

Fatty and Resin Acids of Spanish *Pinus pinaster* Ait. Subspecies

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The qualitative and quantitative composition of the acid fraction of wood extractives from three Spanish *Pinus pinaster* Ait. subspecies (Atlantic, mountain Mediterranean and plain Mediterranean) were studied. Seven samples of each subspecies were prepared according to Technical Association of Pulp and Paper Standards, extracted with petroleum ether (b.p. 40–60°C) in a Soxhlet apparatus and saponified with ethanolic 0.4N potassium hydroxide. The acid fraction was methylated with diazomethane and studied by combined gas chromatography/mass spectrometry. The major constituents found were palmitic, oleic and linoleic among the fatty acids and pimmaric, sandaracopimaric, levopimaric, isopimaric, abietic and dehydroabietic among the resin acids. Neoabietic and palustric acids were not found. Variations in the quantitative composition of the acid fraction enable us to distinguish the three subspecies. Main quantitative differences are found between the Atlantic subspecies and the two Mediterranean subspecies.

KEY WORDS: Fatty acids, *Pinus pinaster* Ait., resin acids, extractives.

Only a few papers about resin composition of Spanish *Pinus pinaster* Ait. are available (1,2). *Pinus pinaster* Ait. covers a surface in Spain of more than 1,500,000 Ha (hectares) and is the main coniferous species for being commercially utilized. In the past, it has been the raw material for many products obtained from its oleoresin.

Wood pine is a potential source for naval stores and fine chemicals. The diterpene acid composition is being evaluated as a chemotaxonomic indicator.

In Spain, two subspecies of *P. pinaster* Ait. can be distinguished—Atlantic and Mediterranean or *mesogeensis*. The Mediterranean subspecies presents two varieties, mountain and plain (3).

In this work the composition of the acid fraction of wood extractives from the three *P. pinaster* Ait. subspecies found in Spain have been studied.

EXPERIMENTAL PROCEDURES

Seven samples of each subspecies were studied: “Xeibos Albos” from the mountains in Tuy (Pontevedra, Spain), which is the Atlantic subspecies; “Pinar Viejo” from Coca (Segovia, Spain), the plain Mediterranean subspecies; and “El Alijar” from Las Navas del Marqués (Avila, Spain), the mountain Mediterranean subspecies.

Samples were prepared according to Technical Association of Pulp and Paper Standards (4). After the bark was removed, samples were chipped and ground to a size of 40–60 mesh, and the moisture content was determined. Powdered wood (800–1000 g) was extracted with petroleum ether (b.p. 40–60°C) in a 2-L Soxhlet apparatus for 24 h.

Extractives were saponified with 100 mL ethanolic 0.4N potassium hydroxide at 70°C for 4 h. After dilution with

water (1:1, vol/vol), the unsaponifiable fraction was extracted with petroleum ether (b.p. 40–60°C), and the aqueous solution was acidified with 0.4N sulfuric acid and extracted with diethyl ether. The acid fraction was methylated with diazomethane, prepared according to American Society for Testing and Materials Standards (5), and analyzed by combined gas chromatography/mass spectrometry. Qualitative analyses were made with a CG-MS 5995 Hewlett-Packard (Palo Alto, CA) instrument equipped with a 50 m × 0.20 mm capillary column (cross-linked 5% Ph Me Silicone High-Performance) under the following conditions: injection temperature, 250°C; carrier gas (N₂) flow rate, 1 mL/min; column temperature, 120–220°C, 20°C/min; electron energy setting, 70 eV. Acids were identified by comparing their methyl ester mass spectra with the existing bibliography (6–11).

Quantitative analyses were made with a CG 5840A Hewlett-Packard instrument, equipped with a 2 m × 1/8" packed column (10% DEGS) and a flame-ionization detector (FID) under these conditions: injection temperature, 200°C; carrier gas (N₂) flow rate, 20 mL/min; column temperature, 180°C; FID temperature, 250°C.

RESULTS AND DISCUSSION

Table 1 summarizes our results. Data presented are the mean value for the seven samples of each subspecies studied.

Percentage of extractive refers to the dry sample. Relative standard deviations are 5.9, 6.6 and 3.9% for the Atlantic, mountain Mediterranean and plain Mediterranean subspecies, respectively. The percentage of acids refers to the total amount of extractive. Relative standard deviations are 4.0, 2.2 and 2.5% for the Atlantic, mountain Mediterranean and plain Mediterranean subspecies, respectively. The percentage of each acid refers to the total

TABLE 1

Acid Fraction Composition of Wood Extractives from Three Spanish *Pinus pinaster* Ait. Subspecies

Percentage	<i>P. pinaster</i> Ait. subspecies		
	Atlantic	Mountain Mediterranean	Plain Mediterranean
Extractive ^a	1.86 ± 0.11	2.13 ± 0.14	1.84 ± 0.07
Acid fraction ^b	72.4 ± 2.9	80.7 ± 1.8	67.9 ± 1.8
Palmitic ^c	2.2 ± 0.1	1.9 ± 0.1	2.1 ± 0.1
Oleic	8.8 ± 0.9	18.7 ± 0.7	21.6 ± 0.7
Linoleic	2.9 ± 0.1	12.0 ± 0.3	13.4 ± 0.4
Pimaric	9.6 ± 0.3	7.0 ± 0.3	5.8 ± 0.1
Sandaracopimaric	2.7 ± 0.2	1.1 ± 0.1	1.4 ± 0.2
Levopimaric	—	2.3 ± 0.3	0.3 ± 0.1
Isopimaric	4.0 ± 0.1	6.9 ± 0.2	5.0 ± 0.1
Abietic	25.3 ± 0.7	24.5 ± 1.8	18.1 ± 0.2
Dehydroabietic	31.6 ± 0.8	20.6 ± 0.9	25.2 ± 0.5
Others	12.9	5.0	7.1

^aPercentage of extractive refers to dry sample.

^bPercentage of acid fraction refers to the total amount of extractive.

^cPercentage of each acid refers to the total amount of the acid fraction.

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amount of the acid fraction. Relative standard deviations are 3–10% for acids in the Atlantic subspecies, 2–16% in mountain Mediterranean and 3–14% in plain Mediterranean.

Major constituents found among the fatty acids were palmitic, oleic and linoleic, and pimaric, sandaracopimaric, levopimaric, isopimaric, abietic and dehydroabietic among the resin acids. Other minor acids detected were 14:0, 10Me-14:0, 12Me-14:0, 15:0, 2Me-15:0, 7-16:1, 2Me-16:0, 17:1, 17:0, 2Me-17:0, 5,9-18:2, 11-18:1, 18:0, 2Me-18:0, 9,12-19:2, 10-19:1, 2Me-19:0, 22:0 and 8, 11, 13, 15 abietatetraenoic, 7-hydroxydehydroabietic, 7-oxodehydroabietic and callitrisic acids.

The percentage of petroleum ether (40–60°C) extractive is about 2% for the three subspecies. This value is similar to other *Pinus* species (12).

Samples of Mediterranean subspecies have similar qualitative and quantitative compositions, they differ mainly in the low percentage of levopimaric acid in the plain subspecies and in the inversion of the percentage ratio of abietic–dehydroabietic between subspecies. Abietic is dominant in the mountain and dehydroabietic is dominant in the plain subspecies.

The Atlantic subspecies differs from the two Mediterranean subspecies. Oleic and linoleic percentages are smaller, the pimaric percentage is greater and levopimaric acid is not found.

Neoabietic and palustric acids were not detected in either Atlantic or Mediterranean subspecies. These acids are reported in other *Pinus* species (1,13–17). Differences may be due to different analytical methods used or different solvents employed.

Quantitative analysis of the acid fraction enables one to distinguish the three Spanish *P. pinaster* Ait. subspecies.

REFERENCES

1. Joye, N.M., and R.V. Lawrence, *J. Chem. Eng. Data* 12:279 (1967).
2. Solis W., F. Isabel and J.L. Zamkorano, *Estudios sobre el empleo de fitocidas para la obtención de productos resinosos*, INIA, Comunicaciones, No. 16, 1982.
3. Nicolás, A., and J.M. Gandullo, *Ecología de los pinares españoles. I. Pinus pinaster* Ait., IFIE, Ministerio de Agricultura, Madrid, 1967.
4. Technical Association Pulp and Paper Industry, *Tappi Test Methods, Method T 11m-59, Sampling and Preparing Wood for Analysis*, Atlanta, 1988.
5. American Society for Testing and Materials, *Annual Book of ASTM Standards*, ASTM Stand. D 3008, Sect. 06.03, Philadelphia, 1988.
6. Bruun, H.H., R. Tyhage and E. Stenhagen, *Acta Chem. Scand.* 12:789 (1958).
7. Genge, C.A., *Anal. Chem.* 31:1750 (1959).
8. Enzell, C.R., and I. Wahlberg, *Acta Chem. Scand.* 23:871 (1969).
9. Chang, T.L., T. E. Mead and D.F. Zinkel, *J. Am. Oil Chem. Soc.* 48:455 (1971).
10. Zinkel, D.F., L.C. Zank and M.F. Wesolowski, U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, Madison, 1971.
11. The Mass Spectrometry Data Centre in Collaboration with Imperial Chemical Industries, PLC, *Eight Peak Index of Mass Spectra*, The Mass Spectrometry Data Centre in collaboration with Royal Society of Chemistry, Nottingham, 1983.
12. Hafizoglu, H., *Holzforchung* 37:321 (1983).
13. Riffer, R., and A.B. Anderson, *Ibid.* 20:36 (1966).
14. Nestler, F.H.M., and D.F. Zinkel, *Anal. Chem.* 39:1118 (1967).
15. Anderson, S.B., R. Riffer and A. Vang, *Holzforchung* 24:182 (1970).
16. Chapman, R.A., H.M. Nugent and H.I. Bolker, *Transactions of the Technical Section. Canadian Pulp and Paper Association* 1:113 (1975).
17. Lloyd, J.Q., *Phytochem.* 14:483 (1975).

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