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THERMAL BEHAVIOR AND STRUCTURAL PROPERITES OF PLANT-DERIVED EUGENYL ACETATE

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Eugenol is an allyl chain-substituted guaiacol in the biosynthesized phenylpropanoid compound class derived from *Syzygium aromaticum* L. and widely used in folk medicine. Nonetheless, its pharmacological use is limited by some problems, such as instability when exposed to light and high temperature. In order to enhance stability, the eugenol molecule was structurally modified, resulting in eugenyl acetate. The eugenyl acetate's thermal behavior and crystal structure was then characterized by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) and compared to a commercial sample.

Keywords: *DSC, essential oil, eugenyl acetate, Syzygium aromaticum L., XRD*

Introduction

The essential oils – as complex mixtures – are one of the most important raw materials used in the food, perfumery and pharmaceutical industries [1]. They are combinations of lipophilic and volatile substances and represent multi-component systems, with embedded key single-component systems [2, 3].

Nevertheless, they have some limitations, such as chemical instability in the presence of air, light, moisture and high temperature that can cause the rapid evaporation and degradation of some active components [4].

Syzygium aromaticum L., popularly known as clove, belongs to the plant family Myrtaceae, and has been used in folk medicine and dental treatment. In dental care, the oil is used as antiseptic and analgesic on gum surfaces to treat toothaches. In addition, it is commonly used in root canal and temporary fillings; it shows antibacterial activity, and helps in dental caries treatment and periodontal disease [5, 6]. Cloves have been successfully used for some breath problems [6].

The major compounds contained in the essential oil extracted by hydrodistillation from dried buds of *Syzygium aromaticum* L. are eugenol (90.1%), eugenyl acetate (8.6%) and its methyl ether derivative (1.3%) [7].

Several pharmacological researchers using eugenol have demonstrated some important medicinal properties, such as anesthetic, bactericidal, fungicidal, analgesic, as well as others [8]. Eugenol has also been reported to participate in photochemical reactions and possess insecticidal, antioxidant and anti-inflammatory activities [9].

However, the efficiency of this compound in therapeutic treatment is limited due to its poor water solubility and chemical instability, and the requirement of high concentrations to achieve a therapeutic effect [10].

Strategies to increase the therapeutic efficacy of the eugenol molecule include adding functional groups to its structure, such as an acetate ion which results in eugenyl acetate, as seen in Fig. 1.

The eugenyl acetate was obtained after the reaction of esterification between the extracted oil from *Syzygium aromaticum* L. and acetic anhydride.

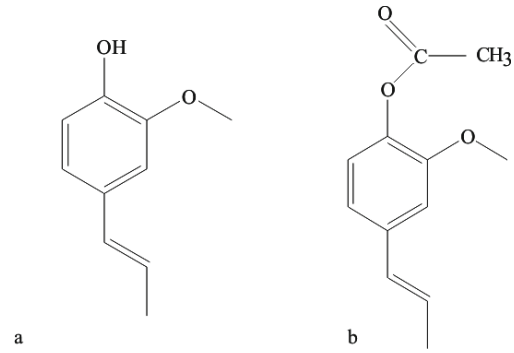

Fig. 1 a – Eugenol structure and b – eugenyl acetate

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DSC analysis has been suggested as a valuable tool for the characterization of oils from their natural source [11]. DSC has some advantages over the more classical detection methods, which is well known and reviewed [12].

Single-crystal XRD is undoubtedly the most important and powerful technique for the elucidation of crystal and molecular structures. It is used mainly as a method to identify and quantify any crystallized phases present in the oil [13].

Therefore, in the present study, the eugenyl acetate synthesized through the extracted oil from *Syzygium aromaticum* L. buds, without previous purification, has been investigated and compared with the standard eugenyl acetate data. Both DSC and XRD were used to determine the eugenyl acetate properties, such as its thermal behavior, structure and stereochemistry. The physical chemical results were compared.

Experimental

DSC curves were made using a DSC 822 Mettler coupled to a TS0801RO sample robot and TS0800GC1 gas controller. The refrigerated cooling system was an EK 90/MT (HAAKE). Samples of approximately 7.5 mg were weighed into 100 μL aluminum pans, and sealed. A sealed empty aluminum pan was used as a reference.

Standard and raw eugenyl acetate samples were subjected to 2°C min^{-1} constant heating rate and a cooling-heating temperature program. The samples were cooled down to –40°C, heated up to 60°C, and then the cooling-heating program was performed twice more, utilizing the same heating rate.

All the measurements were made under dynamic nitrogen atmosphere which flowed at approximately 50 mL min^{-1}.

The equipment was verified using indium and dodecane; the later was used due to the lower melting point which is similar to the eugenyl acetate's thermal characteristics. All the experiment parameters were the same such as for all the samples. The indium values for the melting point and heat of fusion were 158.35°C and –24.54 J g^{-1}, which the standard deviation was less than 5%.

A second set of DSC experiments was used to better understand the thermal behavior of standard eugenyl acetate sample. All experimental parameters conformed in every detail to those previously described; except for the addition of an isothermal step at 60°C between each segment in the scan, over 20 min, that guaranteed the crystal thermal degradation.

XRD analysis was conducted on an Enraf-Nonius Kappa-CCD diffractometer with graphite monochromatic MoKα (λ=0.71073 Å) radiation. The final unit cell parameters were based on all reflections. Data collections were made using the COLLECT® program [14]; integration and scaling of the reflections were performed with the HKL Denzo-Scalepack system of programs [15]. The structure was resolved by direct methods with SHELXS-97 [16]. The model was refined by full-matrix least squares on F^2 by means of SHELXL-97 [17]. All hydrogen atoms were stereochemical positioned and refined with the ridging model.

Results and discussion

Oils do not have a specific melting temperature, rather, they melt over a temperature range, as long as a dynamic method is used to measure the melting process, and often exhibit multiple endotherms. This change may not be visible to the eye, but it can be studied by physical means such as in a DSC instrument. The application of the DSC method for studying the melting behavior of oils has proved very useful [18].

One of the standard and raw eugenyl acetate thermograms using the DSC cooling-heating program is shown in Fig. 2. The line shapes are very similar with some slight differences, and their temperature characteristics can be seen due to the slow rate of scanning.

The DSC for the standard and raw eugenyl acetate experiments exhibited two well defined and repetitive events, a minor and a major peaking, respectively. The former is an exothermic and less defined shoulder peak, associated with the crystallization event; while the latter showed a sharp well

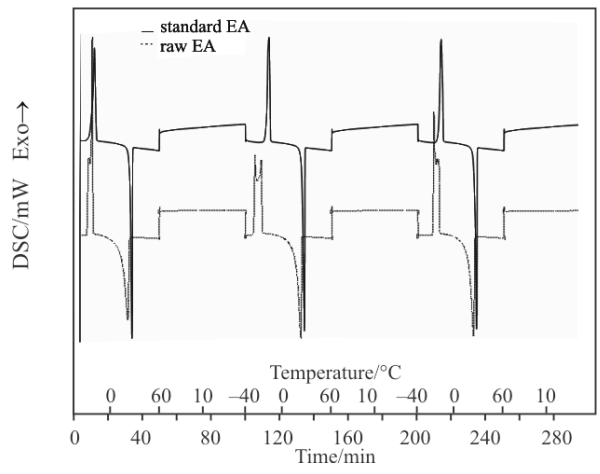

Fig. 2 DSC using the cooling-heating program for standard and raw eugenyl acetate

Table 1 Average of three measurements in the thermal studies for standard and raw eugenyl acetate

Sample	Heat/cool program	T_{oc}/°C	T_{pc}/°C	ΔH_c/J g^{-1}	T_{om}/°C	T_{pm}/°C	ΔH_f/J g^{-1}
Standard eugenyl acetate	Cycle 1	–13.9	–9.6	83.7	25.9	28.1	–97.8
	Cycle 2	–13.7	–8.7	84.3	26.6	28.2	–100.7
	Cycle 3	–11.7	–9.7	83.8	26.1	28.1	–97.8
	Standard dev.	2.0	1.6	0.5	0.3	0.1	1.7
Raw eugenyl acetate	Cycle 1	–27.4	–25.9	69.6	12.5	20.7	–71.8
	Cycle 2	–33.2	–31.5	68.1	13.8	21.3	–73.2
	Cycle 3	–29.5	–27.9	69.3	13.6	21.1	–71.5
	Standard dev.	2.9	2.8	0.8	1.8	1.0	1.7

delineated shape that was attributed to the melting process.

The experimental measurements for the thermal events showed in Fig. 2 above can be seen in Table 1, which was built using the average of three different experiments for each compound replacing the sample, with three cycles of heat/cool program.

Crystallization and melting are the main thermal events and always present, but they exhibited some important differences when comparing their results.

According to standard deviation (SD) the repeatability of the thermal events for both samples may be confirmed.

The standard eugenyl acetate crystallization begins at a higher temperature than for the raw, as showed by the onset temperature (T_{oc}). The former`s peak crystallization temperature (T_{pc}) is above that of the raw compound, indicating the crystallization process takes place over a longer time period at higher temperatures. In addition, even with high temperatures for the crystallization, the energy required for all process is superior, characterized by the higher heat of crystallization (ΔH_c).

The melting processes follow the same results as the crystallization. The onset, peak temperature and heat of fusion are higher for the standard component. This means the process onset and completion occurs later than for the raw product and with higher energy.

The peak temperatures for the raw sample are different from the standard, and each range is broader than that observed for the pure compound. Crystallization and melting enthalpy for the raw eugenyl acetate was significantly decreased by the multicomponent system. This may be due to the impurities (approximately 2%), which decrease the raw eugenyl acetate solubility and initiates the crystallization. In other words, the raw complex blend needs a lesser quantity of energy to reach the transitions than in the standard compound.

DSC experiments with the isothermal step were performed just for the standard eugenyl acetate due to its purity degree.

The latter method was carried out to qualify the crystal growth, and to eradicate any type of particle present in the solution, which may be capable of initiating the crystal growth. Consequently, isothermal DSC provides the natural crystallization process during the increase in temperature in the step where the temperatures are negative. So, there are no unknown particles to initiate the crystallization event; consequently the eugenyl acetate reaches the more stable structure by organizing itself in crystal arrangement after go through determined temperature.

The crystallization process is not affected by including the isothermal step, thus the other scan portions happen in the same way, showing the same characteristic events and thermal properties, as the first DSC program.

The XRD experiment was performed just for the standard eugenyl acetate due to its solid physical state at room temperature, since it is a necessary condition to isolate a single crystal for the development of the XRD experiment. The raw compound is liquid at room temperature, and the experiment can not be performed lower than at room temperature, condition required to obtain the crystal form.

The XRD results defined in this procedure proved that the crystallization was at a high degree of purity for eugenyl acetate. The crystal structure and stereochemistry for the standard eugenyl acetate was determined and can be seen in Fig. 3.

The crystallization event for the raw eugenyl acetate was observed during the DSC experiment,

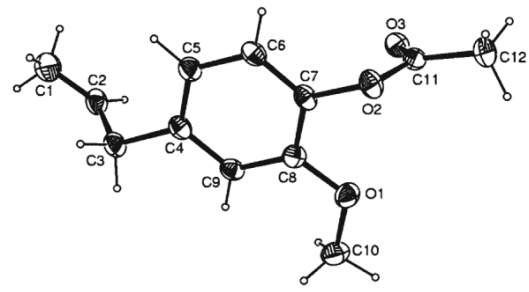

Fig. 3 Crystal structure for standard eugenyl acetate

which was able to reach the low temperatures required for crystallization of the sample.

This change in physical characteristics is due to the presence of impurities in the raw compound obtained during the extraction process, which results in a multi-component system. This mixture affects all the properties, including the thermal and physical characteristics. Consequently, the crystallization process is influenced by the impurities, such as the lower temperature characteristics and the wider crystallization potential.

However, the crystallization for the raw compound was demonstrated using the DSC experiments. DSC experiments were capable of determining the eugenyl acetate thermal behavior, even in the presence of other constituents.

Conclusions

This work has shown that both the standard and raw eugenyl acetate undergo the crystallization process, with significant changes in the raw compound's thermal properties. Even at a concentration as low as 2%, the enthalpy variation, onset and peak temperatures change, as a result of the presence of the impurities.

DSC profiles may offer a qualitative tool for investigating the presence of impurities in extracted essential oils and synthetic compounds using natural resource in some reactions. Thermal analysis is not extensive technique used to investigate the thermal behavior for natural compounds due to their complexity, especially a non purified sample such as the essential oil extracted from *Syzygium aromaticum* L.

The XRD analysis confirmed the formation of a more stable structure for the standard eugenyl acetate, determining its stereochemistry and crystal structure. Nevertheless, the raw eugenyl acetate crystallization was established based on the DSC analysis.

In conclusion, the DSC results suggest that eugenyl acetate of relatively high purity may crystallize at room temperature, providing a relationship between the thermal behavior and the degree of purity. In addition, the crystal structure and stereochemistry were determined, providing significant information about the stability of crystal structures obtained from natural plant extracts.

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