SEVEN
publicações acadêmicas

LABDANO DITERPENE DERIVATIVES AND THEIR POTENTIAL CYTOTOXIC ACTIVITIES

DERIVADOS DE DITERPENOS LABDANOS E SUAS POTENCIAIS ATIVIDADES CITOTÓXICAS

DERIVADOS DE DITERPENO LABDANO Y SUS ACTIVIDADES CITOTÓXICAS POTENCIALES

https://doi.org/10.56238/sevened2025.029-025

Vladimir Constantino Gomes Heleno, Ana Laura Matias Resende, Jadson da Silva Gomes, Raquel Alves dos Santos, Jonas Joaquim Mangabeira da Silva, Julia Mirian Paulino

ABSTRACT

Esterifications were performed with labdane-type diterpenes, initially with polyalctic acid using alkyl and aryl halides as reagents, yielding five semi-synthetic derivatives, three of which presented yields greater than 70%. The derivatives were subjected to 1H and 13C NMR and identified by comparison with the starting material. Ent-copalic acid and ent-β-acetoxycopalic acid were reisolated from copaiba oleoresin (a commercial sample) and identified by 1H NMR in comparison with the literature. For ent-copalic acid, a reaction study was performed to improve yields. Both were subjected to esterifications using the DCC/DMAP system, yielding three new semi-synthetic derivatives. After this, all derivatives and their respective starting materials were subjected to cytotoxic evaluation against a breast tumor cell line and against normal mammary gland cells. The results were very interesting, with the halogenated derivatives in the polyhaltic group showing great promise, with one of them showing similar toxicity to the positive control (activity parameter for the assay). For the copaltic group, the results showed that the active molecule fusion technique can lead to excellent results.

Keywords: Diterpenes. Labdanes. Structural Modification. Cytotoxic Activity.

RESUMO

Foram realizadas esterificações com diterpenos do tipo labdanos, primeiramente com o ácido poliáltico onde foram utilizados haletos de alquila e arila como reagentes, obtendo 5 derivados semissintéticos, sendo que 3 deles apresentaram rendimentos maiores que 70%. Os derivados foram submetidos a RMN de 1H e 13C e identificados por comparação com o material de partida. O ácido ent-copálico e o ácido ent-β-acetóxi copálico foram reisolados do oleorresina de copaíba (amostra comercial) e identificados por RMN de 1H em comparação com a literatura, para o ácido ent-copálico foi realizado um estudo reacional buscando melhorar os rendimentos, ambos foram submetidos a esterificações utilizando o sistema DCC/DMAP, obtendo 3 novos derivados semissintéticos. A partir disso, todos os derivados e seus respectivos materiais de partida, foram submetidos a avaliação citotóxica frente a uma linhagem celular tumoral de mama e frente a células normais de glândula mamária. Os resultados faram muito interessantes, sendo que no grupo poliáltico os derivados halogenados se mostraram muito promissores, um deles apresentou toxicidade semelhante ao controle positivo (parâmetro de atividade para o ensaio). Para o grupo

copálico os resultados mostraram que a técnica de fusão de moléculas ativas pode levar a ótimos resultados.

Palavras-chave: Diterpenos. Labdanos. Modificação Estrutural. Atividade Citotóxica.

RESUMEN
Se realizaron esterificaciones con diterpenos tipo labdano, inicialmente con ácido poliálctico utilizando haluros de alquilo y arilo como reactivos, obteniéndose cinco derivados semisintéticos, tres de los cuales presentaron rendimientos superiores al 70%. Los derivados se sometieron a RMN de 1H y 13C y se identificaron por comparación con el material de partida. El ácido ent-copálico y el ácido ent-β-acetoxicopálico se reaislaron de la oleorresina de copaiba (una muestra comercial) y se identificaron por RMN de 1H en comparación con la literatura. Para el ácido ent-copálico, se realizó un estudio de reacción para mejorar los rendimientos. Ambos se sometieron a esterificaciones utilizando el sistema DCC/DMAP, obteniéndose tres nuevos derivados semisintéticos. Posteriormente, todos los derivados y sus respectivos materiales de partida se sometieron a evaluación citotóxica frente a una línea celular de tumor de mama y frente a células normales de glándula mamaria. Los resultados fueron muy interesantes, ya que los derivados halogenados del grupo poliháltico mostraron un gran potencial, y uno de ellos mostró una toxicidad similar a la del control positivo (parámetro de actividad del ensayo). En el grupo copaltico, los resultados mostraron que la técnica de fusión de moléculas activas puede producir excelentes resultados.

Palabras clave: Diterpenos. Labdanos. Modificación Estructural. Actividad Citotóxica.

1 INTRODUCTION

1.1 NATURAL PRODUCTS

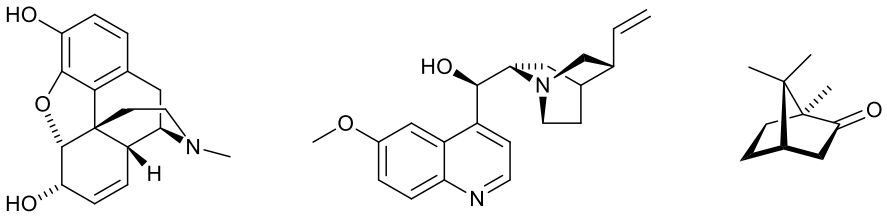
Natural products are compounds or substances originating from living beings, and can be found in plants, animals and microorganisms. Plants are considered the first and largest source of natural products for the discovery of new medicines, while terrestrial and marine organisms are sources that began to be explored later, compared to plants, but which have also proven their potential (NOGUEIRAS *et al.*, 2010) element.

In recent decades, ethnopharmacological studies of medicinal plants have gained more space in the scientific community, aiming at the search for alternatives for the treatment and combat of certain diseases that affect humans and animals (MOORE *et al.*, 2017). Through popular knowledge about the use of medicinal herbs and scientific studies based on them, a relevant role has been played in the discovery and elucidation of new bioactive principles (MOORE *et al.*, 2017).

In this way, the special metabolites produced by plants played a fundamental role in the development of modern synthetic organic chemistry, especially from the nineteenth century onwards, when the first studies on plants were recorded, based on science. This resulted in the isolation of some active principles of plants, already known as medicinal. From these studies, some substances were obtained that have been established as effective active ingredients, and that to this day, are still widely used due to their actions, such as morphine (analgesic), quinine (antimalarial) and camphor (antimicrobial) (EIFER-LIMA *et al.*, 2010; MONTANARI; BOLZANI, 2001).

Figure 1

Substances that have been established as effective active ingredients



Morfina Quinina Cânfora

The great variety and complexity of special metabolites biosynthesized by plants would have been formed during evolution, as a defense mechanism of these plants to environmental conditions rich in microorganisms, insects, animals and also to the conditions of adaptation (BARREIRO, 2002).

It is important to note that Brazil, according to the CBD (Convention on Biological Diversity) hosts 20% of all world biodiversity, making it the country with the largest number of endemic species, estimated at more than 46,000 species, (BARBOSA; VEGA, 2017; CLAY PIT; BOLZANI, 2009), therefore, it is essential to preserve our biodiversity, both due to its immense biological wealth and the immense source of new bioactive compounds.

Aiming at this principle, the study of bioactive natural products is important not only for the need to characterize the pharmacological properties of the molecules in question, but also for the knowledge of new substances that may be active for a certain biological activity, in order to obtain new drugs to treat a specific disease or to serve as models (prototypes) to originate structural analogues with more promising pharmacological properties (ARNESANO; NARDELLA; NATILE, 2018; BARREIRO, 2002; VUORELA *et al.*, 2012).

Among the immense Brazilian biodiversity, the *Copaifera stands out*, a medium-sized tree genus, which can reach 40 meters in height and 4 meters in diameter. Belonging to the family Leguminosae Juss, sub-family Caesalpinioideae consists of 72 species, popularly known as copaiba, copaibeira, paus-de-oleos, balsams of the Jesuits, and are commonly found in Latin America and West Africa (PIERI; MUSSI; MOREIRA, 2009). In Brazil, these species are distributed in the Southeast, Midwest, and mainly in the Amazon region (YAMAGUCHI; GARCIA, 2012), and more than 20 species can be found in this territory (CASCON; GILBERT, 2000), being *C. langsdorffii*, *C. officinalis*, *C. guianensis*, *C. reticulata*, *C. multijuga*, *C. confertiflora*, *C. duckei*, *C. coriacea* and *C. cearenses* the most common (LEANDRO *et al.*, 2012)

The oleoresin produced by these species is usually extracted through incisions or perforations in the trunk of these trees, which demonstrates a wide variety of pharmacological properties, since they have a rich source of active substances. This oleoresin is commonly used in the cosmetics industry due to its emollient, antibacterial and anti-inflammatory properties,(PIERI; MUSSI; MOREIRA, 2009).

In addition, it has wide medicinal use, being indicated as an anti-inflammatory, antiseptic, antiasthmatic, analgesic, antidiarrheal, healing, aphrodisiac, antioxidant, antitetanus, antiherpetic, antimicrobial, anticancer, antitumor and leishmanicidal, among others (LEANDRO *et al.*, 2012; YAMAGUCHI; GARCIA, 2012). Even the wood extracted from the copaibeira has desirable properties for use in carpentry, carpentry,

and is also used in civil and naval construction (PIERI; MUSSI; MOREIRA, 2009), in addition to the manufacture of charcoal (VEIGA, VALDIR F.; PINTO, 2002).

A review of the special metabolites present in *Copaifera* species demonstrates that sesquiterpenes and diterpenes of the labdane and clerodane classes are the major constituents of the oleoresin-resin of these trees (NETO; GRAMOSA; SILVEIRA, 2008; TAPPIN *et al.*, 2004; VEIGA, V.F. *et al.*, 2007; VEIGA, VALDIR F.; PINTO, 2002).

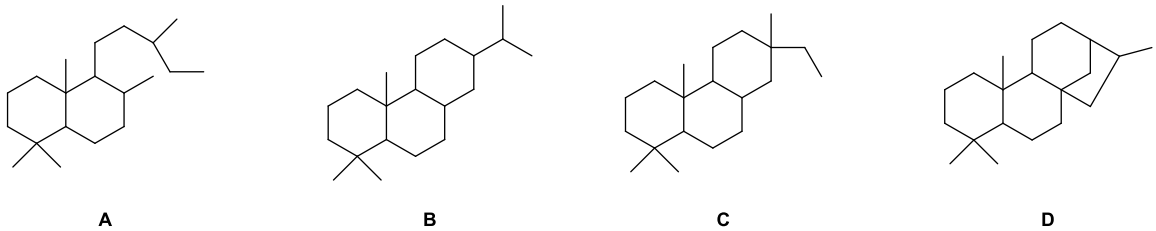
2.2 DITERPENES

Diterpenoids are made up of 20 carbon atoms, produced from the condensation of four isoprene units, and can be found mainly in fungi and plants. This group of substances has interesting biological and ecological activities. The following are biological: antimicrobial, antiparasitic (*Trypanosoma cruzi*), cytotoxicity with relative selectivity for cancer cells, anti-HIV, hypotensive, anti-inflammatory, antispasmodic, cardiovascular diseases, among others (AMBROSIO *et al.*, 2006). Ecological activities were proven by the reduction of larvae of herbivorous insects (NASCIMENTO *et al.*, 2012), larvicidal activity against *A. aegypti* (GERIS *et al.*, 2008) and insecticidal activity, demonstrated by some clerodan-type diterpenes in *Leptinotarsa decemlineata* (potato beetle), a pest that has developed resistance to a large number of synthetic insecticides (BOZOV; GEORGIEVA, 2017).

According to the literature, diterpenes have a wide variety of chemical structures, so they are divided into classes, such as some of the cyclic diterpenes labdans (**A**), abietanes (**B**), pimarans(**C**) and caurans (**D**), as shown in figure 2.

Figure 2

Basic skeletons of cyclic diterpenes



The class of labdan-like diterpenes are among the main chemical constituents of copaifera oleoresin, this class mainly, have demonstrated pharmacological actions such as anti-inflammatory (JIANG *et al.*, 2015), anticancer (NIE *et al.*, 2015),

antibacterial (KIM *et al.*, 2012), antifungal (XU *et al.*, 2015), antiviral (WANG *et al.*, 2015), in smooth muscles, such as antispasmodic activity, antihypertensive activity in rats, relaxing activity in rat aorta alone (RIBEIRO *et al.*, 2007) and both hypotensive effect and relaxing vessel in superior mesenteric artery in normotensive rats (OLIVEIRA, ANDREZZA BEATRIZ; OLIVEIRA, 2007).

2.2.1 Polyaltic acid

Polyaltic acid (**1**), **figure 3**, is a diterpene of the labdane class found in the genus *Copaifera*. This compound is mainly known for its antimicrobial activity (ABRÃO *et al.*, 2018), antiparasitic and antitumor (BORGES *et al.*, 2016; MIZUNO *et al.*, 2015). Unlike the antiparasitic activity, which has been investigated in several studies, the antitumor capacity is little known, so the possibilities for investigation in this area are great.

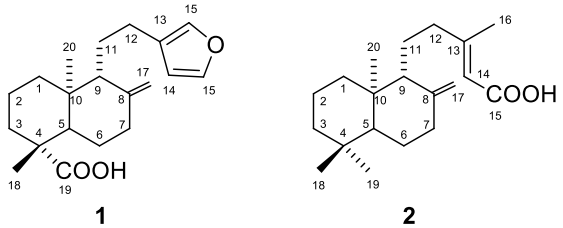
2.2.2 Copalic acid

Copalic acid (**2**), **figure 3**, is also a diterpene of the labdane class, it is mainly isolated as major components of the oleoresin of *the copaifera*, being a marker of such oleoresin, in view of the fact that it was the only one found in all the oleoresins analyzed (VEIGA, VALDIR F.; PINTO, 2002).

Studies show that the isolated substance has proven activities as an analgesic (TAPPIN *et al.*, 2004), antimicrobial (SOUZA *et al.*, 2011), anti-inflammatory with some selectivity for COX-2 and antitumor against lung, colon, stomach, central nervous system and breast cancer strains (MALEK *et al.*, 2011).

Figure 3

Structures of polyaltic acid (1) and copalic acid (2)



1 **2**

β-sitosterol.

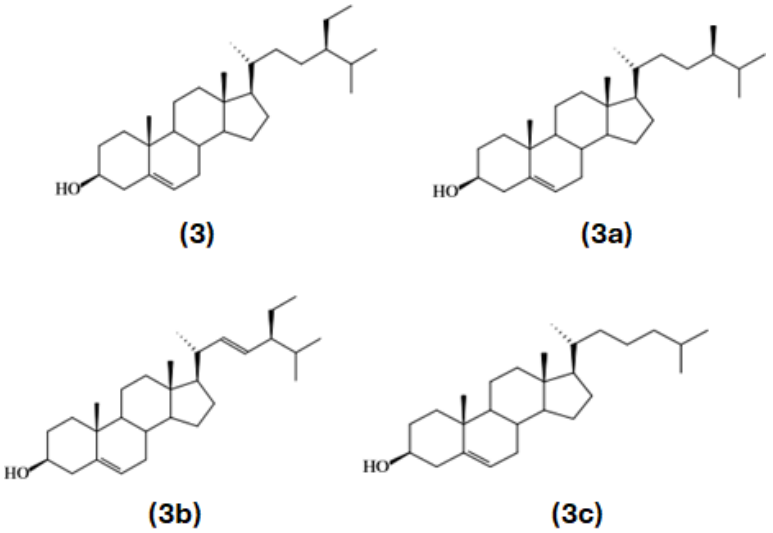
β-sitosterol (**3**) is a phytosteroid, which are steroidal substances or tetracyclic terpenoids extracted from plant species, the most common, shown in **figure 4**, being

β-sitosterol (**3**), campesterol (**3a**), and stigmasterol (**3b**). In animals, the main steroid is cholesterol (**3c**), which has a function analogous to phytosteroids, but is differentiated by the presence of a methyl or ethyl group and/or the presence of a double bond in the side chain (OLIVEIRA, ANDREZZA BEATRIZ; OLIVEIRA, 2007).

β-sitosterol is an amorphous crystalline solid, with low solubility in water and good solubility in organic solvents, and which has in its structure an unsaturation in C-5, secondary alcohol group (carbon C-3), allowing the double bond to be halogenated or oxidized. (MISHARIN *et al.*, 2008).

Figure 4

Example of the most common phytosteroids and their differences



Source: (SANTOS, 2010).

Scientific studies show that β-sitosterol improves symptoms and prevents urinary incontinence caused by an enlarged prostate gland (benign prostatic hyperplasia). The same line of research also shows anticancer activity in prostate cancer cells and has shown that β-sitosterol decreased the growth of cancer cells (BY STEPHEN B. STRUM, MD, 2005).

Based on the promising data of such a substance, β-sitosterol is often being found in the form of more complex derivatives such as fatty acid, aromatic, or glycosylated esters. These esters can confer peculiar and interesting properties to β-sitosterol from a biological point of view, such as: allelopathic agent, aggregation pheromone, develops pharmacological activities, acts against the hepatitis virus (in vitro assays) and also presents selective cytotoxic activity against some types of cancer cells, has antiviral and anti-inflammatory action, in addition to acting to reduce

total cholesterol in blood plasma (CUI *et al.*, 2008; DROZDOV *et al.*, 2007; OLIVEIRA, ANDREZZA BEATRIZ; OLIVEIRA, 2007).

2.3 STRUCTURAL MODIFICATION

Products of natural origin have been an important tool in modern medicine, and can provide some extremely useful medicines, the synthetic production of which is very difficult, if not impossible. Or even serving as models for obtaining synthetic medicines. Semisynthetic drugs and synthetic derivatives of natural products have become increasingly prevalent, making up 28% of approved drugs, while unmodified natural products are only 9.5% (PATRIDGE *et al.*, 2016). Bioactive metabolites are also isolated from natural metabolism that can be slightly modified to become more effective and less toxic (BALUNAS; KINGHORN, 2005).

Several publications show that labdanus-type diterpenes are favorable to structural modifications, and can serve as starting materials for the production of important synthetic and semisynthetic intermediates with great potential (FRIJA; FRIAR; AFONSO, 2011). Based on the interesting biological activities of diterpenes, many synthetic studies have been reported so far and much effort is still being devoted to the development of new, more efficient approaches (AWEN; NOZAWA; HAGIWARA, 2008).

Based on studies of labdan diterpene derivatives, carried out in our research group (AGUIAR, 2019; SOUZA *et al.*, 2011) it is interesting to deepen the studies of modifications for labdane diterpenes.

3 GOALS

- Promote structural modifications by esterification with polyaltic acid alkyl halides.
- Studies of ester formation by the DCC-DMAP system of ent-copalic acid and ent-β-acetoxy copalic acid, from *Copaifera langsdorffi*, using □-sitosterol as alcohol, to evaluate the variation of cytotoxic activity of the products.
- Evaluation of the cytotoxic potential of the starting materials and semi-synthetic derivatives obtained.

4 MATERIALS AND METHODS

4.2 EQUIPMENT AND MATERIALS

The NMR experiments were carried out at the Department of Chemistry of USP in Ribeirão Preto, recorded in a Bruker AVANCE DRX400 spectrometer, operating at 400MHz. The samples were prepared in deuterated chloroform (Aldrich®) at a concentration of 20 to 25 mg/mL. The spectra were studied in our laboratory.

All analyses by Comparative Thin Layer Chromatography (CCDC) were performed on Merck® aluminum plates (art 107730), covered with a layer of silica gel 60 GF254 thick 0.25 mm.

Separations by classical column chromatography (CCC) were performed on glass columns, containing Merck® silica gel 60, 70-230 mesh (0.0630-0.200 mm) as stationary phase. The sizes of the columns were selected according to the sample quantity and equipped with a silicone stopcock at the bottom. The samples incorporated into the silica were applied to the top of the column.

For the separations by Vacuum Liquid Chromatography (CLV) were performed on glass columns, containing silica gel 60, 70-230 mesh (0.063-0.200 mm) and silica gel 60H, 70-230 mesh (0.040-0.063 mm) of the Merck® brand as stationary phase, their sizes were also selected according to the sample quantity. Each sample was applied to the top of the column and incorporated into the silica.

The solvents were distilled by our group as a purification process and were used as mobile phases in the analyses by CCDC, CLV and CCC.

Sulfuric vanillin and ultraviolet light irradiation were used for the development of chromatographic plates, prepared as described. In a 1 L glass bottle, 450 mL of distilled water was added to 100 mL of H_2SO_4 and, subsequently, 450 mL of EtOH, the mixture was stirred. Then, 30 mL of this solution was used for the solubilization of 0.3 g of vanillin. This system was stirred vigorously until complete solubilization of the vanillin crystals.

For the structural modification reactions, ester formation, 1,3-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), potassium hydroxide powder (KOH), anhydrous acetone and alkyl and aryl halides were used, all of which were commercially obtained by the company *Sigma,* all from Aldrich® maca.

At the end of the reactions, distilled water was used and the organic phases were dried using Mg_2SO_4 (anhydrous magnesium sulfate), from Merck®.

4.3 OBTAINING THE POLYALGIC ACID OF *COPAIFERA DUCKEI* DWYER.

The polyaltic acid extracted from the oleoresin of the species *Copaifera duckei* Dwyer, collected in Amazonas, was provided by Prof. Dr. Jonas Joaquim Mangabeira da Silva, one of the researchers who is part of our group of collaborators.

Obtaining β-sitosterol.

The β-sitosterol, used as an alcohol for the esterification reactions from ent-copalic acid and ent-β-acetoxy copalic acid, was provided by Prof. Dr. Wilson R. Cunha, one of the researchers who are part of our Natural Products Research Laboratory.

4.4 REISOLATION OF *THE* ENT-COPALIC ACID AND ENT-B-ACETOXY COPALIC ACID, CONSTITUENTS OF *THE COPAIFERA LANGSDORFFI OLEORESIN.*

Common chromatographic methods were used for the isolation of the starting materials, such as CLV, CCD and CCC. A part of this work was directed to the isolation of substances already known and previously isolated, ent-copalic acid and ent-β-acetoxy copalic acid, so the detection was performed through comparative thin layer chromatography (CCDC), by comparison with standards of these substances, previously obtained by our research group.

A sample (20 g) of Copaífera *langsdorffii oleoresin* (coded as O.C.), acquired from the herbal medicine company Apis Flora, located in the city of Ribeirão Preto (lot n° 0790310), was incorporated into 31 g of silica gel 60, forming a wafer and fractionated by vacuum liquid chromatography (CLV) according to adaptations of the methodology (SOUZA *et al.*, 2011). A glass column and 482 g of silica gel 60 and 60H, in a 1:1 ratio, were used, packaged with the aid of vacuum and hexane. A gradient elution Figure of increasing polarity was used as the mobile phase, as can be seen in **Table 1**, collecting a total of 12 fractions.

From the analysis by CCDC of the patterns of the substances, together with the fractions obtained, it was found that O.C5, O.C6, O.C7 and O.C9 presented major stains, being O.C5 and O.C6 identical to the patterns, these were then chosen to continue the process.

Table 1

Elution Figure used in O.C. fractionation

Solvent utilized	Volume collected (L)	Fraction	Dough Obtained (g)
Hex	0,5	O.C1	0,17
Hex/AcOEt 9:1	0,5	O.C2	12,97
Hex/AcOEt 8:2	0,5	O.C3	1,38
Hex/AcOEt 7:3	0,5	O.C4	1,21
Hex/AcOEt 6:4	0,5	O.C5	1,79
Hex/AcOEt 1:1	0,5	O.C6	0,82
Hex/AcOEt 4:6	0,5	O.C7	0,90
Hex/AcOEt 3:7	0,5	O.C8	0,37
Hex/AcOEt 2:8	0,5	O.C9	0,19
Hex/AcOEt 1:9	0,5	O.C10	0,06
AcOEt	0,5	O.C11	0.05
EtOH	0,5	O.C12	0.03

Hex: n-hexane; AcOEt: ethyl acetate; EtOH: ethanol

4.4.1 Fraction O.C5

This fraction was analyzed by CCDC using the hexane/ethyl acetate mobile phase (8:2) and showed that it consisted of a major stain. This fraction was analyzed by NMR ¹H, confirming that it was entcopalic acid.

4.4.2 Fraction O.C6

This fraction was analyzed by CCDC using the hexane/ethyl acetate mobile phase (6:4) and showed that it was also constituted by a major stain. This fraction was analyzed by NMR ¹H, where it was confirmed that it was *the acid* ent-β-acetoxy copal.

4.5 STRUCTURAL MODIFICATION.

4.5.1 Reactional study for the formation of esters from entecholic acid.

First, an adaptation of the article by FARSHORI et al. was used as a procedure. (2010), **(methodology 1)**: In a 25mL flask, a 30mg solution of ent-copalic acid was prepared in 10mL of DCM, to which 23mg of DCC was added, leaving it in agitation for 5 minutes. Then, 41mg of β-sitosterol was added and 8mg of DMAP was added to this solution after 10 minutes under constant magnetic stirring and room temperature for 48 hours. The development of the reaction was monitored by CCDC periodically. To finish the reaction, the mixture was placed in a separation funnel

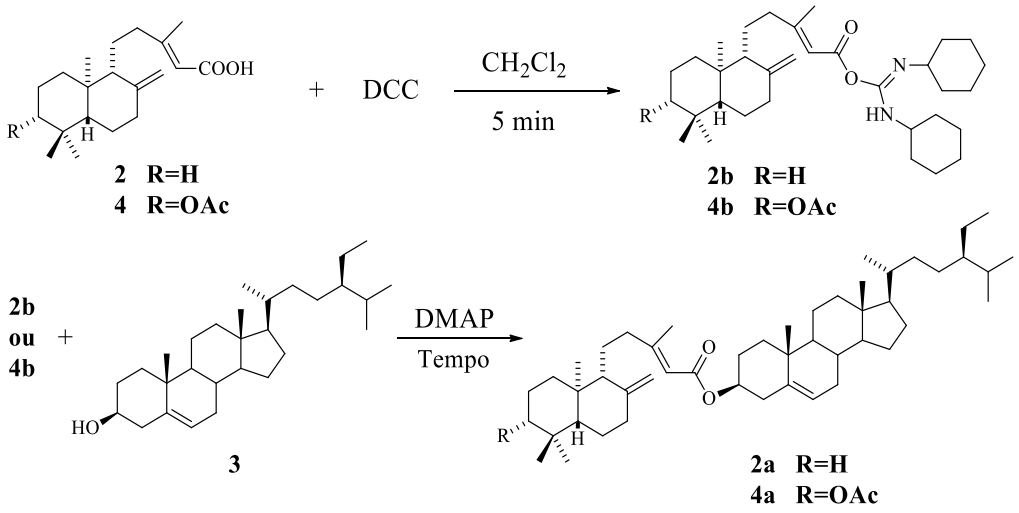
where it was extracted using 4 portions of 10mL of distilled water. The resulting organic phase was dried with anhydrous magnesium sulfate and filtered. The organic residue obtained was purified by means of CCC packaged with 16g of silica gel 60 and 1g of silica gel 60H, as mobile phase Hex/AcOEt was used in a ratio 9.75: 0.25. The product of interest was identified by NMR **(test 1).** The next test **(test 2)** was performed based on this same methodology, with variations only in volume, which changed to 5mL of solvent, and in reaction time, which became 24 hours.

In the next procedure **(methodology 2),** an adaptation of the article by Sheikh et al. (SHEIKH *et al.*, 2010): In a 10mL flask, 0.1mmol (approximately 37mg) of ent-copalic acid, together with 0.2mmol (approximately 42mg) of DCC, were solubilized in 3mL of DCM, with the mixture already under agitation. After 5 minutes of reaction, 0.2mmol (approximately 83mg) of β-sitosterol and 0.2mmol (approximately 25mg) of DMAP were added, maintaining the reaction under constant magnetic agitation at room temperature for 27 hours. The progress of the reaction was monitored by CCDC periodically. To finish the reaction, the mixture was poured into a separation funnel where it was extracted using 4 portions of 10mL of distilled water. The resulting organic phase was dried with anhydrous magnesium sulfate and filtered. The organic waste obtained was purified by means of

CC packed with 14g of silica gel 60 and 1g of silica gel 60H, as the mobile phase Hex/AcOEt was used in a ratio 9.75 : 0.25. The product of interest was identified by NMR **(test 1).** The next tests were carried out based on this same methodology, with variations. In test 2, the solvent volume changed to 5mL, the reaction time became 23 hours and the system was kept in reflux at 45°C for 4 hours. In test 3, the volume remained at 5mL, but the redction time became 49 hours and the system was kept in reflux at 45°C during the entire reaction.

Figure 5

Methodologies 1, 2 and 3 for ent-copalic acid and methodology for 3-acetoxy-ent-copalic acid



Metodologia 1 - ácido copálico R=H

Teste 1:	**Teste 2:**
V_{DCM} = 10 mL	V_{DCM} = 5 mL
Tempo reacional = 48h	Tempo reacional = 24h
Temperatura ambiente	Temperatura ambiente

Metodologia 2 - ácido copálico R=H

Teste 1:	**Teste 2:**	**Teste 3:**
V_{DCM} = 3 mL	V_{DCM} = 5 mL	V_{DCM} = 5 mL
Tempo reacional = 27h	Tempo reacional = 49h	Tempo reacional = 23h
Temperatura ambiente	Aquecimento a refluxo (45°C)	Aquecimento a refluxo (45°C)/4h

Metodologia 3 - ácido copálico R=H	**Esterificação do ácido acetóxi-copálico R=OAc**
Teste 1:	**Teste 1:**
V_{DCM} = 5 mL	V_{DCM} = 3 mL
Tempo reacional = 48h	Tempo reacional = 46h
Temperatura ambiente	Temperatura ambiente

Methodology 3 was based on the following procedure: In a 25mL flask, 73mg of ent-copalic acid and 101mg of DCC were solubilized in 5mL of DCM. After 5 minutes, 46 mg of β-sitosterol and 61 mg of DMAP were added, the reaction was maintained under constant magnetic agitation at room temperature for 48 hours and

its development was monitored by CCDC periodically. To finish the reaction, the mixture was filtered in order to remove the formed precipitate and washed with 3 portions of DCM to remove all organic residue and had its solvent removed by rotary evaporation. The organic residue obtained was purified by means of CC packaged with 13g of silica gel 60 and 3g of silica gel 60H, as a mobile phase Hex/AcOEt was used in a ratio 9.75: 0.25. The product of interest was identified by NMR.

4.5.2 Formation of esters from ent-β-acetoxy copalic acid.

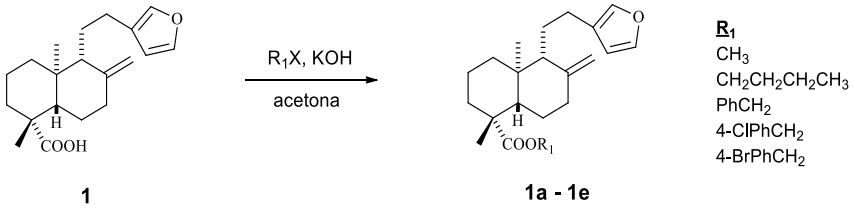
Based on the methodology that presented the best yield in the reactional study for the formation of esters from *entcopalic acid,* (methodology 2), **an adaptation of the article SHEIKH** et al. (2010), following this procedure, 0.1mmol (approximately 43mg) of ent-β-acetoxy copalic acid, along with 0.2mmol (approximately 42mg) of DCC, were solubilized in 3mL of DCM. After 5 minutes of reaction, 0.2mmol (approximately 83mg) of β-sitosterol and 0.2mmol (approximately 25mg) of DMAP were added, maintaining the reaction under constant magnetic agitation at room temperature for 46 hours and its development was monitored periodically by CCDC. To finish the reaction, the mixture was placed in a separation funnel where it was extracted using 4 portions of 10mL of distilled water.

4.5.3 Formation of esters from polyaltic acid.

The esterification reactions with polyaltic acid were adapted from the article by BOECK *et al.* (2005), where the following alkyl and aryl halides were used as reagents: iodomethane, benzyl bromide, 4-bromobbenzyl bromide, 4-chlorobenzyl bromide and bromobutane; all followed according to the general procedure below.

Figure 6

Polyaltic acid esterification reactions with different alkyl and aryl halides



1 1a - 1e

KOH powder (0.0192 g, 0.343 mmol) was added to anhydrous acetone (2.0 ml) and the mixture was stirred for 5 minutes at room temperature. The starting material (0.03 g; 0.1 mmol) was added to the reaction, followed by the alkyl or aryl

halide (0.102 mmol). It remained agitated for the time stipulated for each of the different reactions at room temperature. After this time, the reaction mixture was transferred to a separation funnel containing 20 mL of distilled water. Ethyl acetate (3 x 20 mL) was used for organic phase extraction . Then, it was washed with water (3 x 10 mL), dried with MgSO4, filtered and removed the solvent by rotary evaporation. The purification of the residue was carried out by classical column chromatography (CCC), and finally, the product of each reaction was obtained.

4.6 BIOLOGICAL CYTOTOXICITY ASSAYS – XTT COLORIMETRIC ASSAY

This is a cytotoxicity test used to indicate cell viability, as these enzymes in viable cells reduce the tetrazolium salt to formazane, forming an insoluble salt, which can be quantified by spectrophotometry (Nofziger *et al*., 1988).

For this assay, 104 cells/well were seeded in complete medium in 96-well plates and left under incubation at 37oC for 24 hours. After this period, treatment with *cis-TMS* was performed at concentrations of 0.3125, 0.625, 1.25, 2.5, 5.0, 10.0 and 20 μM. The cells were also treated with doxorubicin hydrochloride or cisplatin at concentrations of 0.5 μM; 5.0 μM; 50 μM; 500 μM in order to define the concentrations of use as a positive control in proliferation, migration and cell death experiments. DMSO 10% (v/v) was used as a positive control, DMSO 1% (v/v) as solvent control, and negative control that did not receive any treatment.

After 24 hours of treatment, the experiment was completed as follows: the plate culture medium was discarded and washed once with non-sterile phosphate salt buffer (PBS); then, 100 μL of XTT solution was added to each well, which was prepared in DMEM culture medium without red phenol from the manufacturer's recommendations (Cell Proliferation Kit, Roche, Manheim). The plates were taken to the 37oC greenhouse protected from light, remaining incubated for 4 hours. Next, the reading was performed in the spectrophotometer (Asys UVM 340, Biochrom, Shanghay, China) using the wavelength of 492 nm and the reference value of 690 nm. Cell viability was calculated in comparison to the negative control considered to have 100% viability, and the values obtained will be in the form of IC50.

5 RESULTS AND DISCUSSIONS

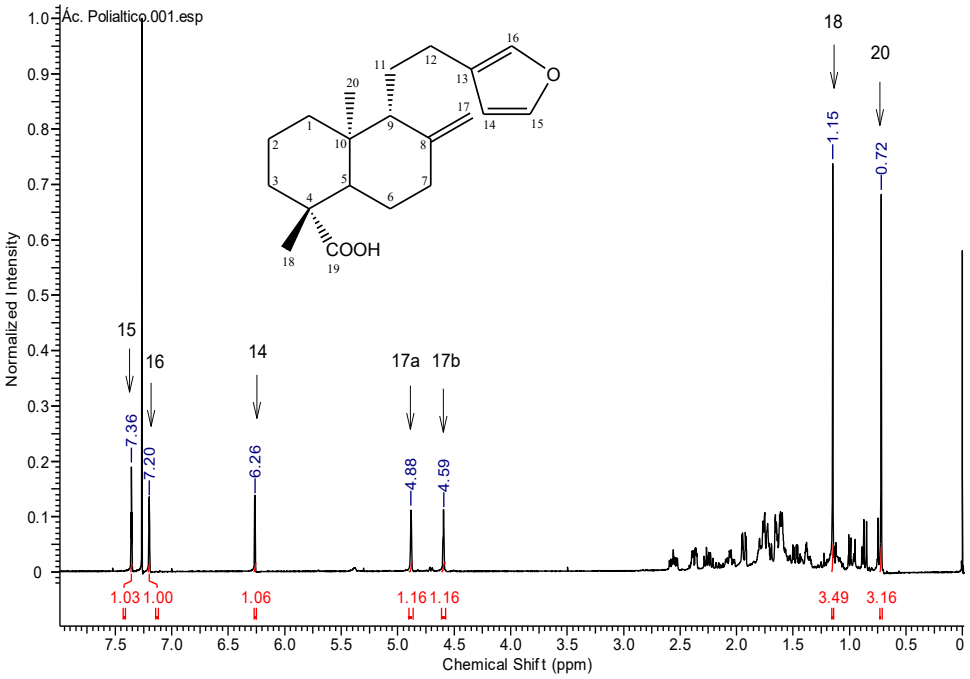
5.4 IDENTIFICATION OF POLYALTIC ACID.

The identification of polyaltic acid was carried out by comparing 1H and 13C NMR data obtained for the isolated substance with 1H NMR data from the literature (CARRERAS; ROSSOMANDO; OSCAR S. GIORDANO, 1998).

The 1H NMR spectrum made from the polyaltic acid sample is shown in (Figure 7).

Figure 7

1H NMR spectrum of polyaltic acid (1). (400 MHz, CDCl3)



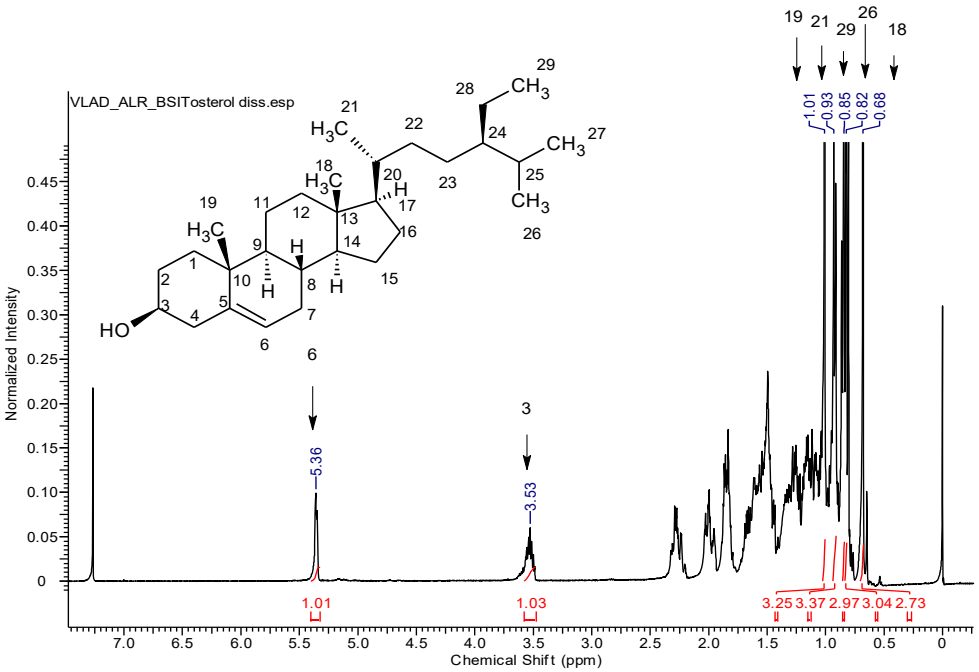
In the 1H NMR spectrum of polyaltic acid, the characteristic signs of the twin hydrogens of the terminal double bond of carbon 17, which presented in the region of 4.59 and 4.88 ppm, and of the hydrogen of the endocyclic double bond presented in the region of 6.26 ppm, were easily perceived. Two other signs of vinyl hydrogens were observed with chemical displacements of 7.20 ppm and 7.36 ppm, attributed to the hydrogens at positions 16 and 15, respectively, and these being neighbors of an electronegative atom, oxygen, this explains a higher than expected chemical displacement for this type of hydrogen. In addition to the two methyl signals of carbons 20 and 18 with chemical displacements at 0.72 and 1.15 ppm. Through these signs, in comparison with the literature, according to Carreras and his collaborators (CARRERAS et al., 1998), the structure of the starting material was confirmed.

In the NMR spectrum of 13C, signs of methyl carbons 18 and 20 present in the structure were observed, with displacements of 18.57 and 14.76 ppm, respectively; methylene carbon at position 17 with displacement of 106.90 ppm and methine carbons referring to carbons 14, 15 and 16 with their respective displacements 110.92, 142.68 and 138.69 ppm.

5.5 IDENTIFICATION OF B-SITOSTEROL

Figure 8

1H NMR spectrum of β-sitosterol ***(2)*** *(400 MHz, CDCl3)*



The substance was confirmed by comparing the data published in the literature (COPETTI et al., 2016) with the spectrum obtained. It was possible to confirm that the spectrum **(Figure 8)** is β-sitosterol, because the signals described in the literature are present in this spectrum, presenting a signal at 3.53 ppm referring to carbon 3 hydrogen due to the electronegative effect of hydroxyl. At 5.36 ppm the signal referring to the hydrogen of carbon 6 is present, which is an olefin hydrogen, both signals present a small change in chemical displacement, but are in agreement with the structure. The signs referring to methyls, identified in the singlets 0.68; 1,01; 0,93; 0.82 and 0.85 ppm, assigned

to the hydrogens of carbons 18, 19, 21, 26 and 29 respectively, were exactly the expected signals for the spectrum in question.

5.6 IDENTIFICATION OF SUBSTANCES OBTAINED BY REISOLATION

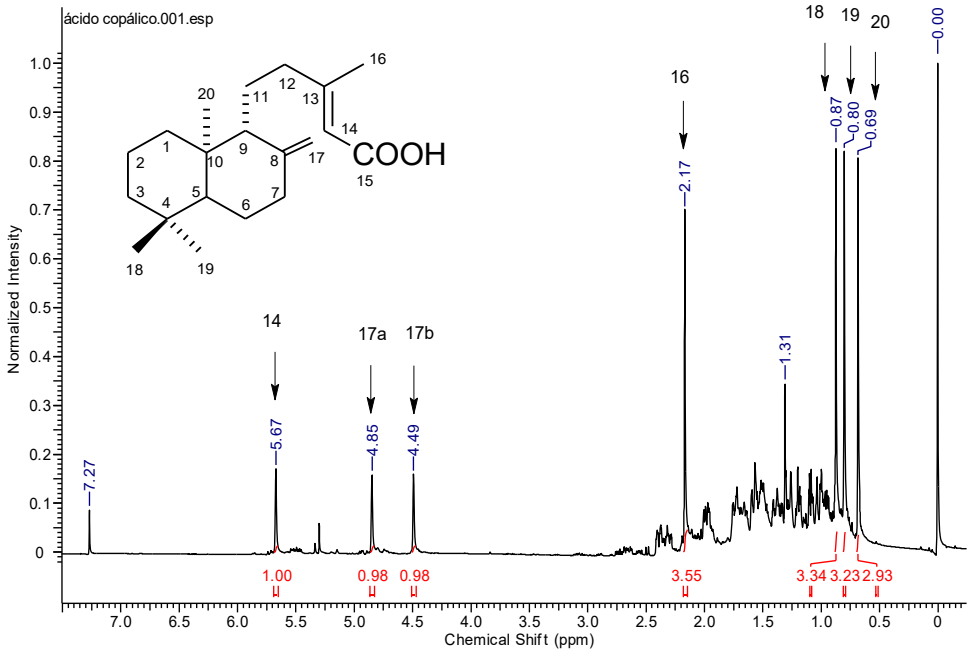
In the case of reisolation, all substances are already known and previously identified natural products, therefore, the identification of such substances was made by comparison with data previously published in the literature. The reisolation process was carried out following the script of Souza *et al*. (2011), as he had already isolated the constituents of *C. langsdorffii.*

During this process, the samples were analyzed by CCDC and then the substances were subjected to 1H NMR analysis . The NMR data obtained for each isolated substance were compared with the NMR data published in the literature. The NMR spectra are in **figures 8 and 9**, so two of the four major constituents of the copaiba oleoresin used were identified.

5.6.1 Identification of ent-copalic acid

Figure 7

1H NMR spectrum of ent-copalic ***acid (3)*** *(400 MHz, CDCl3)*



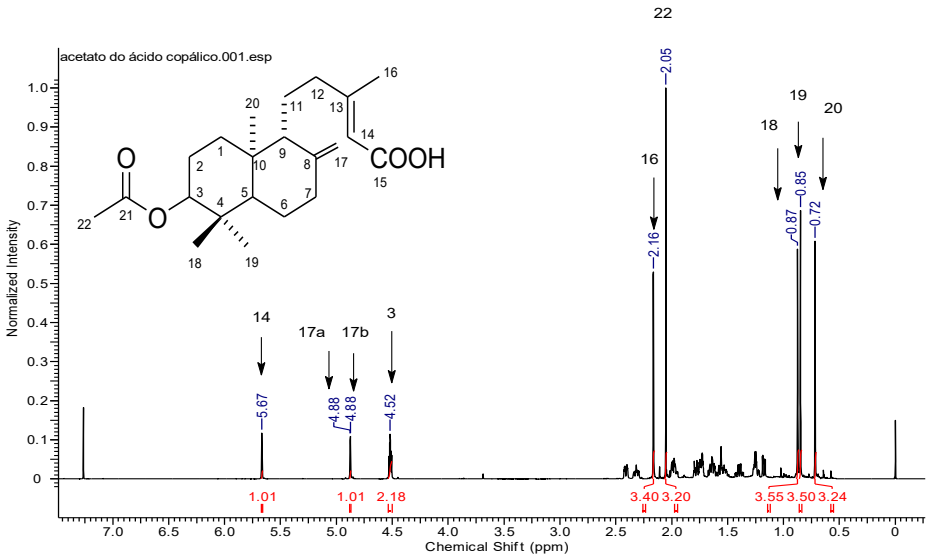
The signals obtained in the spectrum above were the expected signals for the **O.C5** fraction, with reference to the data in the article (VARGAS; ALMEIDA; ARANHA, 2015). The most displaced signal, in the region of 5.67 ppm, refers to carbon 14 hydrogen, since this hydrogen is olefin and neighbors of a carboxyl group, so its signal

is in perfect agreement with the structure. The signals at 4.49 ppm and 4.85 ppm refer to the olefin hydrogens 17b and 17a, respectively, the chemical displacement is in line with what is expected for this group. The signal at 2.17 ppm corresponds to the methyl of carbon 16, characteristic of methyls attached to olefin carbons, and is in accordance with what should be expected for this part of the structure. Finally, the three least displaced signals in the spectrum in the region of 0.69; 0.80 and 0.87 ppm correspond to methyls 20, 19 and 18 as indicated in the spectrum. Thus, the data obtained in this work in comparison with the data previously published in the literature, proves the attribution made

5.6.2 Identification of ent-β-acetoxy copalic acid

Figure 8

*1H NMR spectrum of copalic ent-β-acetoxy acid **(4)** (400 MHz, CDCl3)*



Comparing the NMR data obtained for the **O.C6** fraction with the data published in the literature, it is possible to prove that the fraction studied refers to the substance 3-acetoxy copalic acid, as had been evidenced when compared with the CCDC standard. All the signals detailed for substance **3** (signals 20,19 and 18) also appear for substance **4**, such signals correspond respectively to the three methyls at 0.72, 0.85 and 0.87 ppm. The methyl of carbon 16 presented at 2.16 ppm and the signals referring to the olefin hydrogens of carbons 17 and 14 presented in the region of 4.88 and 5.67 ppm respectively. The difference of such a structure when compared to ent-copalic **acid (3)** is evidenced by the appearance of two new signals, one of these signals is present at 2.05 ppm and refers to the hydrogens of carbon 22 which is attached to the carbonyl of the acetate group according to what should be expected

for this part of the structure. The second signal is present at 4.52 ppm and corresponds to carbon 3 hydrogen, which in turn is bound to an ester oxygen, so it showed more displaced. Therefore, the attribution carried out is in perfect harmony with the data previously published in the literature (VARGAS et al., 2015).

5.7 STRUCTURAL MODIFICATION BY ESTERIFICATION.

The structural modification of natural products plays a large role in modern medicine, as described in the introduction. Previously, a methodology for the esterification of caurano and pimaran-type diterpene skeletons was tested by the group (BOECK *et al.*, 2005), based on the idea that some methodologies for the esterification of labdan-type diterpenes were also tested (AGUIAR, 2019), but using small-chain alcohols, esterifications for the same class of diterpenes are being studied in this work, using β-sitosterol as alcohol in the first reactions, this substance being a long-chain phytosteroid, with many proven biological activities. Initially insisting on the same methodology, the results obtained had a very low yield, so it was necessary to invest in the search for conditions and methodologies that could increase the yield of the product of interest. Subsequently, alkyl and aryl halides were used for esterifications of polyaltic acid, following the same line of labdan-type diterpenes.

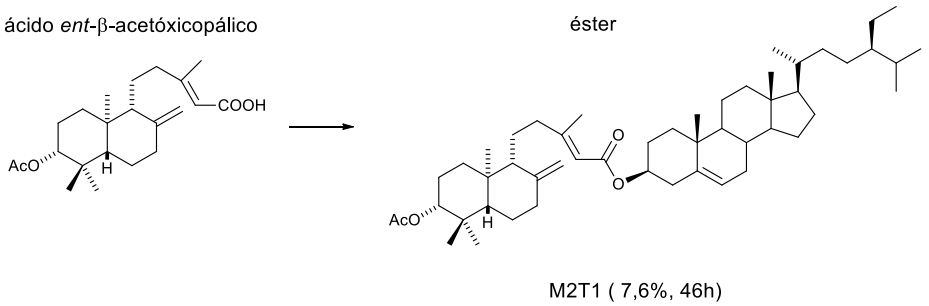
5.7.1 Reactional study to obtain esters from entopalic acid

5.7.2 Esterification reaction from ent-β-acetoxy copalic acid

Based on the best result obtained with the reactional study, since the substance to be esterified is similar to the previous one, belonging to the same class, of labdanus-type diterpenes, with only one acetate group added to its molecule, the reaction was developed following the methodology described in (SHEIKH *et al.*, 2010), making an adaptation only in the reaction time, where the need for such a change was proven by CCDC. However, the reaction obtained a yield that can be considered satisfactory, of 7.6%, but this result can be improved in the next projects.

Figure 10

Esterification with ent-β-acetoxy copalic acid

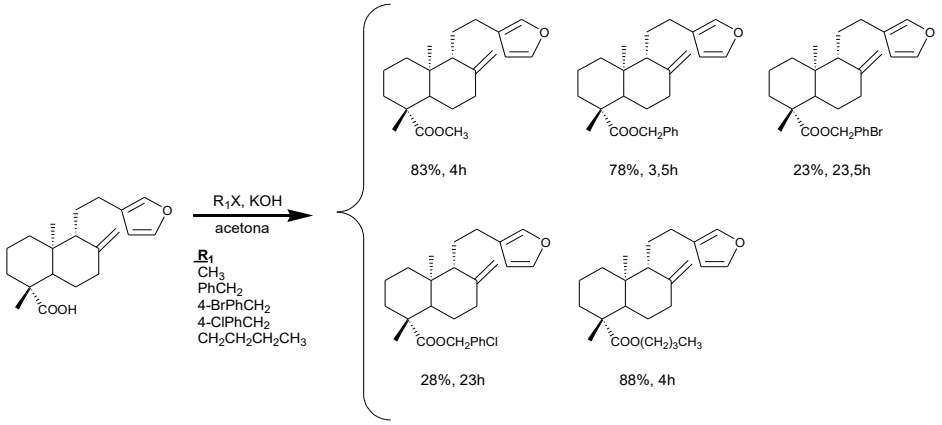


5.7.3 Esterifications reactions from polyaltic acid

Below, in a summarized way, are the transformations performed by esterification of polyaltic acid, presenting the halides used, the reaction time and the yield for each reaction. All reactions showed good results, emphasizing the reactions with iodomethane and bromobutane that showed excellent yields.

Figure 11

Esterification with polyaltic acid



5.8 IDENTIFICATION OF SUBSTANCES OBTAINED BY ESTERIFICATION USING THE DCC/DMAP SYSTEM.

The esterification reactions using the DCC/DMAP system were carried out according to the protocol described in the previous sections (**items 4.4.1 and 4.4.2**) and underwent a purification process by classical column chromatography (CCC). The analogous structures obtained from the starting materials, ent-copalic acid and ent-β-acetoxy- acid, are described and confirmed below.

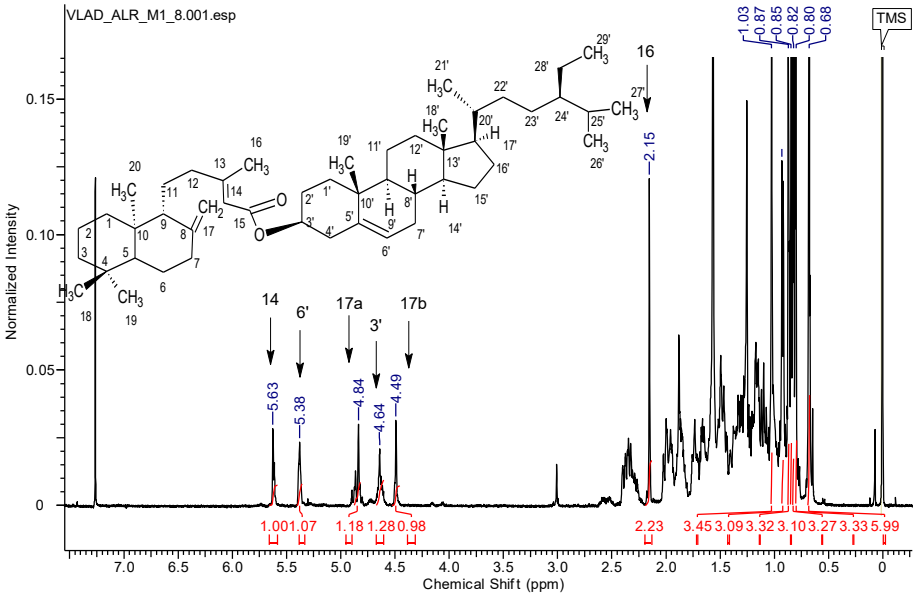
Copalic through 1H NMR analysis of the patterns compared to spectra of the derivatives presented (Figure 10) and (Figure 14). It was also possible to describe and confirm the structure of the intermediate, o-acilurea, through 1H NMR analysis of ent-copalic acid in comparison with the spectrum presented (Figure 13).

5.8.1 Identification of ent-copalic acid + β-sitosterol (5)

Through the spectrum obtained (figure 12), it was possible to observe the formation of the desired product.

Figure 12

H NMR spectrum of formed ester, substance (5) (400 MHz, CDCl3)



A comparison was made with the 1H NMR spectrum of ent-copalic acid (3) (Figure 8), β-sitosterol (2) (Figure 9) and the ester formed (5) (Figure 12). Thus, we can observe the characteristic signs of ent-copalic acid, in the region of 5.63 ppm referring to the olefinic hydrogen of the carbon

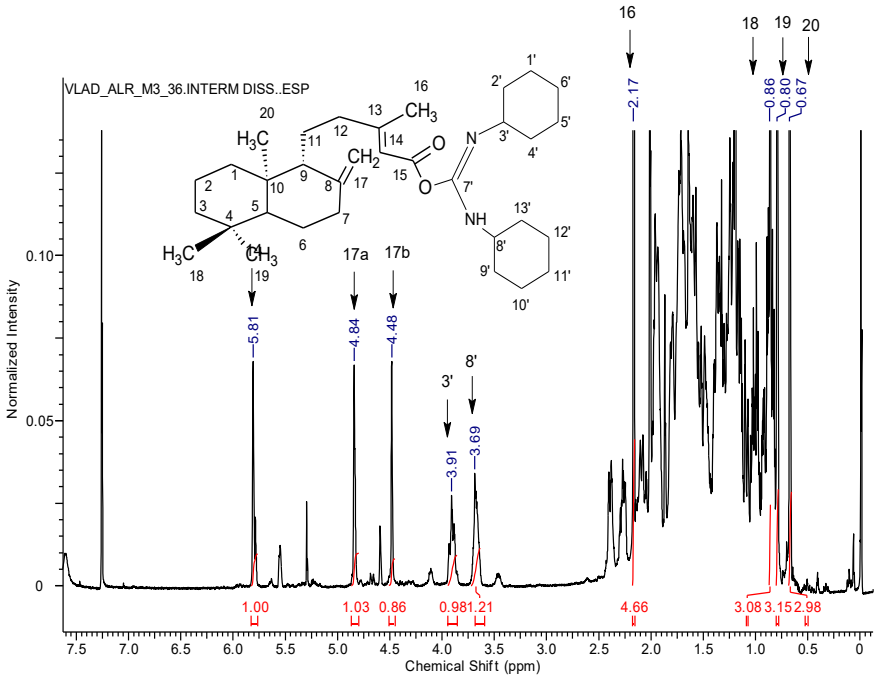
14. It also presents a signal in the region of 2.15 ppm referring to carbon 16 hydrogens, characteristic of methyls linked to olefin carbons. In the region between 4.49 and 4.84 ppm, they present signs referring to the hydrogens of the double bond carbon 17, and the characteristic signs of the methyls of carbons 18 and 19, which are in the regions, respectively, 0.87 and 0.80 ppm. The characteristic signs of β-sitosterol can also be observed, which presents in the region of 4.64 ppm, referring to the hydrogen of carbon 3', which in turn was more displaced, confirming the formation of the ester. To conclude the confirmation, the signals in the regions of 5.38 ppm, referring to the hydrogen of the 6' carbon duo and the signals of the methyl carbons 19', 21', 26' and 29' that are in the 1.03 regions; 0,93; 0.82 and 0.85 ppm respectively. The methyl signals of the 20 and 18' carbons appeared coupled in the region of 0.68 ppm.

5.8.2 Identification of the o-aciluréia intermediate (6)

Through the spectrum obtained (figure 13), it was possible to observe the formation of the intermediate.

Figure 13

1H NMR spectrum of the ester formed, substance (6). (400 MHz, CDCl3)



To confirm the intermediate, a comparison was made with the 1H NMR spectrum of ent-copalic acid (3), (Figure 7), with the obtained spectrum, o-acilurea (6), (Figure 10), where it was possible to observe the characteristic signs of both the acid in question and the intermediate. First, the signal presented in the region of 5.81 ppm referring to the oleofinic hydrogen of carbon 14, which was more displaced due to the presence of electronegative atoms in the molecule. The signals of the germinal hydrogens of carbon 17 presented in the region of 4.48 and 4.84 ppm respectively and the signals of methyls 16, 18, 19 and 20 presented with

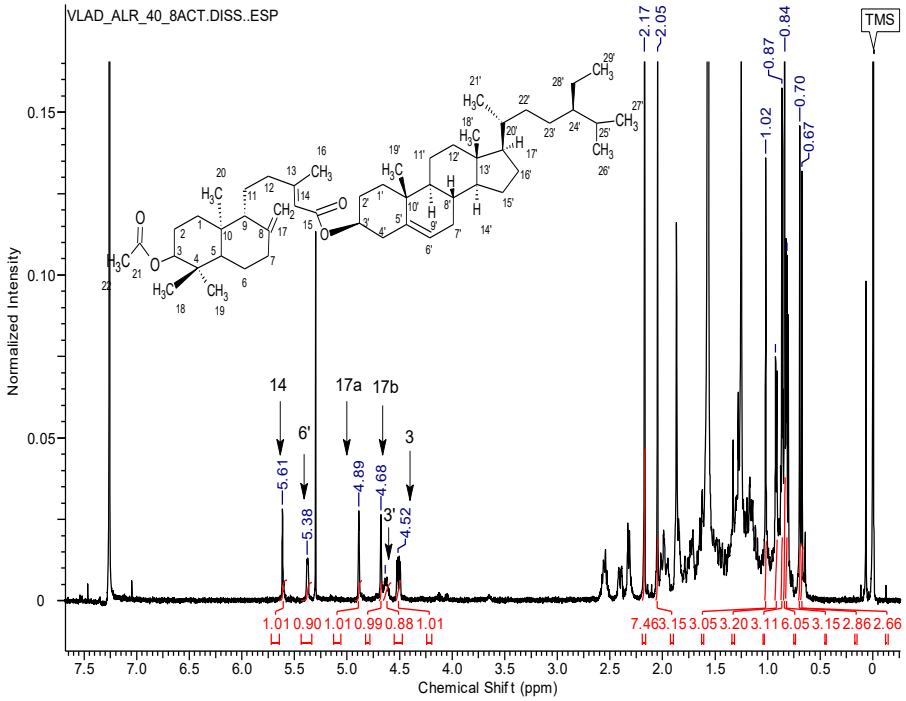
chemical displacement of 2.17; 0,86; 0,80; 0.67 being in agreement with the structure. Concluding the confirmation, the spectrum showed a signal at 3.91 ppm with an integral of approximately 1, confirming what is expected for a dual-nitrogen-bonded ring CH, referring to the hydrogen of carbon 3'. Finally, the signal at 3.69 ppm also with an integral of approximately 1, referring to the hydrogen of carbon 8' which presented a little less displaced because it was not influenced by the duo.

5.8.3 Identification of ent-β-acetoxy copalic acid + β-sitosterol (7)

Through the spectrum obtained (figure 11), it was possible to observe the formation of the desired product.

Figure 14

1H NMR spectrum of the ester formed, substance (7). (400 MHz, CDCl3)



Again, a comparison was made with the 1H NMR spectrum of the copalic ent-β-acetoxy acid (4), (Figure 8), β-sitosterol (2), (Figure 6) and the ester formed (Figure 11). Being able to observe the characteristic signs of the ent-β-acetoxy copalic acid, in the region of 4.52 ppm referring to the hydrogen of carbon 3, which is bound to an oxygen of acid, with this its signal is in perfect agreement with the structure. In the region of 5.61 ppm it presents the sign of oleopine hydrogen referring to carbon 14. At 2.17 ppm it shows the sign of hydrogens referring to carbon 16 and in the region between 4.68 and 4.89 ppm referring to germinal hydrogens of the double bond carbon 17. The characteristic signs of the methyls of carbons 18, 20 and 22 are in the regions, respectively, 0.87; 0.70 and 2.05 ppm. The characteristic signs and β-sitosterol are also present, presenting the signal in the region of 4.64 ppm, referring to the hydrogen of carbon 3', which presents a more displaced multiplet than the characteristic signal of alcohol, confirming the formation of the ester. To conclude the confirmation, the signals in the regions of 5.38 ppm, referring to the hydrogen of the 6' carbon duo and the signals of the methyl carbons 18', 19', 21' and 26' that are in

the 0.67 regions; 1,02; 0.92 and 0.82 ppm respectively. The methyl signals of carbons 19 and 29' appeared coupled in the region of 0.84 ppm.

5.9 IDENTIFICATION OF SUBSTANCES OBTAINED BY ESTERIFICATION USING HALIDES

To obtain the ester derivatives from polyaltic acid labdanus, the alkyl and aryl halide reagents were used: iodomethane, benzyl bromide, 4-bromobbenzyl bromide, 4-chlorobenzyl bromide and bromobutane.

The reactions were carried out according to the protocol described in the previous section (item 4.4.3) and from the CCDC it was verified that the products obtained were impure, therefore, it was necessary to use chromatographic methods, such as CCC, for purification purposes.

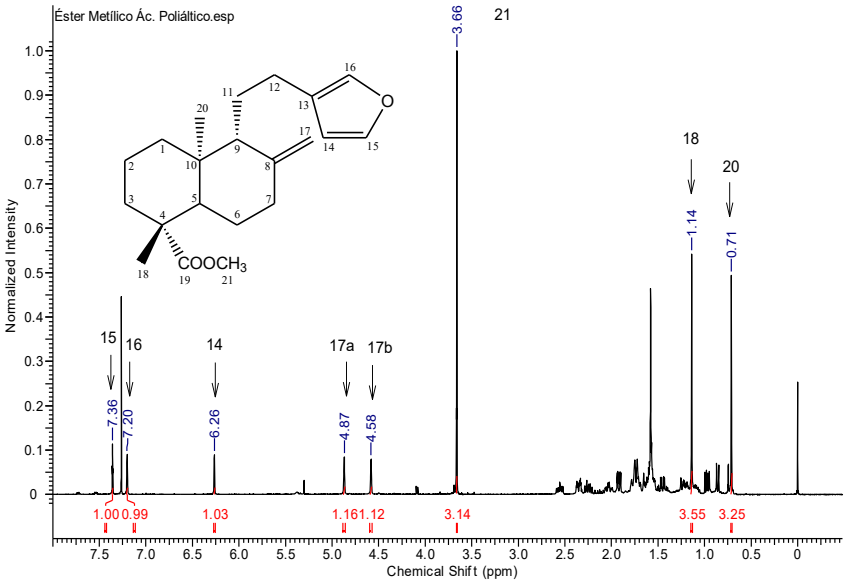
In this section, the discussion of the 1H and 13C NMR spectra of each obtained derivative was presented, after being compared with the spectra of their respective source material.

5.9.1 Identification of the methyl ester of polyaltic acid (8)

Through the spectra obtained, it was possible to observe the formation of the desired product.

Figure 15

1H NMR spectrum of the methyl ester of polyaltic acid (8). (400 MHz, CDCl3)



To confirm the structure, comparative analyses were performed between the 1H NMR spectra of the source material (1), (figure 5), with the spectrum obtained from the sample (8), (figure 15), as well as its 13C NMR spectrum.

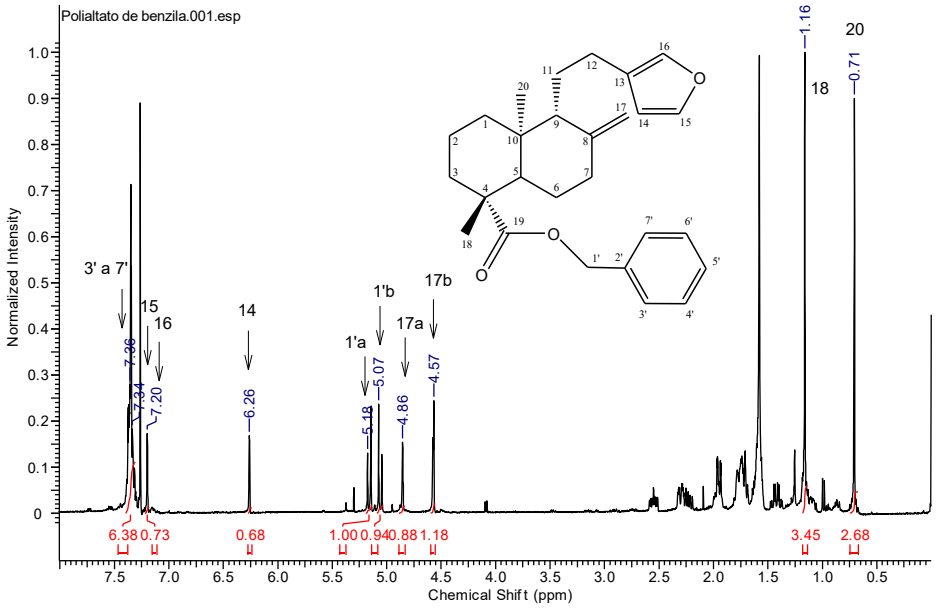
In both the 1H and 13C NMR spectra of the substance, as a product, they showed additional signals referring to the methyl hydrogens at position 21, with a displacement of 3.66 ppm; as well as its carbon present at 51.90 ppm.

5.9.2 Identification of benzyl polyaltote (9)

Again it was possible to observe the formation of the desired product, through the spectra obtained.

Figure 13

1H NMR spectrum of benzyl polyaltate (9). (400 MHz, CDCl3)



One more confirmed product was obtained after analysis between its NMR spectra of 1H (Figure 16) and 13C NMR with the NMR spectra of 1H (Figure 5) and 13C of polyalgic acid (1).

It was noted in the figure above, 1H NMR spectrum , that the differences when compared with the spectrum of the starting material, were exactly those expected for the product initially proposed. One of them linked to the presence of characteristic signs of aromatic hydrogens (from 7.3 to 7.4 ppm), whose relative integral was five hydrogens. The other difference referred to the presence of signals at 5.07 and 5.18 ppm, with doublets attributed to hydrogens of the CH2 group, position 1', connected to the aromatic ring. In this case, both the chemical displacement and the multiplicity

corresponded to the desired structure. In addition, this coupling of twin hydrogens presented itself with a coupling constant value of 12.4 Hz, perfectly normal for this case.

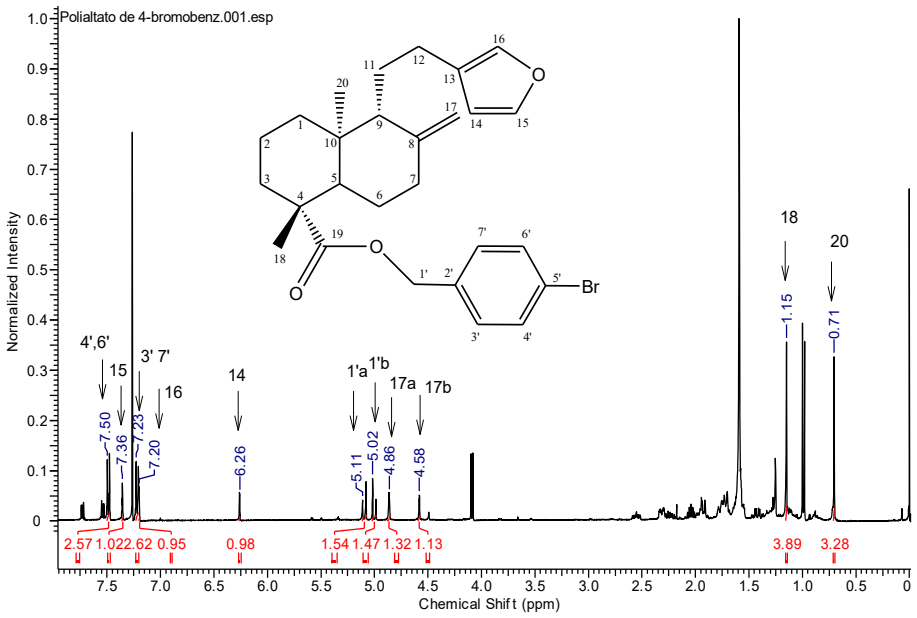
When analyzing the 13C NMR spectrum, it was noted the presence of five new signals (136.38 ppm; 128.83 ppm; 128.48 ppm; 127.90 ppm; 65.22 ppm) related to carbons from 1' to 7' of the ester-bound side chain. These are absent in the 13C NMR spectrum of the parent substance. Thus, the obtaining of benzyl polyaltate (9) was confirmed.

5.9.3 Identification of 4-Bromobbenzyl polyalta (10)

In the spectra below are presented the formation of the desired products.

Figure 16

1H NMR spectrum of 4-bromobbenzyl polyalta (10). (400 MHz, CDCl3)



One more derivative was confirmed from its 1H (Figure 16) and 13C NMR spectra, related to the 1H (Figure 5) and 13C NMR spectra of the source material (1).

The fact that the aromatic hydrogen signals (at 7.23 and 7.50 ppm) were divided into two groups with integral two for each confirmed the structure of the ring replaced in para. In addition, the appearance of signals at 5.02 and 5.11 ppm in relation to the spectrum of the starting material also indicated the presence of the CH2 group, position 1', attached to the ring.

The appearance of five new signals in the 13C NMR spectrum (135.87 ppm; 131.63 ppm; 129.68 ppm; 122.05 ppm; 65.45 ppm) also confirmed the expected structure.

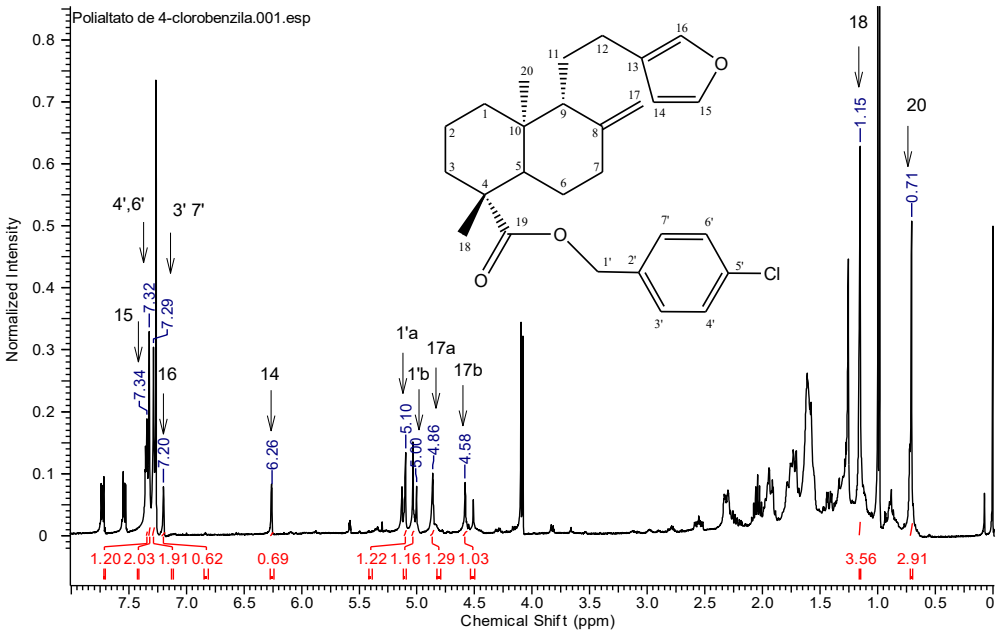
5.9.4 Identification of 4-chlorobenzyl polyaltate (11)

By comparing the 1H (Figure 15) and 13C NMR spectrum of the product, with the spectra of the starting material (1), 1H (Figure 5) and 13C, one more product was confirmed.

What was presented in this case was identical to the previous case, where 4-bromobbenzyl bromide was used as a reagent. In this reaction there was only the substitution of the element bromine (Br) by chlorine (Cl) in the position for the aromatic ring. It was noted that the aromatic hydrogen signals in the 1H NMR spectrum of (Figure 17) have a slightly different distribution from that of the starting material. In addition, in the same way, signs were observed in the region at 5.0 and 5.10 ppm, referring to the CH2 group linked to the ring

Figure 17

1H NMR spectrum of 4-chlorobenzyl polyalta (11). (400 MHz, $CDCl_3$)



And in the 13C NMR spectrum, the new signals were presented with displacements of 134.88 ppm; 133.92 ppm; 129.39 ppm; 128.67 ppm and 65.42 ppm; attributed to their respective carbons.

5.9.5 Identification of butyl polyalto (12)

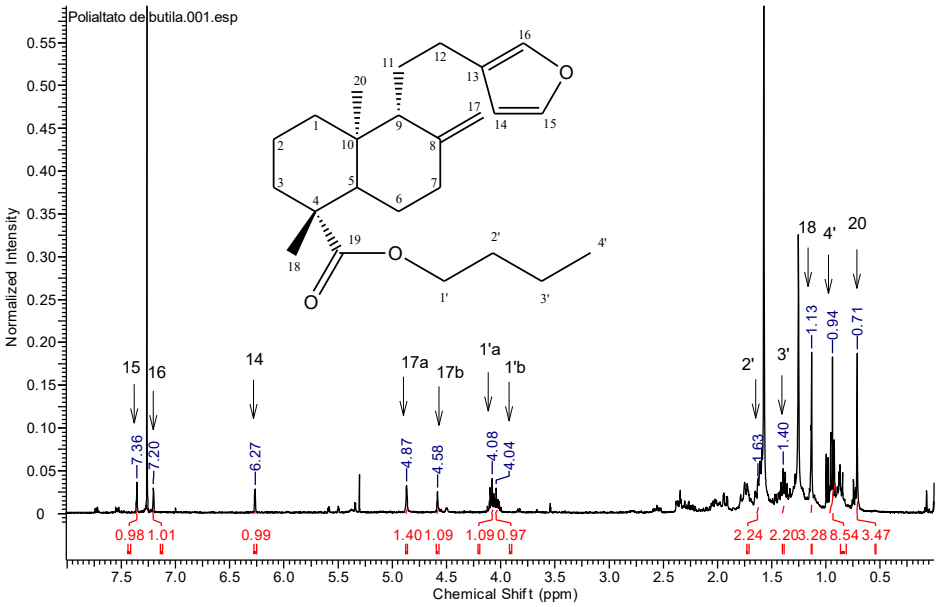
Once again, it was proven that one of the products initially proposed had been obtained. Their NMR spectra of 1H (Figure 18) and 13C were used in the attribution. A comparison was made with the 1H (Figure 5) and 13C spectrums of the source material (1).

Two new signals (double triplets) at 4.04 and 4.08 ppm, with integral to one hydrogen each, were assigned to CH2 (two different hydrogens), 1' position in the structure, directly connected to the oxygen of the ester formed, and the displacement of this signal was explained.

Another easily attributed signal was found at 0.94 ppm, referring to the methyl of the ester chain, defined as a triplet, which was already expected to show a CH2 as a neighbor. In addition, it was noted the intensification of signals in the region between 1.3 - 2.0 ppm attributed to the other signals of CH2 of the ester, with displacements of 1.63 ppm (CH2 bound to CH2 ester) and 1.40 ppm (CH2 bound to methyl).

Figure 18

1H NMR spectrum of butyl polyaltate (12). (400 MHz, $CDCl_3$)



The presence of these new signals was verified when the 13C NMR spectrum was analyzed , which brought four additional signals (64.39 ppm; 30.65 ppm; 19.21 ppm; 13.70 ppm), in addition to the signals of the starting material. Therefore, the structure of another derivative initially proposed, attributed to their respective carbons, was confirmed.

5.10 CYTOTOXICITY TEST RESULTS

For the presentation of the results of the biological tests, (Figures 19 and 20), all the substances tested with their codes are shown.

Figure 19

Polyaltic acid (1) and its derivatives with the test codes

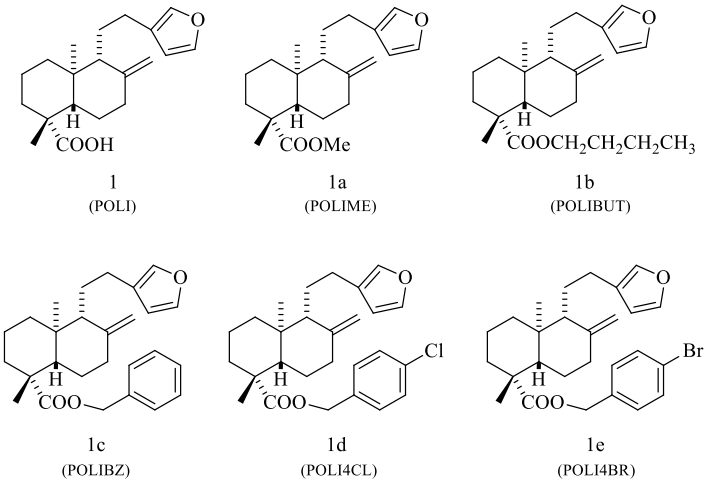
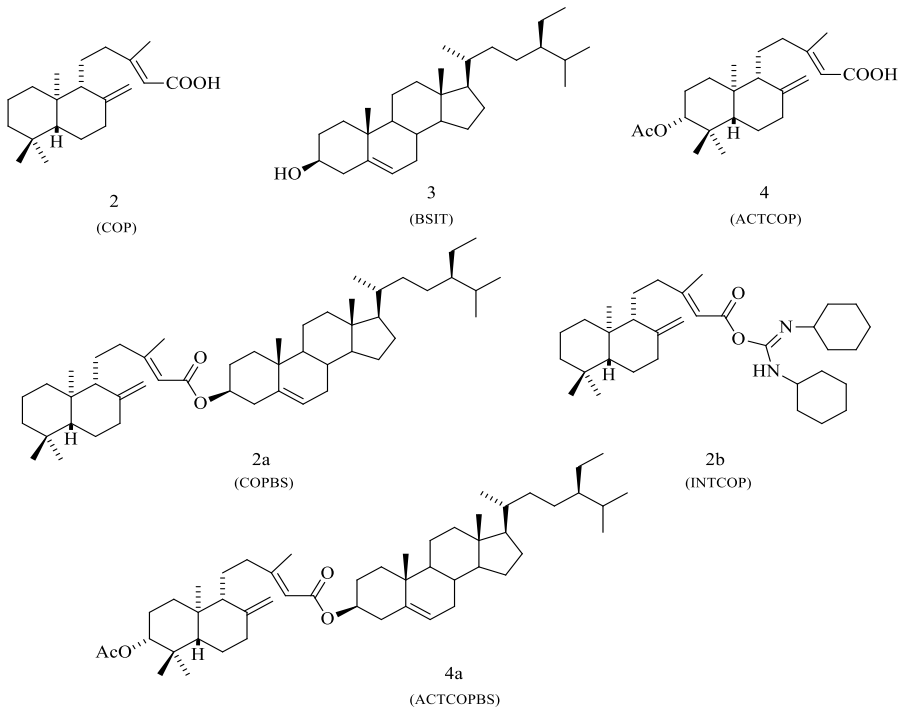


Figure 29

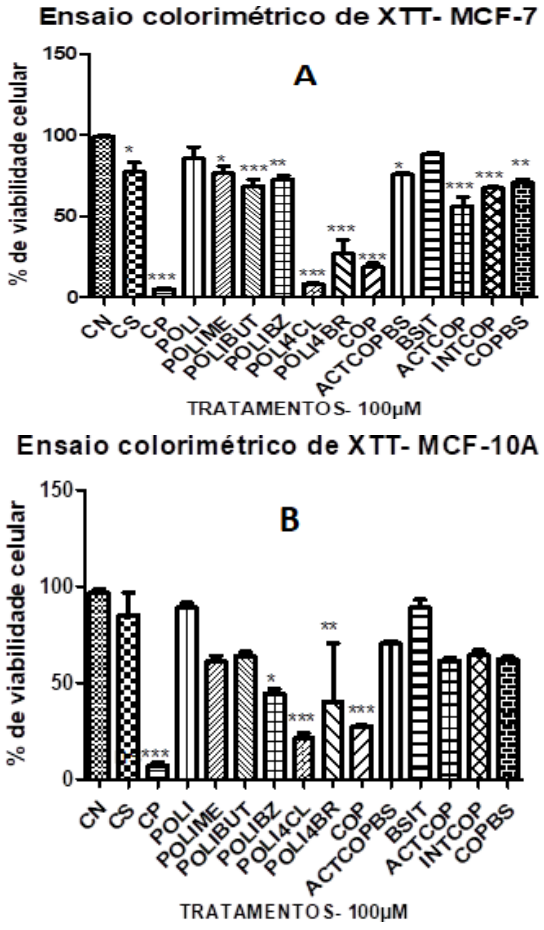
Copalic acid (2), □-sitosterol (3), 3-acetoxy-copalic acid (4) and their derivatives with assay codes



The twelve substances were submitted to the cytotoxicity test and the results were organized in the form of cell viability, being shown in the graphs below (figure 19). Initially, the results obtained against a tumor cell line of breast adenocarcinoma (MCF-7), (Figure 21 A), and then against normal cells of the human mammary gland (MCF-10A), (Figure 21 B), are shown.

Figure 21

*Percentage of cell viability of the following substances: POLY (1), POLIME (1a), POLIBUT (1b), POLIBZ (1c), POLI4CL (1d), POLI4BR (1e), COP (3), ACTCOPBS (4a), BSIT (2), ACTCOP (4), INTCOP (3b) and COPBS (3a), after 24 hours of treatment against MCF-7 (A) and MCF-10A (B) cell lines. NC: negative control; CS: solvent control (DMSO 1%); NC: positive control (DMSO 10%). * P < 0.05. **p < 0.01. P < 0.01 in relation to the NC*



The results of the polyaltic acid group and its derivatives can be evaluated as very interesting. Initially, it is perceived that there are two results that can be classified as promising, due to their activities against tumor cells, which are the results of halogenated substances. Both have a significantly more intense cytotoxicity than their

precursor, polyaltic acid (1), showing that the transformation performed considerably increased this activity. In addition, substance 1d showed toxicity similar to the positive control, an activity parameter for the assay, which places it as really promising. It should also be considered that, for this substance, the cytotoxicity against normal cells (MCF-10A) is clearly lower, showing a certain degree of selectivity, which is also very interesting when it comes to toxicity. It can also be highlighted that, for normal cells, substance 1d is considerably less toxic than the positive control. It can be stated that this is the best result of the present work, in relation to the search for active compounds.

For substance 1e, the toxicity is lower than that of substance 1d, but still of promising value. In addition, the experiment against normal cells suggests that we may have even greater selectivity than that shown in this experiment. This is because the standard deviation of this result in graph B is too high and out of the acceptable range, suggesting that there was a problem in the experiment. Certainly this evaluation has to be redone and, if a lower toxicity is obtained, the selectivity index would be even higher. Thus, the biological reassessment of 1e, which will be carried out soon, may lead to an even more interesting result.

Other than that, these results show very interesting evidence also according to what was obtained for the other derivatives. It can be clearly seen that polyaltic acid (1) is the substance with the lowest cytotoxicity of all (from 1 to 1e), proving that the structural modification performed improves the cytotoxicity of the molecules of this skeleton. Even if promising results had not been achieved, esterification would be a good research path to seek them.

The activity results achieved with the second group of substances in this work, the derivatives of copalic with □-sitosterol, on the other hand, show the opposite. That is, the cytotoxic activity of the precursor, copalic acid (2), is the highest of all, including the derivatives obtained. Thus, in relation to copalic acid (2), all the transformations performed decreased the activity. However, when we evaluate the situation from the point of view of having the □-

sitosterol (3) as a precursor, the evaluation is the opposite. B-sitosterol is the least cytotoxic of all, showing that, from the point of view of this precursor, the modifications improved the activity. More than that, the activity of the derivative is always in a value among the activities of each precursor. For example, compound 4a has its activity at a higher value than compound 3 and less than compound 4, its precursors. The same happens with derivative 2a, when compared to precursors 2

and 3. This shows that the technique of promoting fusion of active molecules can lead to very interesting results of activity. In this group, finally, the most promising substance is copalic acid itself, which also shows relative selectivity. This leads us to conclude that it is likely that another type of transformation with this substrate can lead to results of great interest.

6 CONCLUSIONS

The part of the production of esters from polyaltic acid to evaluate the cytotoxicity of the derivatives was the one that presented the greatest success, both in the yield of obtaining derivatives and in the biological results. The yield of the reactions was above 75% for three derivatives and below 30% for only two of them. The biological results led to the indication and two derivatives as promising, one of them close to the positive control.

The reactional study of the production of copalic acid esters and their acetylated analogue by the DCC/DMAP system using □-sitosterol as alcohol, presented more modest results. The use of the esterification methodology of these compounds already worked on in previous projects did not guarantee good yields of the tested reactions, but there was production of the desired derivatives, with the molecules of each of the two labdans linked to the structure of □-sitosterol (products 2a and 4a). In addition, a reaction intermediary was also isolated, increasing the amount of derivatives obtained. Biological assays of these compounds have not led to promising values, but they have provided interesting results. The activity of all derivatives was at an intermediate value, considering the molecules "condensed" in the process. This shows that this methodology is indeed promising for the search for compounds with different activities and toxicities.

Thus, the present work can be considered as an important contribution to the research of the group in which it was developed.

REFERENCES

Abrão, F., Costa, L. D., Alves, J. M., Siqueira, T. S., de Souza, T. B., de Almeida, G. C., ... & de Carvalho, J. E. (2018). Antibacterial effect of Copaifera duckei Dwyer oleoresin and its main diterpenes against oral pathogens and their cytotoxic effect. Frontiers in Microbiology, 9, 1–11. https://doi.org/10.3389/fmicb.2018.00230

Aguiar, G. de P. (2019). Estudo reacional com diterpenos naturais isolados de Copaifera langsdorffii e avaliação da variação do potencial leishmanicida dos derivados obtidos [Unpublished master's thesis].

Ambrosio, S. R., Tirapelli, C. R., da Costa, F. B., & de Oliveira, A. M. (2006). Kaurane and pimarane-type diterpenes from the Viguiera species inhibit vascular smooth muscle contractility. Life Sciences, 79(10), 925–933. https://doi.org/10.1016/j.lfs.2006.05.015

Arnesano, F., Nardella, M. I., & Natile, G. (2018). Platinum drugs, copper transporters and copper chelators. Coordination Chemistry Reviews, 374, 254–260. https://doi.org/10.1016/j.ccr.2018.07.003

Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. Life Sciences, 78(5), 431–441. https://doi.org/10.1016/j.lfs.2005.09.012

Barreiro, E. J. (2002). Estratégia de simplificação molecular no planejamento racional de fármacos: A descoberta de novo agente cardioativo. Química Nova, 25(6B), 1172–1180. https://doi.org/10.1590/S0100-40422002000700018

Barreiro, E. J., & Bolzani, V. da S. (2009). Biodiversidade: Fonte potencial para a descoberta de fármacos. Química Nova, 32(3), 679–688. https://doi.org/10.1590/S0100-40422009000300012

Boeck, P., Leal, P. C., Yunes, R. A., & Cechinel Filho, V. (2005). Antifungal activity and studies on mode of action of novel xanthoxyline-derived chalcones. Archiv der Pharmazie, 338(2–3), 87–95. https://doi.org/10.1002/ardp.200400913

Borges, C. H. G., da Silva, M. L. A., Viana, G. S. B., & de Almeida, R. A. (2016). Copaifera duckei oleoresin and its main nonvolatile terpenes: In vitro schistosomicidal properties. Chemistry and Biodiversity, 13(10), 1348–1356. https://doi.org/10.1002/cbdv.201600014

Bozov, P. I., & Georgieva, Y. P. (2017). Antifeedant activity of neo-clerodane diterpenoids from Scutellaria altissima against Colorado potato beetle larvae. Natural Product Communications, 12(3), 327–328. https://doi.org/10.1177/1934578X1701200303

Carreras, C. R., Rossomando, P. C., & Giordano, O. S. (1998). Ent-labdanes in Eupatorium buniifolium. Phytochemistry, 48(6), 1031–1034. https://doi.org/10.1016/S0031-9422(97)00918-2

Cascon, V., & Gilbert, B. (2000). Characterization of the chemical composition of oleoresins of Copaifera guianensis Desf., Copaifera duckei Dwyer and Copaifera multijuga Hayne. Phytochemistry, 55(7), 773–778. https://doi.org/10.1016/S0031-9422(00)00284-3

Copetti, D. (2016). Estudo da casca da raiz da espécie Maytenus ilicifolia Mart. ex Reissek, modificação estrutural do composto B-sitosterol e análises das atividades antimicrobianas [Unpublished master's thesis].

Cui, J., Huang, L., & Fan, J. (2008). A facile and efficient synthesis of some (6E)-hydroximino-4-en-3-one steroids, steroidal oximes from Cinachyrella spp. sponges. Steroids, 73(3), 252–256. https://doi.org/10.1016/j.steroids.2007.10.005

Drozdov, F. V., Gamaley, I. A., & Novikova, T. I. (2007). Cytotoxic derivatives of (22R,23R)-22,23-dihydroxystigmastane. Bioorganicheskaia Khimiia, 33(3), 349–356. https://doi.org/10.1134/S1068162007030136

Eifer-Lima, V. L., Albuquerque, E., & Lima, M. E. (2010). Highlights in the solid-phase organic synthesis of natural products and analogues. Journal of the Brazilian Chemical Society, 21(8), 1401–1423. https://doi.org/10.1590/S0103-50532010000800003

Farshori, N. N., Banday, M. R., Ahmad, A., Khan, A. U., & Rauf, A. (2010). DCC/DMAP mediated esterification of hydroxy and non-hydroxy olefinic fatty acids with β-sitosterol: In vitro antimicrobial activity. Chinese Chemical Letters, 21(6), 646–650. https://doi.org/10.1016/j.cclet.2010.01.003

Filho, V. C., & Yunes, R. A. (1998). Estrategies for obtaining pharmacologically active compounds from medicinal plants. Concepts about structural modification for improve the activity. Química Nova, 21(1), 99–105. https://doi.org/10.1590/S0100-40421998000100016

Geris, R., Silva, I. G., Silva, H. H. G., & Furtado, N. A. J. C. (2008). Diterpenoids from Copaifera reticulata Ducke with larvicidal activity against Aedes aegypti (L.) (Diptera, Culicidae). Revista do Instituto de Medicina Tropical de São Paulo, 50(1), 25–28. https://doi.org/10.1590/S0036-46652008000100006

Jiang, J. H., He, Z., Peng, Y. L., Jin, W. D., Wang, Z., & Han, R. W. (2015). Phoenixin-14 enhances memory and mitigates memory impairment induced by Aβ1-42 and scopolamine in mice. Brain Research, 1629, 298–308. https://doi.org/10.1016/j.brainres.2015.10.018

Leandro, L. M., Vargas, F. de S., Barbosa, P. C. S., Neves, J. K. O., Silva, J. A., & Veiga-Junior, V. F. (2012). Chemistry and biological activities of terpenoids from copaiba (Copaifera spp.) oleoresins. Molecules, 17(4), 3866–3889. https://doi.org/10.3390/molecules17043866

Mizuno, C. S., Souza, A. B., Tekwani, B. L., Ambrosio, S. R., & Veneziani, R. C. S. (2015). Synthesis and biological evaluation of polyalthic acid derivatives for the treatment of neglected diseases. Bioorganic & Medicinal Chemistry Letters, 25(23), 5529–5531. https://doi.org/10.1016/j.bmcl.2015.10.062

Montanari, C. A., & Bolzani, V. da S. (2001). Planejamento racional de fármacos baseado em produtos naturais. Química Nova, 24(1), 105–111. https://doi.org/10.1590/S0100-40422001000100018

Moore, N., Hamza, N., Berke, B., & Umar, S. (2017). News from Tartary: An ethnopharmacological approach to drug and therapeutic discovery. British Journal of Clinical Pharmacology, 83(1), 33–37. https://doi.org/10.1111/bcp.13094

Neto, J. de S. L., Gramosa, N. V., & Silveira, E. R. (2008). Constituintes químicos dos frutos de Copaifera langsdorffii Desf. Química Nova, 31(5), 1078–1080. https://doi.org/10.1590/S0100-40422008000500025

Nie, S., Liu, X., Wu, Z., Zhang, M., & Li, J. (2015). Removal of hexenuronic acid by xylanase to reduce adsorbable organic halides formation in chlorine dioxide bleaching of bagasse pulp. Bioresource Technology, 196, 413–417. https://doi.org/10.1016/j.biortech.2015.07.115

Nofziger, H., Currens, M. J., Seniff, D., & Boyde, M. R. (1988). Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Research, 48(1), 4827–4833.

Nogueiras, C., Martinez, A., & Perez, C. (2010). Contribution to the phytochemical study and biological activity of plants of Cuban flora. Biotecnología Aplicada, 27(4), 315–318.

Oliveira, A. B. (2007). Microencapsulamento de estigmasterol proveniente de Musa paradisiaca L., Musaceae [Unpublished master's thesis].

Oliveira, C. A., Santos, L. S., & de Almeida, M. R. (2014). Synthesis of fruity flavor esters: An experiment for undergraduate courses within one of the principles of green chemistry. Revista Virtual de Química, 6(1), 152–167. https://doi.org/10.5935/1984-6835.20140016

Pieri, F. A., Mussi, M. C., & Moreira, M. A. S. (2009). Óleo de copaíba (Copaifera sp.): Histórico, extração, aplicações industriais e propriedades medicinais. Revista Brasileira de Plantas Medicinais, 11(4), 465–472. https://doi.org/10.1590/S1516-05722009000400016

Ribeiro, L. A. A., Almeida, R. N., & de Sousa, D. P. (2007). O Ácido (8)17,12E,14-labdatrieno-18-óico (labdano302), diterpeno tipo labdano isolado de Xylopia langsdorffiana St. Hil. & Tul. (Annonaceae) relaxa a traquéia isolada de cobaia. Brazilian Journal of Pharmacognosy, 17(2), 197–203. https://doi.org/10.1590/S0102-695X2007000200010

Santos, R. A. F. (2010). Avaliação das propriedades biológicas dos derivados sintéticos do B-sitosterol e triterpenos [Unpublished master's thesis].

Sheikh, M. C., Takagi, S., & Ogasawara, H. (2010). Mechanistic studies of DCC/HOBt-mediated reaction of 3-phenylpropionic acid with benzyl alcohol and studies on the reactivities of "active ester" and the related derivatives with nucleophiles. Tetrahedron, 66(36), 7272–7278. https://doi.org/10.1016/j.tet.2010.07.011

Souza, A. B., de Souza, M. G. M., Fernandes, M. A., Santos, R. A. F., & de Almeida, M. C. (2011). Antimicrobial activity of terpenoids from Copaifera langsdorffii Desf. against cariogenic bacteria. Phytotherapy Research, 25(2), 215–220. https://doi.org/10.1002/ptr.3244

Strum, S. B., & Faloon, W. (2005). Beta-sitosterol and the aging prostate gland. Life Extension Magazine, June, 1–4. https://www.lifeextension.com/magazine/2005/6/report_prostate/Page-01

Tappin, M. R. R., Pereira, J. F. G., & Lemos, T. L. G. (2004). Análise química quantitativa para a padronização do óleo de copaíba por cromatografia em fase gasosa de alta resolução. Química Nova, 27(2), 236–240. https://doi.org/10.1590/S0100-40422004000200012

Vargas, F. de S., Almeida, P. D. O. de, & Aranha, E. S. P. (2015). Biological activities and cytotoxicity of diterpenes from Copaifera spp. oleoresins. Molecules, 20(4), 6194–6210. https://doi.org/10.3390/molecules20046194

Veiga, V. F., Rosas, E. C., & Carvalho, M. V. (2007). Chemical composition and anti-inflammatory activity of copaiba oils from Copaifera cearensis Huber ex Ducke, Copaifera reticulata Ducke and Copaifera multijuga Hayne—A comparative study. Journal of Ethnopharmacology, 112(2), 248–254. https://doi.org/10.1016/j.jep.2007.03.005

Veiga, V. F., & Pinto, A. C. (2002). The Copaifera L. genus. Química Nova, 25(2), 273–286. https://doi.org/10.1590/S0100-40422002000200016