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Chromatography-mass spectrometry characterization of Peru Balsam

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KEYWORDS

Myroxylon balsamum; Balsam of peru; GC-MS.

1. INTRODUCTION

Myroxylon Balsamum (L.) Harms (family Leguminosae, subfamily Papilionoideae) is a tree native to Central and South America, and it is used in folk medicine, as shade for cultivated crops, ornaments, and for fine timber. Balsam and its essential oil extracted from this tree are used to flavor baked goods, candy, chewing gum, gelatin, ice cream, pudding, soft drinks and syrups, and as incense in churches. Balsam oil is also used in perfume, cosmetic and soap industries. Seeds are used to flavor aguardiente, a popular alcoholic beverage in Latin America[1-8]. Common names in the region include: Bálsamo, palo de bálsamo (Spanish-speaking America, in general), cedro chino, nabal (Brazil), incienso, and quina (Argentina)[2]. It can be found in a very extended area, from southern Mexico, Central America and even as southbound as Argentina. It has been introduced to southern Florida, Indonesia, India and West Africa[1,9-14].

Taxonomical description

Myroxylon balsamum is a large and beautiful tree with a valuable mahogany-like wood and a straight smooth trunk; the last is coarse grey, compact, heavy granulated and of a pale straw color, containing a resin which changes from citron to dark brown, which smells and tastes as balsamic and aromatic. The process of extraction produces three grades of balsam; the title "Balsam of Peru" is derived from the fact of it was originally shipped from Peru. There are several fake Peruvian balsams offered in the world market, but they do not have the same properties. A white balsam is made from the fruit of Myroxylon Pereirae, which has a peculiar resinous body and none of the chemical constituents of Balsam of Peru; this is termed Myroxocarpin. Another substance obtained from the same tree and widely used in Central America is known as Balsamito, an alcoholic extract of the young fruit.

Actions

Anti-inflammatory, antiseptic, balsamic, expectorant, anti-parasite, stimulant, promotes growth of epithelial cells. Peru Balsam has been reported in use from beginning of 17th century[3] and it is description[4,15-17] .The wider use that was being made of Peru Balsam is depicted on December 1912 in Merck's Report. It possesses stimulant, stomach, vulnerary, protective and disinfectant properties[5]. In syrup form various formulas are advised.

Topical use

When diluted in carrier oil, Balsam of Peru may relieve dry chapped skin, eczema, rashes, sores and wounds, rheumatism, diaper rash, bedsores, prurigo, and sore nipples. It can be applied to the chest to relieve asthma, colds, coughs and bronchitis. It may also be effective to combat skin parasites such as scabies. Balsam of Peru has been used to relieve symptoms of nervous tension and stress. It is often used as a fixative and fragrance component in cough syrups, soaps, detergents, creams, lotions and perfumes. Balsam of Peru is also used as a flavoring agent in most food categories and in alcoholic and soft drinks. Orally ingested it stimulates the heart, increases blood pressure, and lessens mucous secretions. Since it contains benzoic acid, Balsam of Peru may cause dermatitis in individuals with sensitivity to benzoin resinoids.

In spite of all the attractive properties of Peru Balsam, basically no scientific reports on neither the composition nor the physico-chemical properties of this substance are available in the specialized literature, for all the above data belong to the folk culture of Latinamerica. Two of the principals constituents of Peru Balsam oil are benzyl benzoate and benzyl cinnamate[6] and it is prepared by extraction with volatile solvents from Peru Balsam. Biological data are reported from experiments in rats, rabbits, volunteers human subjects[7] and in guinea pigs[8]. Also no phototoxic effects were reported in some samples of Peru Balsam oil[9]. Conventional hyphenated techniques for the analysis of natural compounds, such as gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS), have proved highly successful in the resolution and identification of volatile and non-volatile substances. Both GC-MS and LC-MS allow separating the original substance (i.e., a mixture of molecules) via the chromatographic step and then to ionize the individual molecules so that they produce characteristic spectra from which the identities of the molecules can be deduced (in conjunction with retention indices). Accordingly, the present study presents the first reports on the composition of Balsam of Peru via the above chromatographic techniques.

2. EXPERIMENTAL

2.1 Materials

Commercial Balsam of Peru was supplied by Farmacia Paris (Mexico City, Mexico). Balsam of Peru was stored in glass jars at room conditions.

2.2 GC-MS analyses

A milligram-worth sample was placed in a tube and dissolved in one ml of ethanol, chromatography degree. Two micrograms are obtained from the resulting solution and injected into the chromatographer, by using helium as the carrier. The temperature rate employed was 60^{0}C per minute, from 60 to 200^{0}C.

The equipment employed was a 6890 HP Gas Chromatography System along with a mass detector Hewlett Packard 5973 Mass Selective Detector, controlled through a PC HP Pentium II.

3. RESULTS AND DISCUSSION

The compounds were identified by GC-MS, as mentioned. Thirteen volatile and non volatile signals were identified in Balsam of Peru. Figure 1 shows a typical chromatogram where the main peaks were identified and quantified by using the database WILEY245.L. Peak S corresponds to the solvent (Ethanol); peaks 1, 8 and 10 correspond to non-volatile compounds: β-farnesene and nerolidol; whereas peaks 2 to 7, 9 and 11 to 13 correspond to volatile compounds. TABLE 1 summarizes the identified signals, their formula and areas under peaks. The three most abundant compounds

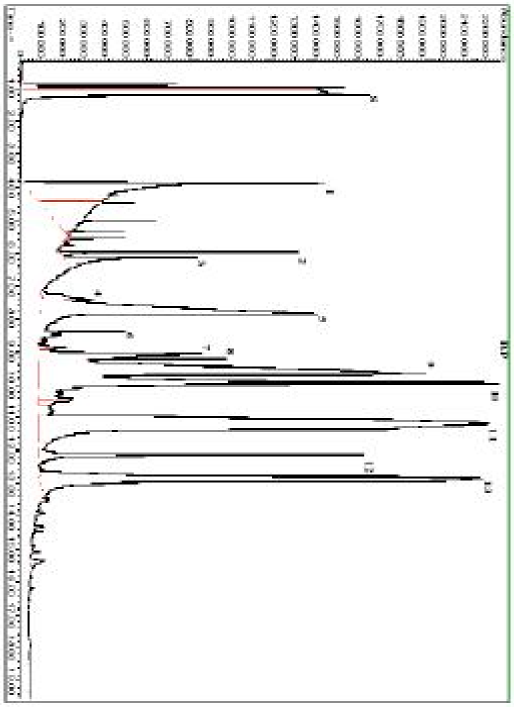


Figure 1: GC-MS of Balsam of Peru (BP sample)

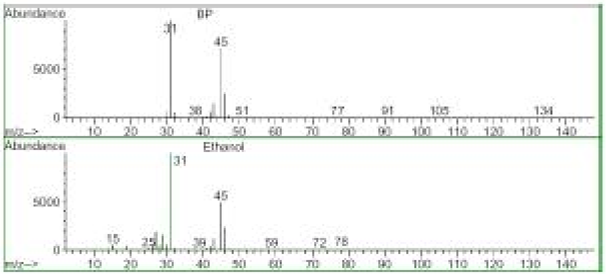


Figure 2: Mass spectrum of peak S, solvent

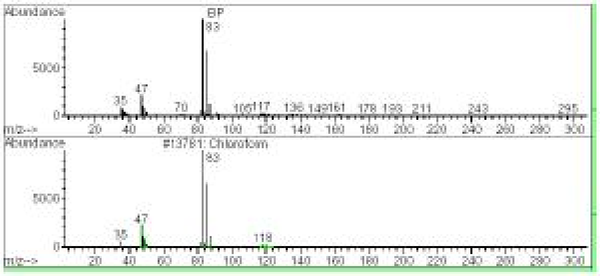


Figure 3: Mass spectrum of peak 1: chloroform

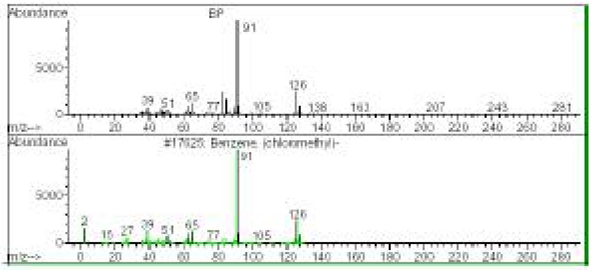


Figure 4: Mass spectrum of peak 2: Benzene, (chloro methyl)

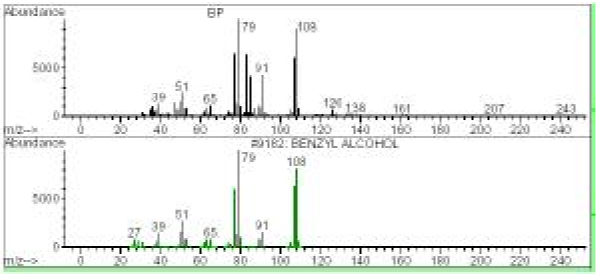


Figure 5: Mass spectrum of peak 3: Benzyl alcohol

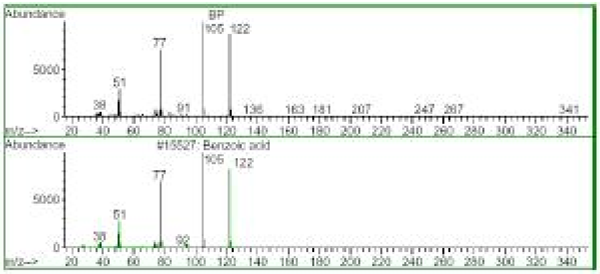


Figure 6: Mass spectrum of peaks 4 and 5: Benzoic acid

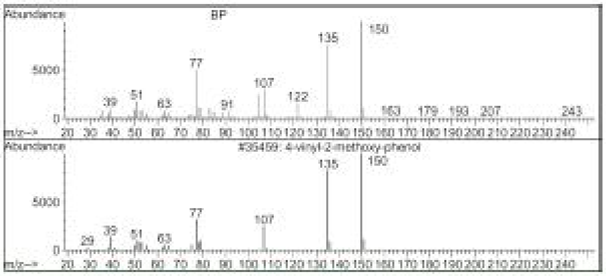


Figure 7: Mass spectrum of peak 6: 4-vinyl-2-methoxy-phenol

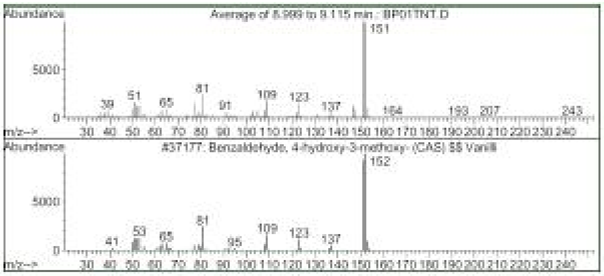


Figure 8: Mass spectrum of peak 7: Vanillin

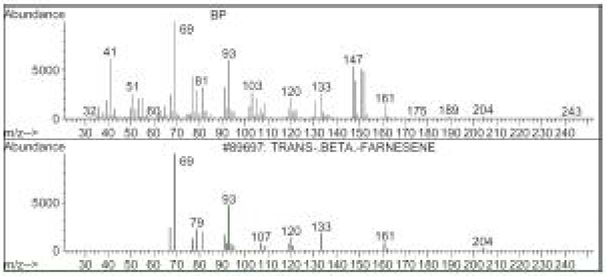


Figure 9: Mass spectrum of peak 8: β-farnesene

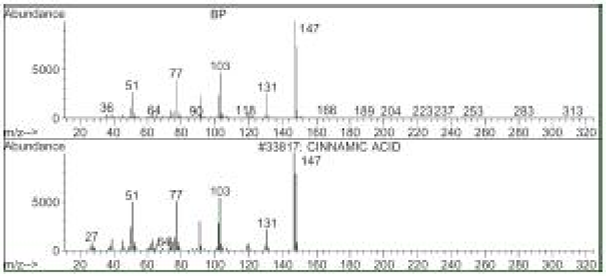


Figure 10: Mass spectrum of peak 9: Cinnamic acid

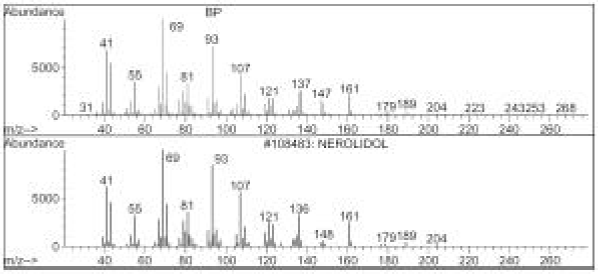


Figure 11: Mass spectrum of peak 10: Nerolidol

TABLE 1: Identification of Peru Balsam by compound

Peaks	Compound	Formula	Quality	Abundance
S	Solvent	C_2H_6O	90	1600000
1	Chloroform	$CHCl_3$	96	140000
2	Benzyl chloride	C_7H_7Cl	93	1275000
3	Benzyl alcohol	C_7H_8O	96	825000
4	Benzoic acid	C_7H_6O	97	350000
5	Benzoic acid	C_7H_6O	97	1350000
6	4-Vinyl-2-methoxy-phenol	$C_9H_{10}O_2$	93	475000
7	Vanillin	$C_8H_8O_3$	96	850000
8	Trans-beta-farnesene	$C_{15}H_{24}$	96	950000
9	Cinnamic acid	$C_9H_8O_2$	97	1800000
10	Nerolidol	$C_{15}H_{26}O$	93	2200000
11	Benzyl benzoate	$C_{14}H_{12}O_2$	98	2125000
12	Benzyl cinnamate	$C_{16}H_{14}O_2$	95	1575000
13	Benzyl cinnamate	$C_{16}H_{14}O_2$	98	2100000

TABLE 2: Extra peaks of compounds found in the in MS spectra

Peaks	Compound	Base peak (m/z)	Extra peaks (m/z)
S	Solvent	31	-
1	Chloroform	83	-
2	Benzene, (Chloromethyl)-	91	83
3	Benzyl alcohol	79	83
4	Benzoic acid	105	83
5	Benzoic acid	105	83
6	4-Vinyl-2-methoxy-phenol	150	83, 91, 122,
7	Vanillin	151	147
8	β-farnese	69	103,147,151
9	Cinnamic acid	147	-
10	Nerolidol	69	-
11	Benzyl benzoate	105	39
12	Benzyl cinnamate	91	-
13	Benzyl cinnamate	91	-

were nerolidol, benzyl benzoate and benzyl cinnamate. All MS spectra presented typical fragments of compounds. Extra peaks in MS spectra and base peak (m/z) are presented in TABLE 2.

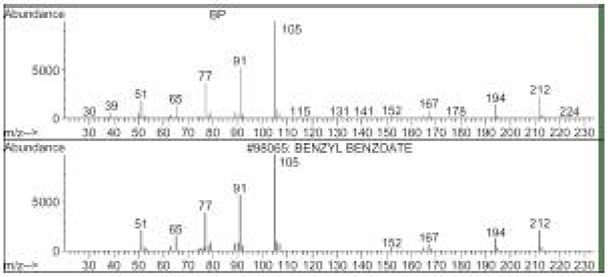


Figure 12: Mass spectrum of peak 11: Benzyl benzoate

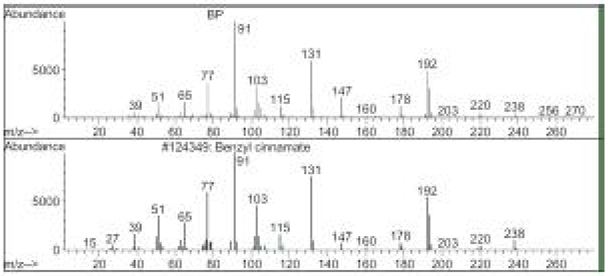
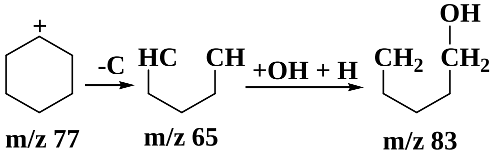


Figure 13: Mass spectrum of peaks 12 and 13: Benzyl cinnamate



SCHEME 1

Benzene, (chloromethyl)-, benzyl alcohol, benzoic acid and 4V2MP presented additional signals, at m/z 83 and others. The ion species of m/z 77, in benzene, (chloromethyl)-, benzyl alcohol, and benzoic acid generated ions at m/z 65 by loss of C (ring rupture) and created an intermediate ion specie at m/z 83 through the gain of an OH radical and a proton. While the ion specie of m/z 75 in 4V2MP generated ions at m/z 63 by loss of C (ring rupture) and created the intermediate ion specie at m/z 83, by gaining an OH radical and a proton, also. Partial fragmentation pathways (from C_6H_5 fragment) are proposed to account for the additional signal observed in benzene, (chloromethyl)-, benzyl alcohol, and benzoic acid, as shown in the SCHEME 1. Partial fragmentation pathways (from base peak) are, in turn, proposed to account for the additional signal observed in 4V2MP, as depicted in SCHEME 1.

For 4V2MP, the ion mass species of m/z 122 was generated by loss of CH from ion m/z 135. And, the ion species of m/z 107 generated ion at m/z 91 by loss of O. This ion species can be identified as C_6H_3O. For β-farnesene, the intermediate ion mass species of m/z 151 was generated by loss of C and the gain of two H from ion m/z 161. While ion species of m/z 151 generated ion at m/z 147 by loss of four H. Finally, the ion species of m/z 107 generated ion at m/z 103 by loss of four H. For vanillin, the ion mass species of m/z 147 was generated by loss of four H from ion m/z 151 (base peak).

4. Concluding remarks

The analysis of such natural substances is rather complicated, since the fraction of aromatic compounds alone of a simple natural product may be composed of 50-200 constituents, and many of them are present in trace quantities. However, we have been able to identify the 11 most relevant components of Peru Balsam, which opens the possibility no only of understanding the structure of this natural product, but also to test the properties of the constituents, both individually and sinergetically combined, as it is currently under way.

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