NATURAL RESIN MATERIALS - SPECTRAL AND CHROMATOGRAPHIC INVESTIGATIONS

**N.M. ŞTIRBESCU[1]   R.M. ION[2,3]   S. TEODORESCU[1]
R.M. ŞTIRBESCU[1]   A.I. BUCURICĂ[1]**

Abstract: *Natural coniferous resins are recognized as raw materials for various products, are liquid hydrocarbon secretions of many plants, and are mainly composed of volatile and nonvolatile terpenes and essential oils. Some resins, particularly in conifers, contain a mixture of diterpenic, organic carboxylic acids, called resin acids. The resins can be found in solid or semi-solid state, it are not soluble in water but are soluble in alcohol. The chemical composition, and structure of different coniferous resins prelevated from Dâmboviţa County, Romania, are investigated and discussed in this paper.*

Key words: *coniferous, natural resins, Raman, FTIR, chromatography*

1. Introduction

In various period of time, the coniferous forests were predominant source of vegetation of the earth with applications in industry, medicine and environmental area [6]. The most popular and widespread of gymnosperms are conifers. Conifers have the property of producing the resin to repair specific injuries or to protect from attack by insects [13]. Studies have revealed that the resin is a highly viscous fluid which over time becomes semi-solid or solid is in the amorphous state, yellow colored translucent and apparently insoluble in water odorless. If they are heated or burned resins have strong odour characteristic [4].

The earliest uses of the resins were in the form of incense, for the manufacture of seals, and as a drug having anti-bacterial properties, healing, soothing and firming and toning of the human body. In Asia for example, resins are used to relieve cough in India to treat boils and Thailand to treat urinary tract. In Greece pine resin is used in white wine production [1].

2. Objectives

The main product obtained from the resin (Figure 1) is turpentine, which by repeated distillation affords the rosin. It was used to obtain paper, soaps, paints and varnishes. Today it is used for adhesives, inks to reduce sliding friction to stringed musical instruments and produces a range of chemicals [11].

---

[1] *Valahia* University of Târgovişte, ICSTM, Romania.
[2] ICECHIM, Nanomedicine Research Group, Bucharest, Romania.
[3] Doctoral School, *Valahia* University of Târgovişte.

Fig. 1. *Natural resins (personal photos)*

The chemical composition of the resins depends on the source from which the resin (such as conifers). In the large majority contain a mixture of resins and volatile acids.

In this paper, some resins, particularly from conifers, Figure 1, contain a mixture of diterpenic, organic carboxylic acids, called resin acids, are identified and analyzed by FTIR, Raman, and gas-chromatography.

3. Material and Methods

The investigated samples have been prelevated from Dâmboviţa County area, as follows: Black pine_Dealu Monastery_1, Swiss pine_Dealu Monastery_2, Douglas green fir_Dealu Monastery_3, Scots pine_Dealu Monastery_4, Silver Fir_Dealu Monastery_5, Jenuper_Dealu Monastery_6, Spruce_Târgovişte_7, Fir_Târgovişte_8, Fir_Vulcana_9.

FT-IR investigations have been achieved with a Vertex 80 FT-IR spectrometer, equipped with several features such as Automatic Accessory Recognition, which facilitate performing spectroscopic measurements. The standard configuration is designed for data acquisition in the mid IR region (8 000 to 350 cm^{-1}). Purging the spectrometer with dry air reduces the content of unwanted atmospheric interferents inside the spectrometer significantly.

For RAMAN spectrometry, Rigaku's Xantus-2^{TM} Handler Raman Analyzer has been used for quickly and accurately identification of chemical substances based on the chemical fingerprint on the molecular level, and is therefore highly specific. The near infrared excitation at 1064 nm provided by a Nd-YAG laser diode is coupled with the spectrometer, based on a Michelson-type interferometer, and equipped Ge detector.

Gas-chromatography with mass spectrometry (GC/MS) has been achieved with an Agilent 6890 gas chromatograph. The measurement parameters were: DB-35 column (60 m x 0.25 mm I.D.), stationary phase (5% phenyl - 95% methylpolysiloxane), 30 m length, connected to a 2 m long deactivated fused silica capillary pre-column, carrier gas: He (purity 99.9995%), constant flow 1.2 mL/min; temperature 50 ^{o}C (isothermal for 2 min) and the final temperature (300 ^{o}C) maintained for 20 min. Small samples of 2 mL were analysed by using hexadecane and tridecanoic acid as internal standards.

4. Results and Discussions

Infrared spectroscopy is widely used in the study of resins, due to its low analytical costs, the common availability of infrared spectrometers, and the non-destructive nature

of spectroscopic analysis. Based on the soft provided by the apparatus, in our cases, based on the deconvolution soft of the FTIR apparatus, many overlapping absorption bands in spectroscopy could be identified and more precisely located.

In fact, the deconvolution corresponds to a multiplication of the interferogram I(x) using exp(a*x) deconvolution function for Lorentzian and (a*x2) for Gaussian shapes, which intensifies the interferogram edges. The deconvolution led to the amplification of the signal and noise. To avoid the increase of the noise, a Blackman-Harris apodization has been performed, with the values between 1 at the beginning and 0 at the end of the interferogram [14].

So, by self-deconvolution program, the difficult and ambiguous absorption bands have been processed and well-resolved lines have been convoluted (broadened). However, some additional information can be used to help constrain the problem.

Considering that for our coniferous trees there aren't any complete report, we consider an original and relevant paper on this area.

The FTIR spectra achieved for the investigated samples put into evidence specific groups revealing the resins composition, as shown in Table 1.

The CH stretching region (2848 cm^{-1}) for the resins is similar for all tree specimens both in band wavenumbers and in band intensities, Figure 2. Meanwhile, a similar range 1690-1750 cm^{-1} is assigned to free carboxylic acid and to esters or to carbonyl group of carboxylic aromatic acids generated by the oxidation of aliphatic chains or from vegetable oils, has been identified at 1715 cm^{-1} by Vasquez et al. [15].

For Pinus and Junipers species the band from 1604 cm^{-1} is higher than 1556 cm^{-1}, however for pinus species this ratio is reverse. The spectral range 1550-650 cm^{-1} contains the largest number of absorption features, but not all absorption features in this range can be assigned to a specific molecular functional group.

In addition to FT-IR spectra, shown above, the Raman spectra, Figure 3.

The resins can be divided into three subfamilies (within the studied corpus): shellacs, diterpenoid resins and triterpenoid resins [5].

For our resins, which have been collected from different coniferous trees, the Raman bands could be classified as follows:

- The band from 3085 cm^{-1} is assigned only to the diterpenoid resins [3].
- The band at 3060 cm^{-1} is assigned to the stretching vibration of the aromatic or olefinic CH groups [2].
- Raman spectra collected from the solid wood samples containing wood resin showed a band at 1650 cm^{-1} due to unsaturated resin components [9]. The decrease of this band is observed between different coniferous trees, this phenomenon being attributed to polymerization and oxidation processes of the olefinic terpenoids, leading to the decrease of the C=CH$_2$ amount [10].
- The 1444 cm^{-1} band to the deformation of CH bonds (CH$_2$ or CH$_3$ groups) [7].
- The bands from 794 and 1001 cm^{-1}, could be assigned to phenolics [12].
- The main components from resin: resin acids, fatty acids, fatty acid ester, sitosterol and sitosterol acetate, have absorption in the region 500-200 cm^{-1} [8].

In all coniferous resins, alpha and beta pinene constituents are present, a wide range of fragrances, flavors, and polyterpene products, together with camphor, linalool, citral, citronellol, citronellal and menthol, present, too in these samples. GC-MS analysis shows slightly more polar constituents of pine resin with a good resolution for these kinds of diterpenoid isomers, Figure 4.

The main FTIR bands for coniferous trees   Table 1

| Nr. crt.	Band or spectral range [cm⁻¹]	Assignment
1.	3700 - 3100	Symmetrical stretching of O-H bonds
2.	3400	Symmetrical stretching of O-H bonds
3.	2800-3200	C-H (methyl and methylene) stretching vibrations of saturated hydrocarbons
4.	3076	C-H stretching of monoalkyl groups.
5.	3050 - 2400	Vibrational frequencies of aliphatic single C-H bonds
6.	2935, 2848	Methylene groups
7.	2960, 2870	Methyl groups
8.	2840	Specific to coniferous resins
9.	2929 - 1456 cm⁻¹	CH_3 and CH_2 vibrations assigned to some hydrocarbonated structures, etheric and alcoholic groups
10.	1800	C-R, C-O and C-C wags and bends.
11.	1729	C=O of fatty acids or from acyl glycerol groups [3]
12.	1604, 1556	Vibrational modes of C-C conjugated with C=O stretching
13.	1693	C=O in carboxyl groups of resin acids.
14.	1640	O-H bending band.
15.	1550, 1170	Carotenoid structures
16.	1460, 1448	Water from coniferous resins
17.	1550-1350	C-H bending of methyl and methylene groups
18.	1300-1100	C-O single bonds
19.	1242 -1178	Acid resins
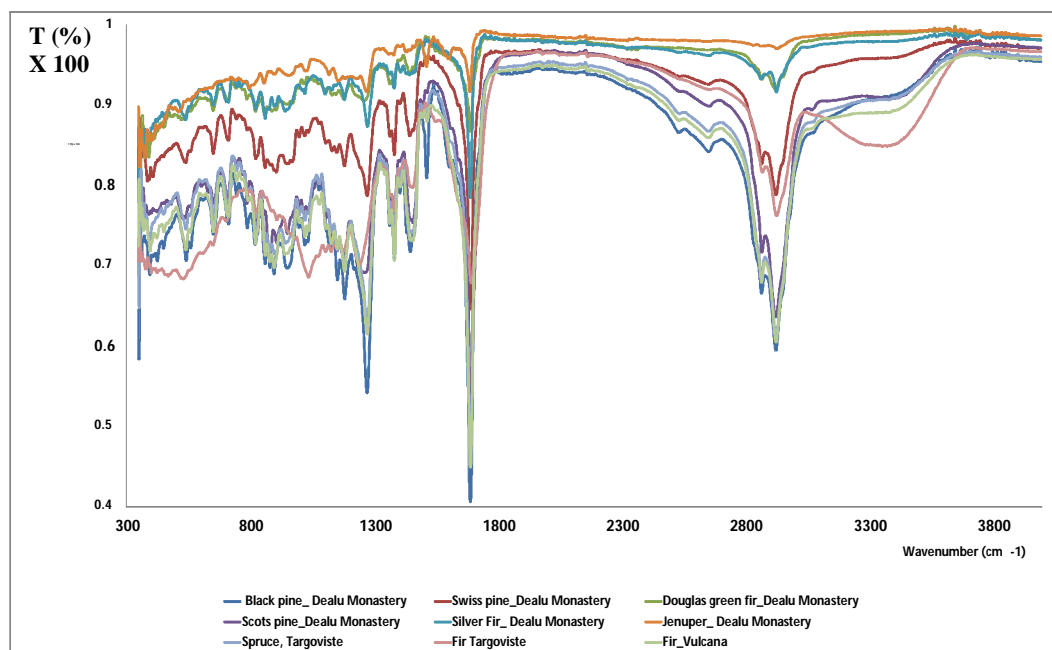
Fig. 2. *FT-IR spectra of natural resins*
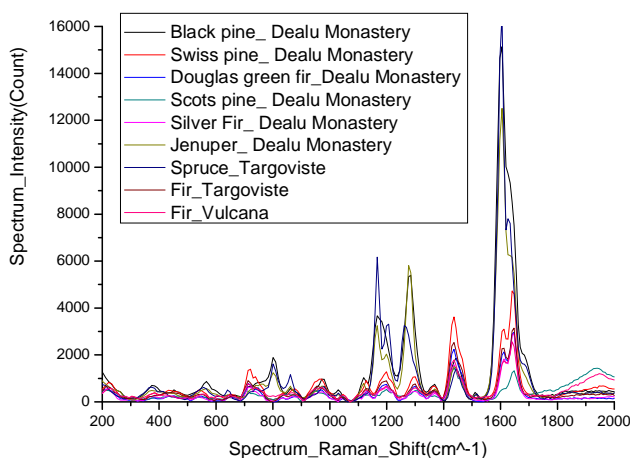
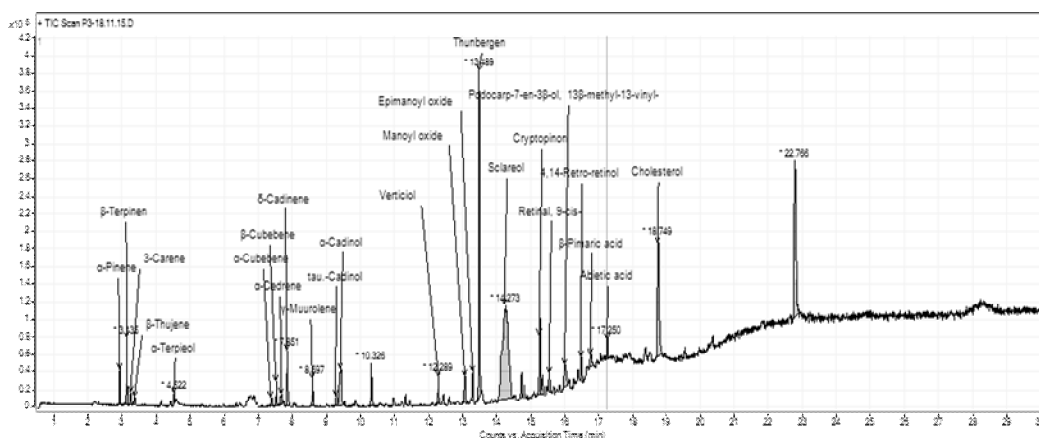Fig. 3. *Raman spectra of natural coniferous resins*

Fig. 4. *GC-MS chromatogram of pine resin*

Gas chromatography together with mass spectrometry is a powerful tool in identifying individual extractive components in coniferous trees, e.g. monoterpenes, resin acids, stilbenes, flavonoids etc., and the obtained extracts were identified with gas chromatograph and mass spectrometry (GC-MS), which led to identification of the terpene compounds: monoterpenes, sesquiterpenes alpha-pinene, 3-carene and limonene.

5. Conclusions

The chemical composition, and structure of different coniferous resins prelevated from Dâmbovița County, Romania, are investigated and discussed in this paper. They have investigated by FTIR, Raman and gas-chromatography methods, identifying resin acids dissolved in mono-, sesqui- and some diterpenoids. Raman analyses of natural organic materials by visible and near infrared wavelengths underline that only Raman allows a proper collection of their Raman spectra, since it avoids totally their fluorescence emission. Gas chromatograph and mass spectrometry (GC-MS), led to identification of the terpene compounds: monoterpenes, sesquiterpenes alpha-pinene, 3-carene and limonene.

Acknowledgements

References

1. Clinovschi, F.: *Dendrologie.* Editura Universităţii Suceava, 2005.
2. Daher, C., Paris, C., Le Ho, A.S., Bellot-Gurlet, L., Echard, J.P.: *A Joint Use of Raman and IR Spectroscopy for the Identification of Natural Organic Media used in Ancient Varnishes*. In: J. Raman Spectroscopy **21** (2010), p. 1204-1209.
3. Ekeberg, D., Flate, P.-O., Eikenes, M., et al.: *Qualitative and Quantitative Determination of Extractives in Heartwood of Scots Pine (Pinus sylvestris L.) by Gas Chromatography*. In: Journal of Chromatography A **1109** (2006), p. 267-272.
4. Graham, A.: *Historical Phytogeography of the Greater Antilles. In: Flora of the Greater Antilles. Vol. 1*. Bronx. Ed. T. Zanoni. New York Botanical Garden Press, 2003.
5. Keeling, C.I., Bohlmann, J.: *Molecules of Interest Diterpene Resin Acids in Conifers.* In: Phytochemistry **67** (2006), p. 2415-2423.
6. Langenheim, J.: *Plant Resins: Chemistry, Evolution, Ecology, and Ethnobotany.* Portland, OR. Timber Press, 2003.
7. Ludley, K.E., Jickells, S.M., Chamberlain, P.M., Whitaker, J., Robinson, C.H.: *Distribution of Monoterpenes between Organic Resources in Upper Soil Horizons under Monocultures of Picea abies, Picea sitchensis and Pinus sylvestris*. In: Soil Biology & Biochemistry **41** (2009), p. 1050-1059.
8. Mumm, R, Hilker, M.: *Direct and Indirect Chemical Defence of Pine against Folivorous Insects*. In: Trends Plant Sci. **11** (2006), p. 351-358.
9. Nuopponen, M., Willför, S., Jääskeläinen, A.-S., Sundberg, A., Vuorinen, T.: *A UV Resonance Raman (UVRR) Spectroscopic Study on the Extractable Compounds of Scots pine (Pinus sylvestris) Wood. Part I: Lipophilic compounds*. In: Spectrochimica Acta Part **A 60** (2004), p. 2953-2961.
10. Nuopponen, M., Willför, S., Jääskeläinen, A.-S., Vuorinen, T.: A UV Resonance Raman (UVRR) Spectroscopic Study on the Extractable Compounds in Scots pine (Pinus sylvestris) Wood. Part II. Hydrophilic compounds. In: Spectrochimica Acta Part **A 60** (2004), p. 2963-2968.
11. Palo, M., Uusivuori, J. (Eds.): *World Forests. Society and Environment*. Dordrecht/ London/Boston. Kluver Academic Publishers, p. 404, 1999.
12. Pan, H., Lundgren, L.N.: *Phenolics from Inner Bark of Pinus Sylvestris*. In: Phytochemistry **42** (1996), p. 1185.
13. Popovici, L., Moruzi, C., Toma, I.: *Atlas Botanic.* Bucureşti. Ed. Didactică şi Pedagogică,1985.
14. Tappert, R., Wolfe, A.P., Mckellar, R.C., Tappert, M.C., Muehlenbachs, K.: *Characterizing Modern and Fossil Gymnosperm Exudates using micro-Fourier Transform Infrared Spectroscopy*. In: Int. J. Plant Sci. (2011), p. 120-138.
15. Vasquez, M.P., Silva, J.N.C., Souza, M.B.J.R., Pereira, N. Jr.: *Enzymatic Hydrolysis Optimization to Ethanol Production by Simultaneous Saccharification and Fermentation*. In: Appl. Biochem. Biotechnol. (2007), p. 136-140.