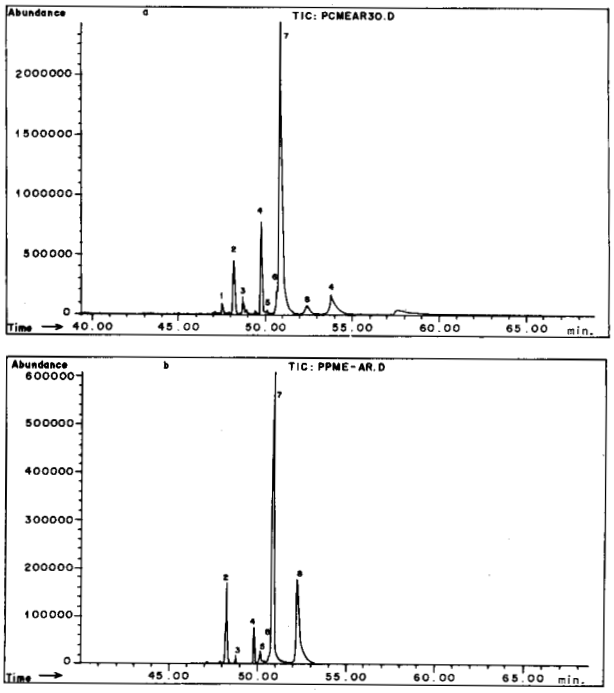

Figure 1. Gas chromatogram of the resinous acid methyl esters of a) *P. caribaea*, and b) *P. pinaster* wood extracts.

Table 3. Resinous acid in wood extracts of *P. caribaea* and *P. pinaster* (% as methyl esters).

Peak	Compound	*P. caribaea*	*P. pinaster*
1	dehydropimaric (?)	1.3	
2	pimaric	7.3	13.1
3	sandaracopimaric	2.0	1.0
4	isopimaric	11.5	4.8
5	palustric	-	2.0
6	8,11,13,15-abietatetranoic	2.6	1.0
7	dehydroabietic	61.1	51.8
8	abietic	2.6	26.3
9	6,8,11,13-abietatetranoic	8.6	-
10	7-oxo-dehydroabietic	1.6	-
	others	1.4	0.1
	total	100.0	100.0

other *Pinus* species[5,8,15]. However, the content of long chain alkanoic acids (arachidic, behenic, and lignoceric) is higher than those usually found in wood extracts[15].

The presence of levopimaric, dehydroabietic, and 7-oxo-dehydro-abietic acids indicates that the saponification was not complete. This does not cause significant error because the total is under 10% and the fatty acid content for pines is low.

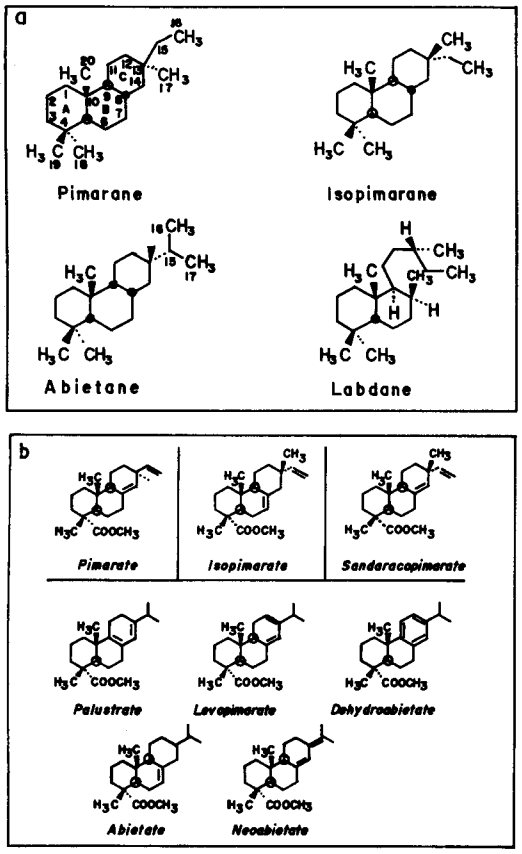

Figure 2. a) Main hydrocarbon skeletons for the resinous acid, and b) resinous acid methyl esters of the abietane, pimarane, and isopimarane skeletons[41].

Table 4. Fatty acids in wood extracts of *P. caribaea* and *P. pinaster* (% as methyl esters).

Peak	Compound	*P. caribaea*	*P. pinaster*
1	nonanedioic	t	0.6
2	7-hexadecenoic (Z)	1.2	
3	palmitic	12.9	31.3
4	heptadecanoic	1.7	2.9
5	linoleic	7.6	14.4
6	oleic	46.1	25.5
7	stearic	4.6	4.3
8	not identified, M^+ 227	2.7	4.7
9	levopimaric	2.4	1.8
10	8,11,13,15-abietatetranoic	0.8	-
11	dehydroabietic	3.5	3.2
12	arachidic	1.3	4.0
13	not identified	M^+	329
14	7-oxo-dehydroabietic	2.1	-
15	behenic	2.7	4.3
16	tricosanoic	0.8	-
17	lignoceric	3.8	2.2
	others	4.6	0.8
	total	100.0	100.0

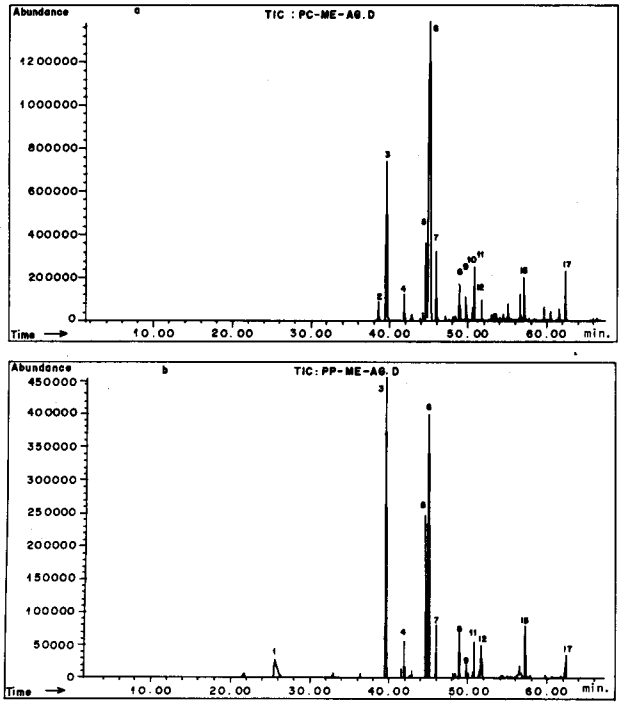

Figure 3. Gas chromatogram of the fatty acid methyl esters of a) *P. caribaea*, and b) *P. pinaster* wood extracts.

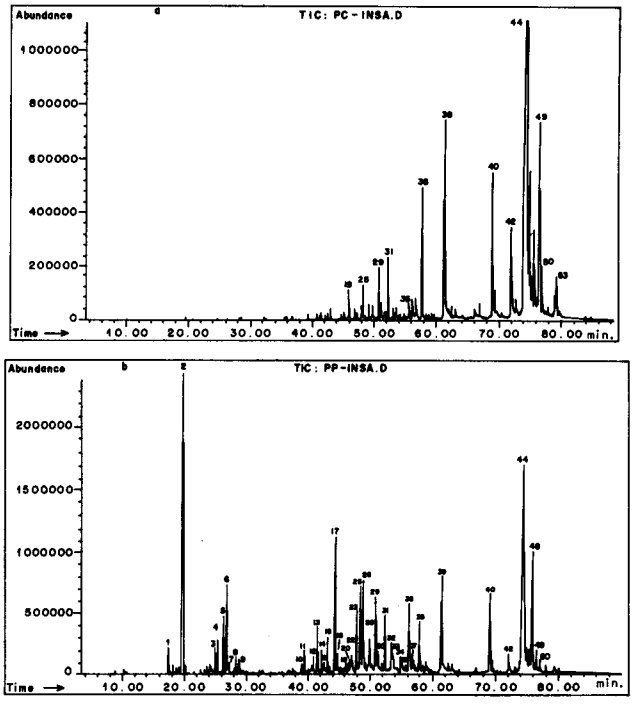

Figure 4. Gas chromatogram of the unsaponifiable constituents of a) *P. caribaea*, and b) *P. pinaster* wood extracts.

Table 5. Unsaponifiable constituents of *P. caribaea* and *P. pinaster* (%).

Peak	Compound	*P. caribaea*	*P. pinaster*
1	longipinene	-	0.5
2	α-longifolene	-	13.5
3	dihydrovalencene	-	0.6
4	sesquit. alcohol, M^+ 220	-	0.7
5	not identified, M^+ 177	-	1.5
6	sesquit. alcohol, M^+ 222	-	2.8
7	not identified, M^+ 138	-	0.3
8	cubenol	-	0.3
9	T-cadinol	-	0.4
10	diterp. hydrocarbon, M^+ 272	-	0.3
11	pimara-8(14),15-diene	t	0.6
12	isopimaradiene	t	0.5
13	norpimara-8(14),15-dien-3β-ol	t	1.4
14	norpimarol	t	0.6
15	norsandaracopimarol	t	0.5
16	norisopimarol	t	1.0
17	19-norpimara-8(14),15-dien-3-one	5.4	
18	norpalustrol	-	0.6
19	nordehydroabietol	0.7	0.3
20	not identified, M^+	222	0.4
21	not identified, M^+	288	0.3
22	2-hydroxi-isopimarene	t	0.7
23	hydroxi-isopimarene	-	1.9
24	not identified, M^+	270	0.4
25	levopimarol	0.8	4.1
26	hydroxi-palustrene	-	3.8
27	abietatetraene (?)	0.3	
28	palustrol		0.5
29	methyldehydroabietate	1.1	2.4
30	dehydroabietol	0.5	1.0
31	methyl-hydroxi-abietatetraenoate	1.3	1.5
32	methylabietatetraenoate	0.5	1.2
33	dehydroabietic acid	-	0.8
34	aliphatic alcohol, M^+ 326	t	0.6
35	abietic acid	0.4	t
36	behenyl alcohol	0.9	2.5
37	methyl-7-oxo-dehydroabietate	0.5	0.6
38	aliphatic alcohol, M^+ 342	3.8	1.9
39	lignoceryl alcohol	6.7	3.8
40	not identified, M^+ 394	4.5	2.9
41	not identified, M^+ 396	0.8	t
42	campesterol	4.4	0.5
43	campestanol	0.9	0.3
44	β-sitosterol	47.4	19.5
45	β-sitostanol	1.7	0.5
46	24-methylpolinastanol	4.8	1.0
47	not identified, M^+ 486	1.1	1.0
48	not identified, M^+ 410	3.3	5.8
49	24-methylpolinasterol	7.4	0.8
50	not identified,M^+ 410	1.4	0.4
51	24-methylen-cycloartanol	0.5	-
52	dihydrocycloeucalenol	0.8	-
53	cycloeucalenol		1.2
	others	1.1	6.9
	total	100.0	100.0

In spite of their moderate presence in wood extracts, the unsaponifiable constituents exhibit a great diversity of components (Fig. 4 and Table 5) which enables a better differentiation between *P. caribaea* and *P. pinaster*. The latter is rich in sesquiterpenoids, which do not appear for the former. As in the essential oils[1], α-longifolene is one of the main constituents of *P. pinaster* wood extracts.

Compound 13, norpimara-8(14),15-dien-3β-ol, was isolated by Lange and Weissmann from *P. pinaster*[27]. Based on its mass spectrum and retention time, we could assign the other norterpenols. The characteristic fragmentation pathway of all norterpenols is: 274-15 $(M\text{-}CH_3)^+$ and 259-18 $(M\text{-}CH_3\text{-}H_2O)^+$. Except for the above mentioned work[27], we could not find any other reference on norterpenols in pine extracts.

Compound 22, 2-hydroxi-isopimarane, as well as hydroxi-isopimarane have already been reported[39].

Compound 27 has not been reported so far, although tetraenditerpenes are natural constituents of wood extracts.

Compounds 29-37, except compounds 30, 34, and 36, are the remaiders of esters or acids. Their content is relatively low and, therefore, they do not affect the overall analysis.

Four alkanols could be detected, but only two (compounds 36 and 39) were identified.

Phytosterols are the most important group of unsaponifiable constituents in *P. caribaea* extracts, being β-sitosterol composing almost 50% of the total mass. It also represents the main component in *P. pinaster* unsaponifiable constituents.

The triterpenoids have a significant presence in *P. caribaea* and appear in small amounts in *P. pinaster*. Five of them could not be identified.

An overall view of Fig. 4 shows that the diterpenoids are the most abundant of the *P. pinaster* unsaponifiable constituents; *P. caribaea* presents β-sitosterols and triterpenoids as its main components.

Finally, the direct determination of wood extract components, as suggested for pine oleoresins[42] (after previous methylation), is temerarious because a great number of constituents have a retention times close to others (perhaps also for oleoresins).

Conclusions

Pinus wood extracts can be analyzed with relatively good accuracy, combining chemical and GC-MS techniques. The extracts must firstly be saponified to separate unsaponifiable constituents from resinous and fatty acids. These can be selectively separated by means of methyl sulfuric acid. The three fractions must be separately analyzed by GC-MS.

Major differences between *P. caribaea var. hondurensis* and *P. pinaster var. mediterranea* wood extracts can be seen in the unsaponifiable constituents. The former presents a high content of phytosterols and triterpenoids (β-sitosterol = 47.4%), while *P. pinaster* is rich in diterpenoids. The latter also presents significant amounts of sesquiterpenes (α-longifolene = 13.5%) which do not appear in the *P. caribaea* unsaponifiable constituents.

Acknowledgments

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References

1. M.C. García Vallejo, E.A. Nascimento, Sérgio A.L. Morais, *Volatile Wood Oils of Brazilian Pinus* caribaea var. hondurensis *and Spanish* Pinus pinaster var. mediterranea. *J. Braz. Chem. Soc.* **30**, (1994).
2. I.D. Suckling and R.M. Ede, *Appita* **43** (1): 77 (1990).
3. Y. Ohtani and T. Shigemoto, *Appita* **44** (1): 29 (1991).
4. B.B. Sithole, *Appita* **45** (4): 259 (1992).
5. A. Quinde and L Paszner, *Holzforschung* **46** (6):513 (1992).
6. I.D. Suckling, H.L. Hua and J.M. Uprichard, *Appita* **43** (3): 217 (1990).
7. W. Lange and G. Weissmann, *Holz als Roh- and Werkstoff* **49**, 476 (1991).
8. C. Arrabal Miguel and M. Cortijo Martinez, *Ponencias y Comunicaciones del Congreso Forestal Español* (Lourizán, Pontevedra, 1993) tomo IV, p. 297.
9. F. Isabel Fernandez-Vega and P. Navarrete Varela, *Ponencias y Comunicaciones del Congreso Forestal Español* (Lourizán, Pontevedra, 1993) tomo IV, p. 1289.
10. W. Lange, H. Kubel and G. Weissmann, *Holz als Roh- and Werkstoff* **47**, 487 (1989).
11. H. Yildirim and B. Holmbom, *Acta Acad. Abo. Ser. B* **37** (3): 9 p. (1978).
12. H. Yildirim and B. Holbom, *Acta Acad. Abo.Ser. B* **37** (4):6 p. (1978).
13. H. Yildirim and B. Holbom, *Acta Acad. Abo.Ser. B* **37** (5):9 p. (1978).
14. H. Hafizoglu, *Holzforschung* **37** (6): 321-326 (1983).
15. H. Hafizoglu, *Holzforschung* **43** (1): 41-43 (1989).
16. A. Caperos Sierra, A. Romero Salvador, and F. García-Ochoa Soria, *Tappi J.* **74** (5): 191 (1991).
17. C.R. Enzel, *Tetrahedron Letters* 1285 (1966).
18. C.R. Enzel, *Tetrahedron Letters* 2135 (1966).
19. C.R. Enzel, *Arkiv Kemi* **26**: 87 (1966).
20. C.R. Enzel and R. Ryhage, *Arkiv Kemi* **27**: 213 (1967).
21. C.R. Enzel and R. Ryhage, *Arkiv Kemi* **26**: 425 (1966).
22. C.R. Enzel and I. Wahlberg, *Acta Chim Scand.* **23**: 871 (1969).
23. H. Audier, S. Bory, G. Defaye, M. Fétizon and G. Moreau, *Bull. Soc. Chim.* 3181 (1966).
24. H. Audier, S. Bory, M. Fétizon and N.-T. Anh, *Bull. Soc. Chim.* 4002 (1966).
25. G. Weissmann and W. Lange, *Holz als Roh- and Werkstoff* **44**, 426 (1986).
26. W. Lange and G. Weissmann, *Holz als Roh- and Werkstoff* **45**, 285 (1987).
27. W. Lange and G. Weissmann, *Holz als Roh- and Werkstoff* **45**, 345 (1987).
28. G. Weissmann and W. Lange, *Holzforschung* **46**, 147 (1987).
29. W. Lange and G. Weissmann, *Holz als Roh- and Werkstoff* **46**, 157 (1988).
30. W. Lange and G. Weissmann, *Holzforschung* **43**, 359 (1989).
31. W. Lange and T. S. Janezié, *Holzforschung* **47**, 207 (1993)
32. R. Ekman, *Acta Acad. Abo. Ser. B* **39** (4) (1979).
33. R. Ekman, *Acta Acad. Abo. Ser. B* **39** (5) (1979).
34. R. Ekman, *Holzforschung* **37** (4) 205 (1983).
35. H. Hafizoglu, *Holzforschung* **41** (1) 27 (1987).
36. H. Hafizoglu and B. Holmbom, *Holzforschung* **41** (2) 73 (1987).
37. H. Hafizoglu and B. Holmbom, *Holzforschung* **41** (3):141 (1987).
38. H. Hafizoglu and M. Reunanen, *Holzforschung* **41** (4): 261 (1987).
39. R.P. Adams, *Identification of Essential Oils by Ion Trap Mass Spectroscopy*. (Academic Press, London, 1989).
40. D.F. Zinkel, *Tappi* **58** (1) 109 (1975).
41. J.S. Han and D. F. Zinkel, *Naval Stores Review* **100** (1) 11 (1990).
42. S. Zhanqian, L. Xing and L. Zhiqin, *Naval Stores Review* **103** (2) 6 (1993).