University of Salamanca

Faculty of Pharmacy

Department of Pharmaceutical Science: Pharmaceutical Chemistry

# Diploma thesis

## Isolation and semi-synthesis of germacranolide derivatives: onopordopicrin

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**Declaration**

I declare that this thesis is my original work. All literature and information sources used are properly cited. This work has not been used to obtain equal or any other degree.

Hradec Králové, 2023 Eliška Cejnarová

**Acknowledgment**

My sincerest gratitude belongs to my supervisor prof. Esther del Olmo, for her valuable advice, all her time and endless patience as well as her support, kindness, and unforgettable experience to work with her in the laboratory. I´m very grateful for this opportunity to work with that special people on such an interesting topic and I give my thanks to all persons in the laboratory, who were every time kind, helpful and supportive to me.

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**ABSTRACT**

This work is focused on onopordopicrin (ONO) as a potent molecule with antiprotozoal activity. ONO is a sesquiterpene lactone of germacranolide structure. Sesquiterpene lactones are typical secondary metabolites of family *Asteraceae*. As a source of ONO *Centaurea ornata* Willd. (*Asteraceae*) was used. It is a thistle-like endemic plant of the Iberian Peninsula. The plant was collected in July 2022, in Zamora region.

First, an ether extract from the aerial flowering part of the plant was obtained (0.26 % of the dried plant weight). The crude plant extract was dissolved in methanolic solution and fractionated by liquid/liquid separation with hexane. Then the methanolic part was concentrated, dissolved in water and fractionated with solvents of increasing polarity (chloroform, ethyl acetate and n-butanol). The chloroform fraction containing ONO was purified by column chromatography to obtain the major compound ONO, which represented 54.8 % of crude extract.

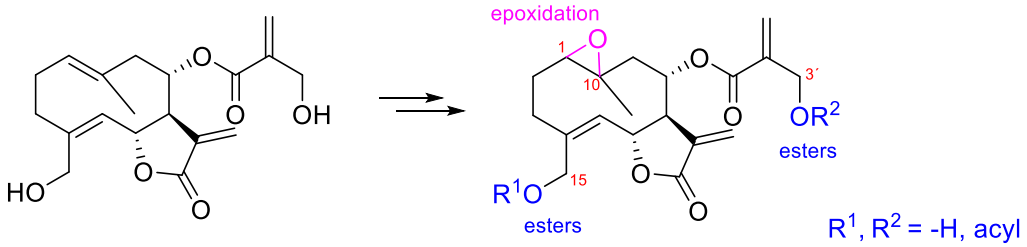



**Figure 1.** Modifications of onopordopicrin

The aim of this work was the synthesis of novel derivatives of ONO – esters and epoxides (Figure 1). The modifications were proposed to enhance pharmacological properties and selectivity of the molecule for an antiprotozoal treatment. For the esterification of ONO, five different methods were used: Steglich esterification, Yamaguchi esterification, Mukaiyama esterification, and treatment with acyl chlorides and anhydrides. The most convenient methods for the preparation of esters were Mukaiyama esterification and treatment with acyl chlorides and anhydrides. The second synthetic part was focused on the epoxidation of the double bond (1,10) of ONO, followed by treatment with Lewis acids. The epoxidation was performed via reaction of ONO with *meta*-chloroperoxybenzoic acid (*m*CPBA) in order to gain the 1,10-epoxide. The epoxide (1,10) is more reactive and can provide the closure of the cycle in positions 5,10 in presence of Lewis acid.

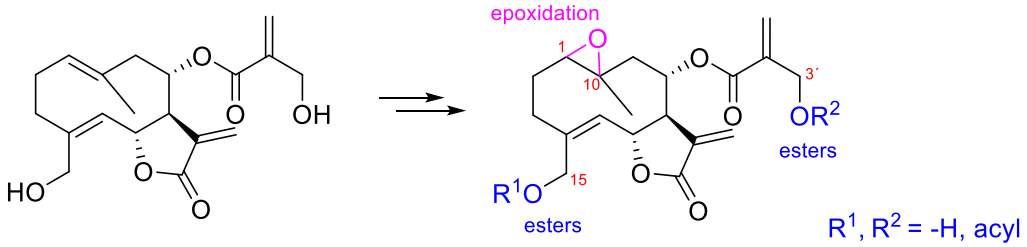
The obtained compounds were sent to bioassays to set the antiprotozoal activity against *Leishmania donovani*, *Trypanosoma cruzi* and *Plasmodium falciparum*.

**Key words**: *Centaurea ornata* Willd., onopordopicrin, antiprotozoal activity.

**ABSTRAKT**

Tato práce je zaměřena na onopordopikrin (ONO) jakožto slibnou molekulu s antiprotozoální aktivitou. ONO je seskviterpenový lakton germakranolidové struktury. Seskviterpenové laktony jsou typické sekundární metabolity čeledi *Asteraceae.* Jako zdroj ONO byla použita *Centaurea ornata* Willd. (*Asteraceae*). Jedná se o endemickou rostlinu bodlákovitého vzhledu z Pyrenejského poloostrova. Rostlina byla sbírána v červenci 2022 v oblasti Zamora.

Nejprve byl získán etherový extrakt z nadzemní části kvetoucí rostliny (0,26 % hmotnosti sušené rostliny). Surový rostlinný extrakt byl rozpuštěn v methanolickém roztoku a rozdělen vytřepáváním s hexanem. Poté byla methanolická část odpařena, rozpuštěna ve vodě a dále byla dělena pomocí vytřepávání s rozpouštědly o vzrůstající polaritě (chloroform, ethylacetát a n-butanol). Chloroformová frakce obsahující ONO byla přečištěna sloupcovou chromatografií, čímž byla získána hlavní sloučenina ONO, která představovala 54,8 % surového extraktu.




**Obrázek 1.** Modifikace onopordopikrinu

Cílem této práce byla syntéza nových derivátů ONO – esterů a epoxidů (Obrázek 1). Modifikace molekuly byly navrženy pro zlepšení farmakologických vlastností a zvýšení selektivity molekuly pro antiprotozoální terapii. Pro esterifikaci ONO bylo použito pět různých metod: Steglichova esterifikace, Yamaguchiho esterifikace, Mukaiyamova esterifikace a reakce za působení acylchloridů a anhydridů. Nejvhodnějšími metodami pro přípravu esterů se ukázaly Mukaiyamova esterifikace a reakce s acylchloridy a anhydridy. Druhá část práce byla zaměřena na epoxidaci dvojné vazby (1,10) ONO s následným působením Lewisovými kyselinami. Epoxidace byla provedena reakcí ONO s *meta*-chlorperoxybenzoovou kyselinou (*m*CPBA) za účelem přípravy 1,10 epoxidu. Epoxid (1,10) je reaktivnější a může poskytnout uzavření cyklu v polohách 5,10 za působení Lewisovy kyseliny.

Získané sloučeniny byly zaslány na biologické testování pro stanovení antiprotozoální aktivity proti *Leishmania donovani*, *Trypanosoma cruzi* a *Plasmodium falciparum*.

**Klíčová slova**: *Centaurea ornata* Willd., onopordopikrin, antiprotozoální aktivita

**Content**

## LIST OF ABBREVIATIONS

| | |
|---|---|
| CDI | 1,1′-carbonyldiimidazole |
| DCM | dichloromethane |
| DMAP | 4-dimethylaminopyridine |
| DMC | dimethyl carbonate |
| DMF | dimethylformamide |
| EDC | 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride |
| EtOAc | ethyl acetate |
| Lut | 2,6-lutidine |
| *m*CPBA | *meta*-chloroperoxybenzoic acid |
| MeOH | methanol |
| MBA | 4-methylbenzoic acid |
| MOBA | 4-methoxybenzoic acid |
| MTBE | methyl *tert*-butylether |
| Muk | Mukaiyama´s reagent |
| n-BuOH | n-butanol |
| ONO | onopordopicrin |
| Pyr | pyridine |
| rt | room temperature |
| SAR | structure-activity relationship |
| STL´s | sesquiterpenic lactones |
| TEA | triethylamine |
| THF | tetrahydrofuran |
| TMS | tetramethylsilane |

# INTRODUCTION

## Genus *Centaurea*

The *Centaurea* genus belongs to the Asteraceae family and comprises a large group of thistle-like plants that includes more than 700 species.[1] These plant species are spread worldwide, although most of them are found in the Mediterranean region. Some of them are very frequent plants in various places, while others are endemic to their location, and some specimens are even in danger of extinction.[2] The diversity among species is very large, even in habitat and appearance. The color of the flowers is very wide, ranging from blue, to red, or yellow, or even white. Its application is also very diverse, some are invasive plants with a negative impact on agriculture, others are rich in nectar showing interest to beekeepers, or used for decorative purposes, and a group of them are used in traditional medicine for their healing properties.[3]

## *Centaurea ornata*

*Centaurea ornata* Willd. also known as "abrepuños", is an endemical plant of the Iberian Peninsula. It is a perennial and axonomorphic plant with a high degree of morphological variations, which are related to the level of ploidy. Thus, its stems reach 80 cm in height, the appendage of the bracts of the involucre ends in a thorn of about 5-24 mm and its corolla also varies in shades ranging from intense yellow to reddish. [4,5] (Figure 2)

**Figure 2.** *C. ornata* aerial part.

As it has been mentioned, this species is located in both Spain and Portugal, practically throughout the entire peninsula, excluding northern coast by Cantabrian Sea. Its typical habitat is land with little humidity, rather arid, on the edges of roads and highways, in latitudes that go from sea level up to 2000 m of altitude.

## *Centaurea ornata* in traditional medicine

The roots of *Centaurea ornata* are commonly called "cardo de arzolla". The infusion of the root is traditionally used in the Extremadura region for gastric disorders, such as

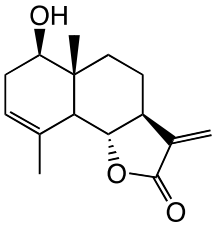
pain and ulcers, and as a laxative and cholagogue. It is also used topically to treat wounds, bites, gangrene, skin cancer, hemorrhoids, inflammation, etc.[3]

However, there is morphological similarity between *Centaurea ornata* and *Atractylis gummifera.* These two plants together with similar name of the drug (the root) - in both cases as "cardo de arzolla"- lead to a frequent confusion by its consumers, and therefore to the intoxication when ingesting the infusion obtained from the roots of *A. gummifera.* An expert with the adequate knowledge for their differentiation is required. This intoxication causes renal, hepatic, or multi-organ failure, in severe cases, could cause the death of the patient.

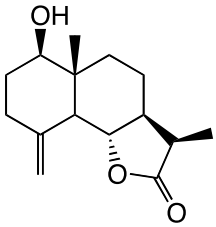
Nevertheless, despite its use in the traditional medicine of Extremadura, this plant is rarely used in the rest of Spain, where it is only used as a diuretic.[6, 7]

**Secondary metabolites isolated from *C. ornate* Wild.**

There are few studies on the phytochemical composition of *Centaurea ornate* Willd. In a work published by Navarro et al. in 1990[8] some sesquiterpenic lactones with eudesmanolide skeleton (santamarine [**1**] and 11-*epi*-dihydroreinosine [**2**]) (Figure 3) and guaianolides related to grosshemin (grosshemin α,β-dihydroxy-isobutyrate [**3**] and 3α-dihydro-4(15)-dehydrogrosshemin α,β-dihydroxy-isobutyrate [**4**]) (Figure 4) were described. Additionally, they described some phenolics compounds: vanillin [**5**], 5-methyl-8-hydroxycoumarin [**6**] and flavonoid salvigenin [**7**] (Figure 5).

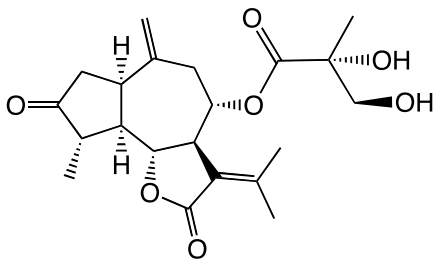



santamarine [**1**]

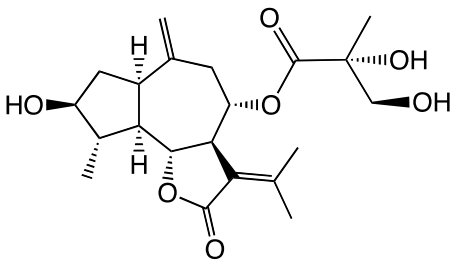



11-*epi*-dihydroreinosine [**2**]

**Figure 3.** Structures of eudesmanolides **1** and **2**



grosshemin
α,β-dihydroxy-isobutyrate [**3**]



3α-dihydro-4(15)-dehydrogrosshemin
α,β-dihydroxy-isobutyrate [**4**]

**Figure 4.** Structures of guaianolides **3** and **4**

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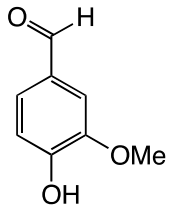
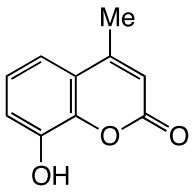
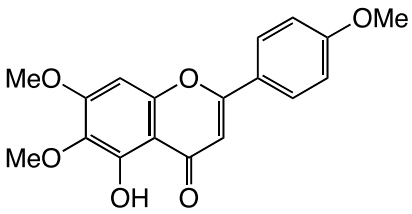


vanillin [**5**]



5-metyl-8-
hydroxycoumarin [**6**]



salvigenin [**7**]

**Figure 5.** Structures of phenolics compounds from *C. ornata*

**Onopordopicrin**

Onopordopicrin (ONO) is a sesquiterpene lactone (STL) with germacranolide skeleton.

The molecule was first isolated from *Onopordum spp*.[9] In last decades, ONO has been also isolated from other plants from the *Asteraceae* family, such as *Arctium lappa,*[10] *A. nemorosum,*[11] *Centaurea tweediei,*[12] *Onopordum acanthium,*[13] *O. Illyricum,*[14] etc. ONO has been tested in several activities, e.g., antibacterial,[15,16] antiviral,[17] antifungal,[18] anti-inflammatory,[19,20] antiprotozoal,[21-23] antioxidative,[24] antiproliferative[25,26] and cytotoxic[27]. The range of activities is quite wide due to molecule´s structure, which is highly functionalized with an α,β-unsaturated γ-lactone at positions 6,7, an α,β-unsaturated ester at position 8, two allylic alcohols at positions 15 and 3´ and two double bonds at positions 1,10 and 4,5 (Figure 6).

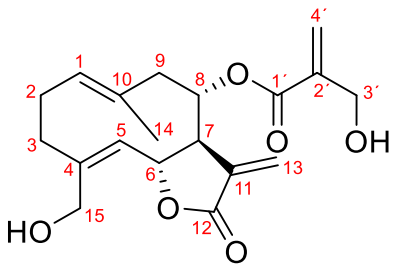



**Figure 6.** Structure of onopordopicrin

As for that, the molecule is less stable and more sensitive to working conditions, e.g. temperature, humidity, pH. It brings some specific requirements for storage as well as reaction methods: ONO should be stored under inert atmosphere in dark and cold; the usage of mild conditions and reagents is preferred. The work up of ONO derivatives can be held in pH ranging 3-8. As it was mentioned above, ONO has displayed wide spectrum of activities, however the highest potency is to become a novel antiprotozoal drug. There are some studies of antiprotozoal activity of natural STL´s[28,29], as well as of ONO, against the protozoa causing severe diseases such as *Plasmodium falciparum* (malaria), *Leishmania donovani* (leishmaniasis) or *Trypanosoma cruzi* (Chagas disease). The protozoa are difficult to treat due to their complicated life-cycle comprising of many development stages and intermediate hosts, so there are many steps and mechanisms that could be inhibited. For the antiprotozoal activity, an α-methylene-γ-lactone has been considered as a crucial part of molecule. The presence of 2-(hydroxymethyl)acrylate in the molecule is advantageous[30]. Also, the presence of *trans-trans* conformation in STL´s with closed cycle and substitution with cyclic or aromatic substituents enhanced the pharmacological properties[31].

**Types of esterification**

The esterification is a chemical reaction, where an ester is formed from an alcohol and an acid (or its derivative). This reaction normally works in acidic conditions, but there are also different methods using coupling agents or more reactive derivatives, that allow the reaction to proceed. Five methods used in this work will be mentioned below.

**The Steglich esterification**[32-34] is standardly used for preparation of esters in natural products. This method is held in mild conditions, ambient temperatures and neutral pH and can afford even sterically difficult reactions to proceed. In this type of reaction,

the coupling agents are used, e. g. *N,N*-dicyclohexylcarbodiimide (DCC), *N,N*-diisopropylcarbodiimide (DIC), 1,1'-carbonyldiimidazole (CDI), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), 4-dimethylaminopyridine (DMAP). The coupling agent activates an acid, which leads to formation of reactive carboxylate, that can directly react with an alcohol or form a symmetric anhydride. The formation of the anhydride can provide the reaction with the alcohol to obtain desired ester.

Another **esterification** method is reaction **with acyl chlorides**[34,35]. Acyl chlorides are more reactive derivatives of carboxylic acids. This type of reaction is not that used in natural products, because there are some limitations. Firstly, standard process of preparation of acyl chloride leads to formation of hydrochloric acid, which could be problematic in case of acid-labile compounds. Secondly, acyl chlorides are less stable and incline to hydrolysis. Nevertheless, this type of reaction can provide esters of hardly reactive substances. Also, DMAP can be added to improve the yields.

**The Yamaguchi esterification**[36,37] provides esters via formation of mixed anhydride. In this reaction, the Yamaguchi reagent (2,4,6-trichlorobenzoyl chloride) reacts with the acid of interest to form mixed anhydride in presence of base (TEA). Then, there are two possible mechanisms of reaction: the first one leads to direct formation of ester, and the second one provides symmetric anhydride of the acid and then the formation of desired ester. As for this reaction, there is possibility to replace the coupling reagent with benzoyl chloride, *p*-toluoyl chloride, 2-methyl-6-nitrobenzoic anhydride (MNBA) and others. This type of reaction can be provided at room temperature but runs better at higher temperatures and leads to good yields.

Also, **treatment of alcohols with anhydrides**[35,38] provides esters. The anhydride in the reaction undergoes alcoholises resulting in one molecule of an ester and one molecule of an acid as a side product. Therefore, the reaction is held in presence of base (pyridine, TEA) to quench forming acid.

**The Mukaiyama esterification**[39,40] is a reaction of an alcohol, an acid and the Mukaiyama´s reagent (2-chloro-1-methylpyridinium iodide). The coupling reagent activates the acid to form an ester and as a side products 1-methylpyridin-2-one and an inorganic acid are formed. Thus, presence of base (e. g. TEA, pyridine, 2,6-lutidine) is required. Also, usage of catalytic amounts of DMAP might be advantageous.

**Types of epoxidations**

The epoxidation is a reaction, where double bond is converted to oxirane cycle (epoxide), using oxidative agents like oxygen, hydrogen peroxide, organic peracids, etc.

The most common method of epoxidation in natural products is epoxidation induced by *m*-chloroperoxybenzoic acid (*m*CPBA)[41,42]. This reaction is standardly held in un-aqueous solvent system (e.g., dichloromethane, tetrahydrofuran) and runs at low or room temperature.

Also, other oxidative reagents might be used to gain epoxides, but there are some limitations in natural products, because of their complex structure. Some of them might be too reactive to destroy the molecule, while others can provide desired products or another side products. As for the epoxidation of double bond on the sesquiterpenoid cycle only some reagents are convenient, e.g. *tert*-butyl hydroperoxide, vanadium triethoxide, vanadyl acetylacetonate[43,44].

## THE AIM OF THE WORK

The aim of this work is to obtain different derivatives of onopordopicrin by semi-synthesis in order to gain novel antiprotozoal drugs. The following partial tasks are established in this work:

1) Preparation and fractionation of *Centaurea ornata* Willd. extract.
2) Isolation and purification of onopordopicrin.
3) Synthesis of esters and epoxide derivatives.
4) Preparation of samples for biochemical evaluation and to establish relationship between the structure and the activity (SAR).

Firstly, an ether extract is prepared, which represents complex mixture of secondary metabolites like flavonoids, terpenoids, phytosterols and others. Separation of these substances is based on their different polarity via extraction with different solvents (hexane, chloroform, ethyl acetate, butanol, methanol, water).

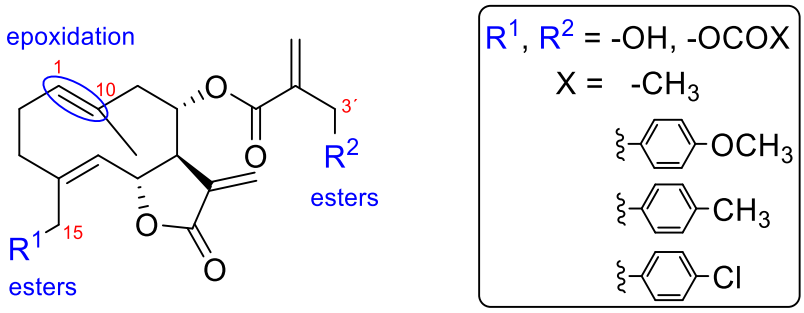



**Figure 7.** Modifications of onopordopicrin

Once ONO is isolated and purified, it is proposed to carry out two types of modifications (Figure 7). Firstly, the formation of esters on the hydroxy groups to obtain the monoesters and/or diesters to be performed. To obtain the esters, and due to the presence of a lactone and an ester in the molecule, it is decided to apply a method under mild conditions such as the Steglich's. Also, other methods of esterification in natural products are explored in order to find more convenient method for the preparation of esters. Secondly, the epoxidation of the double bond in position 1,10 is performed to subsequently treat the obtained epoxide with different Lewis acids. The epoxidation is performed with *meta*-chloroperoxybenzoic acid (*m*CPBA). The obtained compounds are identified and sent for evaluation of their antiprotozoal activity to determine structure-activity relationship and define the lead structure.

# MATERIALS AND METHODS

## INSTRUMENTATION

### INFRARED SPECTROSCOPY (IR)

The spectra were obtained at Perkin Elmer FT-IR System BX, as a solid crystal (sample including KBr) or oil on NaCl crystal. The values of frequency of absorption are expressed in $cm^{-1}$.

### HIGH RESOLUTION MASS SPECTROSCOPY (HR-MS)

The spectra were made by Themo Orbitrap Qexactive Focus. The ionization was carried by mediate electrospray 5500 V and the time of flight were detected (ESI-Q-TOF). The ions were characterized by *m/z*.

### GAS CHROMATGRAPHY WITH MASS SPECTROSCOPY DETECTION (GC-MS)

The spectra were made by Agilent 7820A GC System with a column Agilent SMS (30 m long; 0,25 mm in diameter; filled with 5% phenyl-methyl silica) followed by mass spectrometer Agilent Technologies 5977B. The ionization was realized under electronic impact (70 eV). The ions were characterized by *m/z*.

### $^{1}$H NMR AND $^{13}$C NMR

The spectra were obtained by spectrometers Varian or Bruker 400 MHz, (400 MHz for $^{1}$H, 100 MHz for $^{13}$C) in $CDCl_3$ or $CD_3OD$ and TMS as internal reference. The values of chemical displacement ($\delta$) were expressed in ppm and the coupling constants (*J*) in Hz.

### COLUMN CHROMATOGRAPHY

Silica gel Merck 60 (0,063-0,2 nm) was used, in a proportion of 40 to 60 g of silica for 1 g of substance to be purified.

### THIN LAYER CHROMATOGRAPHY (TLC)

Prefabricated Merck $SiF_{254}$ silica gel with $UV_{254}$ fluorescence indicator 0.25 mm thick on aluminium support were used. The plates were visualized with a UV lamp with light of $\lambda$ = 254 and 336 nm, and then revealed with a 10% solution of phosphomolybdic acid in ethanol, with subsequent heating (100-110 °C) for five minutes, or in a chamber with $I_2$.

## EXPERIMENTAL SECTION

### Preparation of the extract. Fractionation. Purification of the major compound.

The flowering aerial part of *C. ornata* Willd. was collected in July 2022 in the Zamora province, Castilla y León, Spain. The drug was identified by Dr. Rico and one sample of the drug has been kept in the herbarium of the University of Salamanca (SALA 173402).

The plant was dried in the dark to give 3.36 kg, triturated and extracted with methyl *tert*-butyl ether (MTBE) (2 × 5 L) at room temperature (2 h). After filtering and evaporating the solvent under reduced pressure, a crude extract was obtained, which made 8.89 g (0.26 % of dry plant). Then, the crude extract was dissolved in MeOH/$H_2O$ (500 mL, 9:1) and washed with hexane (7 × 250 mL). The hexane fraction was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. The aqueous-methanolic fraction was concentrated and then, dissolved in $H_2O$ and washed with $CHCl_3$ (7 × 250 mL). The chloroform fraction was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure. The aqueous part was washed with EtOAc (5 × 250 mL), followed by n-BuOH (1 × 250 mL). Both organic fractions were dried over anhydrous $Na_2SO_4$ and the solvents were removed under reduced pressure. The $^1$H NMR and $^{13}$C NMR spectra of all fractions were made and it was identified that ONO was present mainly in the chloroform fraction. The chloroform fraction was purified by column chromatography, using eluents of increasing polarity: hexane/EtOAc 9:4; $CHCl_3$; $CHCl_3$/MeOH 9:1; $CHCl_3$/MeOH 1:1 and MeOH. The $CHCl_3$/MeOH 9:1 fraction containing ONO was dried over anhydrous $Na_2SO_4$ and the solvent was removed under reduced pressure, obtaining 4.87 g of ONO (54.8 % of crude extract) (Figure 8).

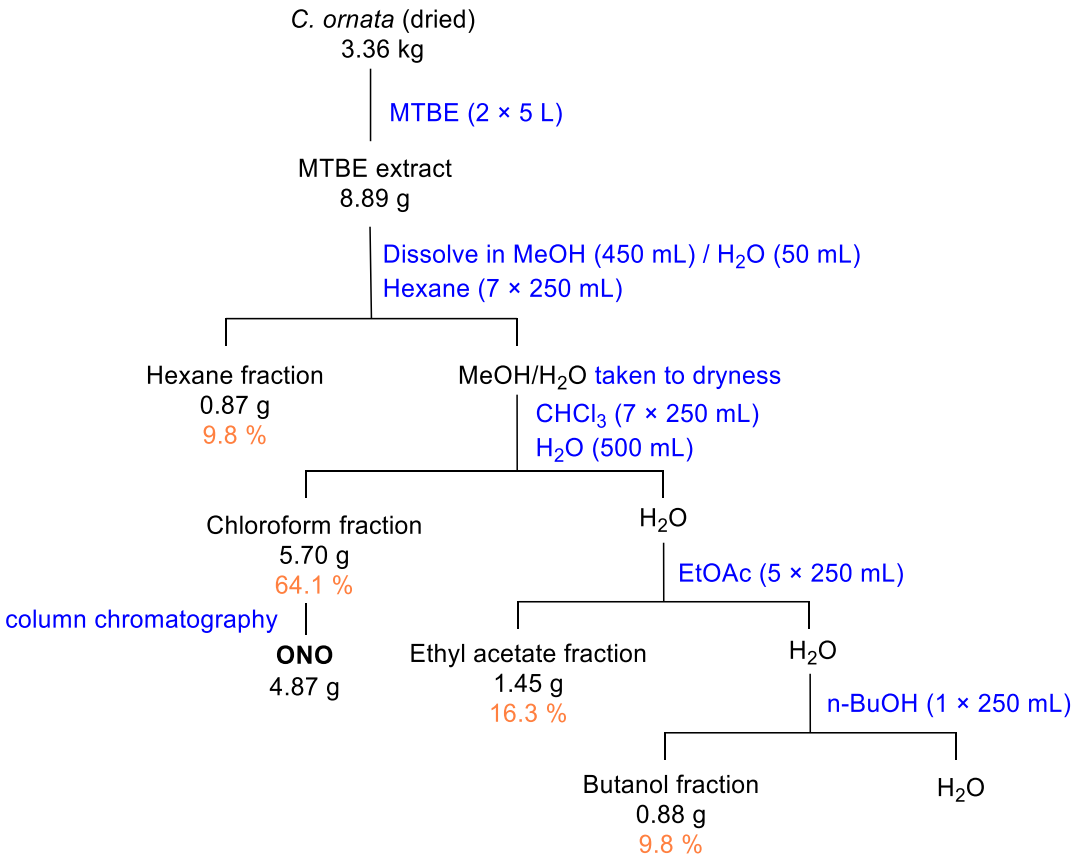



**Figure 8.** Scheme of extract fractionation and isolation of onopordopicrin.

**Synthesis of esters**

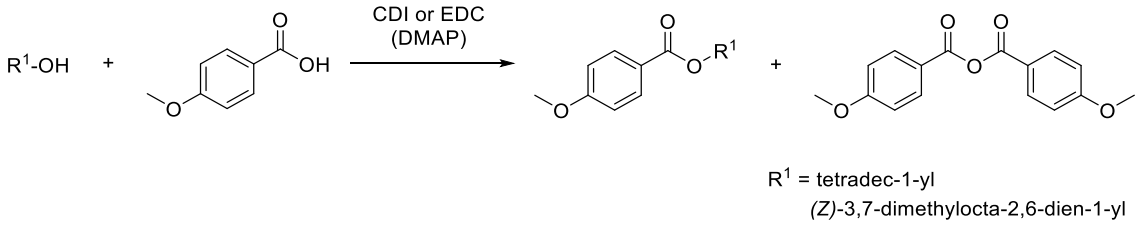
***a. Steglich esterification***

In order to standardize the reaction protocol, the process was first applied to aliphatic alcohol (1-tetradecanol) and 4-methoxybenzoic acid (MOBA). Then, the type of coupling agent (CDI, EDC), its relationship with respect to the reagents, type and amount of solvent in the reaction, the proportion between alcohol and acid, as well as the convenience of using DMAP, were analysed. The optimized conditions were also applied to nerol and MOBA, see table 1.

*General esterification procedure under Steglich conditions*

To a solution of 4-methoxybenzoic acid in the selected solvent (table 1), the appropriate coupling reagent was added at 0 °C under argon atmosphere, and the mixture was stirred for 1 h at room temperature (rt) to activate the acid. Then, a solution of the corresponding alcohol in chosen solvent was added dropwise.

The mixture was stirred for 1-24 hours at rt under argon atmosphere. The reaction was diluted with ice water and concentrated under reduced pressure. The resulting crude mixture was diluted with $CHCl_3$ (10 mL), and the organic layer washed with 1M HCl (3 × 10 mL), and then with brine until neutral pH. The organic layer was dried over anhydrous $Na_2SO_4$, concentrated under reduced pressure, and the crude mixture was purified by column chromatography [$CHCl_3$/MeOH (98:2)]. The formation of ester was observed as well as unreacted starting material and anhydride as a side product were isolated.




**Scheme 1.** Scheme of alcohol esterification under Steglich conditions.

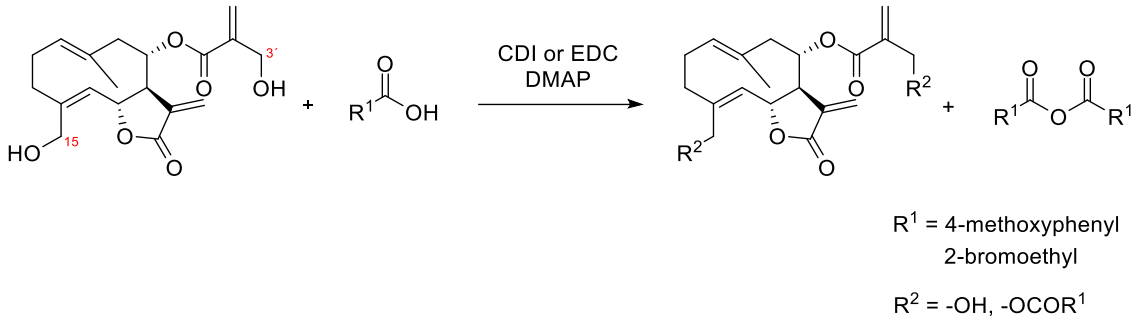
**Table 1. Optimalization of the esterification process under Steglich conditions**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Coupling (equiv.) | Time (h) | Ester | Side product (anhydride) | Starting material (alcohol / acid) |
|---|---|---|---|---|---|---|---|---|
| **1** | 14C-OH 206 (0.96) | MOBA (1.0) | THF (2.0) | CDI (1.0) | 24 | - | + | + / + |
| **2** | 14C-OH 177 (0.83) | MOBA (1.0) | THF (3.0) | CDI / DMAP (1.0 / 1.0) | 24 | - | + | + / + |
| **3** | 14C-OH 126 (0.59) | MOBA (2.0) | DCM / DMF (2.0 / 0.5) | EDC / DMAP (3.0 / 0.3) | 1 | + | + | + / + |
| **4** | 14C-OH 126 (0.59) | MOBA (3.0) | DCM / DMF (3.0 / 0.5) | EDC / DMAP (5.0 / 5.0) | 24 | ++ | + | - / + |
| **5** | nerol 91 (0.59) | MOBA (3.0) | DCM / DMF (2.0 / 0.3) | EDC / DMAP (5.0 / 5.0) | 2 | ++ | - | + / + |

14C-OH = tetradecanol; MOBA = 4-methoxybenzoic acid

**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

Once the reaction conditions were found (table 1, *items* 4 and 5), they were applied to the ONO with the same experimental protocol, see table 2. However, it was necessary to elongate the reaction time, modify reagents ratio and change the solvent (table 2, *items 2* and *3*).




**Scheme 2.** Scheme of onopordopicrin esterification under Steglich conditions.

**Table 2. Onopordopicrin esterification under Steglich conditions**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Coupling (equiv.) | Time (h) | Diester (yield %) | Monoester (yield %) | Side product (anhydride) | Starting material (ONO / acid) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ONO 152 (0.44) | MOBA (3.0) | DCM / DMF (2.5 / 0.5) | EDC / DMAP (5.0 / 5.0) | 24 | - | - | + | + / ++ |
| 2 | ONO 158 (0.45) | MOBA (2.2) | THF (10.0) | EDC / DMAP (2.4 / 2.4) | 72 | + | 5.1 | ++ | + / + |
| 3 | ONO 147 (0.42) | MOBA (2.2) | THF (10.0) | CDI / DMAP (2.2 / 2.2) | 24 | - | + | + | + / ++ |
| 4 | ONO 174 (0.50) | 3-Br-PA (3.0) | DCM (2.5) | EDC / DMAP (5.0 / 5.0) | 24 | - | - | - | - |

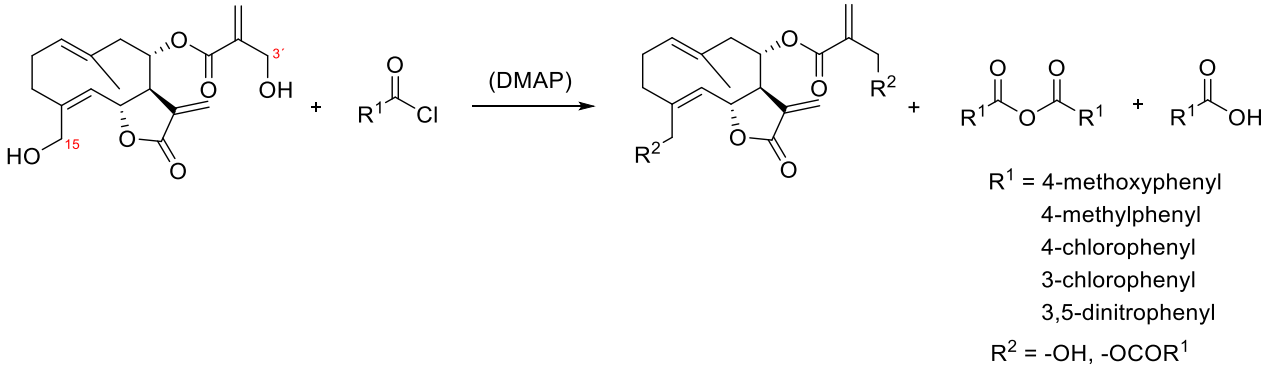
MOBA = 4-methoxybenzoic acid; 3-Br-PA = 3-bromopropionic acid

**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

### *b. Reaction with acyl chlorides*

*General procedure*

To a solution of ONO in the appropriated solvent (table 3), the coupling reagent (in some cases) was added. Then, the acyl chloride was added dropwise at 0 °C under argon atmosphere. The mixture was stirred for appropriate time at rt, and in the dark. After completion of the reaction (followed by TLC), the solvent was removed under reduced pressure. The resulting crude mixture was dissolved in $CHCl_3$ (10 mL), and the organic layer was washed with 0.1M HCl (3 × 10 mL) to remove pyridine and DMAP, 0.05M NaOH (2 × 10 mL) to eliminate formed acid, and brine until neutral pH. The organic layer was dried over anhydrous $Na_2SO_4$ and the solvent evaporated under reduced pressure. The residue was purified by column chromatography [$CHCl_3$/MeOH (98:2) or hexane/EtOAc (3:1)].




**Scheme 3.** Scheme of ONO esterification via acyl chlorides.

**Table 3. Onopordopicrin esterification with acyl chlorides**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Coupling (equiv.) | Time (h) | Diester (yield %) | Monoester (yield %) | Side product (anhydride) | Starting material (ONO / acid) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | ONO 174 (0.50) | MOBCl [a] (4.0) | DCM / DMF (5.0 / 0.3) | / | 24 | - | - | + | + / + |
| **2** | ONO 166 (0.48) | MOBCl [b] (4.0) | THF / TEA (10.0 / 1.0) | / | 72 | - | - | ++ | + / + |
| **3** | ONO 86 (0.25) | MOBCl (3.0) | Pyr (1.0) | / | 44 | 1.3 | 4.2 | ++ | + / ++ |
| **4** | ONO 183 (0.53) | MOBCl (3.0) | Pyr (2.0) | DMAP (0.25) | 24 | 6.0 | 16.1 | + | + / ++ |
| **5** | ONO 69 (0.20) | MBCl (2.5) | Pyr (1.0) | / | 24 | - | 2.2 | ++ | + / ++ |
| **6** | ONO 67 (0.19) | MBCl (3.0) | Pyr (1.3) | DMAP (0.25) | 24 | 3.0 | 3.6 | + | + / - |
| **7** | ONO 123 (0.35) | MBCl (2.0) | THF / TEA (3.5 / 0.2) | DMAP (0.25) | 24 | 12.6 | 17.8 | - | - |
| **8** | ONO 68 (0.20) | 4-Cl-BCl (2.2) | Pyr (1.0) | / | 3 | 20.3 | 8.0 | + | + / ++ |
| **9** | ONO 90 (0.26) | 3-Cl-BCl (3.0) | Pyr (1.0) | / | 24 | - | 7.7 | + | + / ++ |
| **10** | ONO 180 (0.52) | 3,5-di$NO_2$-BCl (4.0) | DCM / DMF / TEA (2.5 / 0.3 / 1.4) | / | 2 | - | + | ++ | ++ / - |

MOBCl = 4-methoxybenzoyl chloride; MBCl = 4-methylbenzoyl chloride; 4-Cl-BCl = 4-chlorobenzoyl chloride; 3-Cl-BCl = 3-chlorobenzoyl chloride; 3,5-di$NO_2$-BCl = 3,5-dinitrobenzoyl chloride

[a] RCOCl obtaining from RCOOH. RCOOH, $SOCl_2$, DCM (4 equiv. / 11 equiv. / 3 mL) 3 h reflux.

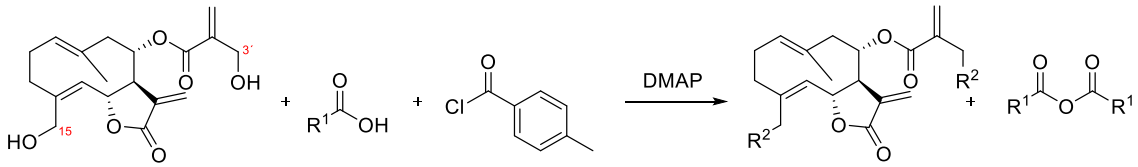
[b] RCOCl obtaining from RCOOH. RCOOH and $SOCl_2$ (4 equiv. / 5 mL) 2 h reflux.

**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

### *c. Yamaguchi esterification*

*General procedure*

To a solution of ONO in THF, the corresponding acid and acyl chloride were added under an argon atmosphere (table 4). Then, TEA was added to the mixture, followed by addition of DMAP. The mixture was stirred for 24 h at rt under an argon atmosphere and in the dark. The solid part was filtered off and the filtrate was concentrated on a rotary evaporator. The resulting crude mixture was dissolved in $CHCl_3$ (10 mL), and the organic layer was washed with 0.1M HCl (3 × 10 mL), 0.05M NaOH (2 × 10 mL) and brine to neutral pH. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography [$CHCl_3$/MeOH (98:2) or hexane/EtOAc (3:1)].




$R^1$ = pentyl
2-bromoethyl
4-methylphenyl
$R^2$ = -OH, -OCOR$^1$

**Scheme 4.** Scheme of esterification under Yamaguchi conditions.

**Table 4. Onopordopicrin esterification under Yamaguchi conditions**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Coupling (equiv.) | Time (h) | Diester (yield) | Monoester (yield) | Side product (anhydride) | Starting material (ONO / acid) |
|---|---|---|---|---|---|---|---|---|---|
| **1** | ONO 105 (0.30) | 3-Br-PA (2.0) | THF / TEA (3.0 / 0.2) | MBCl / DMAP (2.00 / 0.25) | 24 | + | + | ++ | - / + |
| **2** | ONO 120 (0.35) | Hexanoic acid (2.0) | THF / TEA (3.5 / 0.2) | MBCl / DMAP (2.00 / 0.25) | 24 | ++ | ++ | - | + / + |

3-Br-PA = 3-bromopropionic acid; MBCl = 4-methylbenzoyl chloride

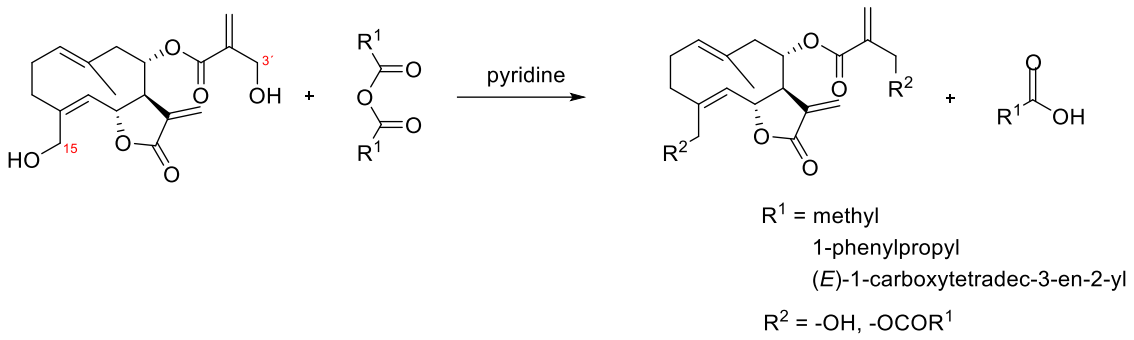
**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

### *d. Reactions with anhydrides*

*General procedure*

To a solution of ONO in pyridine (1 mL) the corresponding anhydride was added dropwise at 0 °C (table 5), and the mixture was stirred for 5 min at 0 °C, followed by 24 h at rt, under argon atmosphere and in the dark. Then, the pyridine was removed under reduced pressure, and the crude mixture was dissolved in $CHCl_3$ (10 mL). The organic

layer was washed with 0.05M NaOH (2 × 10 mL) to eliminate the acid formed (not in case of dodecenylsuccinic anhydride), 0.1M HCl (3 × 10 mL) to remove the residual pyridine, and brine to neutral pH. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The residue was purified by column chromatography, [5.1) $CHCl_3$; 5.2) hexane/EtOAc (2:1); 5.3) $CHCl_3$/MeOH/$CH_3COOH$ (98:2:1)].




**Scheme 5.** Scheme of ONO esterification via anhydrides.

**Table 5. Onopordopicrin esterification with anhydrides**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Time (h) | Diester (yield %) | Monoester (yield %) | Side product (acid) | Starting material (ONO / anhydride) |
|---|---|---|---|---|---|---|---|---|
| 1 | ONO 199 (0.57) | $Ac_2O$ (2.5) | Pyr (1.0) | 24 | 26.0 | 1.8 | - | - |
| 2 | ONO 170 (0.49) | Ph-$but_2O$ (5.0) | Pyr (1.0) | 24 | 44.2 | - | ++ | - / ++ |
| 3 | ONO 121 (0.35) | Dodecenylsuc. anh. (2.5) | Pyr (1.5) | 24 | - | 21.7 | ++ | - |

$Ac_2O$ = acetic anhydride; Ph-$but_2O$ = 2-phenylbutyric anhydride; dodecenylsuc. anh. = 2-(dodec-2-en-1-yl)succinic anhydride

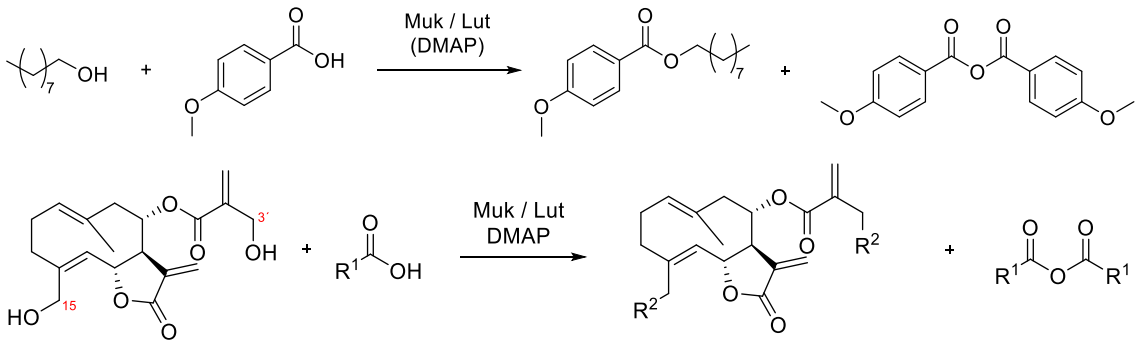
**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

### *e. Mukaiyama esterification*

*General procedure*

To a solution of the corresponding alcohol (ONO or nonanol) in the selected solvent, the Mukaiyama´s reagent (2-chloro-1-methylpyridinium iodide) and 4-methoxybenzoic acid or 2,5-dichlorobenzoic acid were added, followed by addition of 2,6-lutidine, and in some cases DMAP (table 6). The mixture was stirred for 6-90 hours at rt or at 60 °C under argon atmosphere. Then, the mixture was diluted with EtOAc (15 mL) and the formed pyridone was filtered off in a Büchner funnel. The organic filtrate was

washed with water (1 × 10 mL), brine (1 × 10 mL), 0.5M HCl (2 × 10 mL), a 50% solution of $NaHCO_3$ (3 × 5 mL) and brine to neutral pH. Then, the organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography [$CHCl_3$/MeOH (98:2)].




**Scheme 6.** Scheme of esterification under Mukaiyama conditions.

**Table 6. Nonanol and onopordopicrin esterification under Mukaiyama conditions**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Coupling (equiv.) | T | Time (h) | Diester (yield %) | Monoester (yield %) | Side product (anhydride) | Starting material (ONO / acid) |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 9C-OH 144 (1.00) | MOBA (1.0) | diethyl ether (2.0) | Muk / Lut (1.05 / 2.4) | rt | 24 | - | + | + | + / ++ |
| 2 | 9C-OH 144 (1.00) | MOBA (1.0) | DMC (2.0) | Muk / Lut / DMAP (1.05 / 2.4 / 0.05) | 60 °C | 90 | ++ | + | + | + / + |
| 3 | ONO 167 (0.50) | MOBA (2.0) | DMC (1.0) | Muk / Lut / DMAP (2.10 / 4.8 / 0.05) | 60 °C | 7 | 13.5 | - | ++ | + / + |
| 4 | ONO 155 (0.45) | 2,5-diCl-BA (2.0) | DMC (1.0) | Muk / Lut / DMAP (2.10 / 4.8 / 0.05) | 60 °C | 6 | 52.0 | 2.1 | - | - |

9C-OH = nonanol; MOBA = 4-methoxybenzoic acid; 2,5-diCl-BA = 2,5-dichlorobenzoic acid;
Muk = 2-chloro-1-methylpyridinium iodide; Lut = 2,6-lutidine

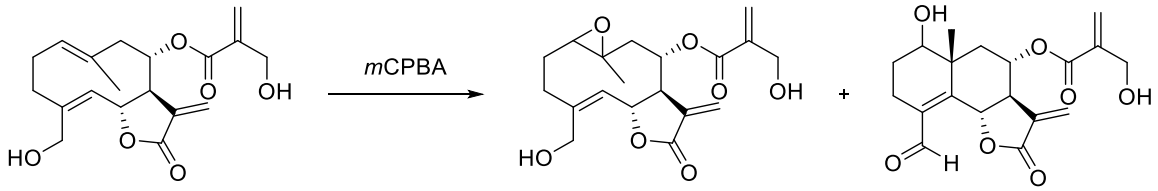
**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

### Synthesis of 1,10-epoxyonopordopicrin

*General procedure*

To a solution of ONO in the selected solvent at 0 °C, *m*CPBA was added. The mixture was stirred under the conditions indicated in table 7, argon atmosphere and in the dark. The reaction was diluted with ice water and 16% $Na_2S_2O_5$ (2 mL) was added to destroy the remaining *m*CPBA. The solvent was evaporated under reduced pressure. The crude

mixture was dissolved in n-BuOH (10 mL) and washed with 50% $NaHCO_3$ (3 × 5 mL), 0.1M HCl (3 × 5 mL) and brine to neutral pH. The organic layer was dried over anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography [$CHCl_3$/MeOH (98:2)].




**Scheme 7.** Scheme of onopordopicrin epoxidation.

**Table 7. Epoxidation of onopordopicrin**

| *Item* | R-OH mg (mmol) | Reagent (equiv.) | Solvent (mL) | Addition | T | Time (h) | 1,10-epoxide (yield %) | Aldehyde (yield %) | Other structure |
|---|---|---|---|---|---|---|---|---|---|
| **1** | ONO 162 (0.47) | *m*CPBA (1.5) | Pyr / DCM (0.1 / 4.5) | At once | rt | 1.5 | - | - | cmix |
| **2** | ONO 127 (0.36) | *m*CPBA (3.6) | Pyr / DCM (0.1 / 3.0) | At once | 0 °C (30´) rt (75´) | 1.75 | - | - | cmix |
| **3** | ONO 215 (0.62) | *m*CPBA (4.0) | Pyr / THF (0.1 / 4.0) | At once | rt | 2.75 | 15.5 | + | cmix |
| **4** | ONO 204 (0.59) | *m*CPBA (4.0) | Pyr / THF (0.1 / 3.0) | At once | 0 °C | 2.75 | + | - | ester of epoxide |
| **5** | ONO 169 (0.49) | *m*CPBA (1.5) | Pyr / DCM / THF (0.1 / 2.0 / 2.0) | 4 times in 45´ | 0 °C | 1.25 | - | - | cmix |
| **6** | ONO 162 (0.47) | *m*CPBA (4.0) | Pyr / DCM / THF (0.15 / 2.0 / 2.0) | 4 times in 15´ | 0 °C (30´) rt (30´) | 1 | - | ++ | cmix |
| **7** | ONO 345 (0.99) | *m*CPBA (4.0) | Pyr / DCM / THF (0.32 / 4.0 / 4.0) | 4 times in 15´ | 0 °C (30´) rt (60´) | 1.5 | 21.4 | 7.7 | - |
| **8** | ONO 220 (0.63) | *m*CPBA (4.0) | Pyr / THF (0.2 / 4.0) | At once | rt | 0.5 | - | + | cmix |

*m*CPBA = 3-chloroperoxybenzoic acid, cmix = complex mixture

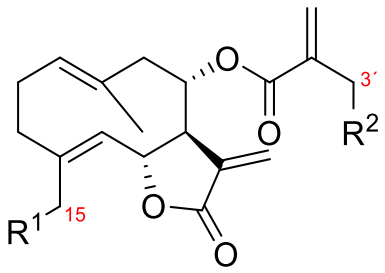
**++** - gained, but not purified; **+** - observed in traces; **-** - not observed

## RESULTS

### Summary of obtained derivatives

Finally, 13 ester derivatives of ONO were synthesized and characterized by NMR, HR-MS, and IR. Also, one epoxy-derivative was prepared and one side product of epoxidation - an aldehyde - was isolated. Below, all obtained compounds including simple esters prepared in process of method optimalization are mentioned. The table 8 shows all obtained ester derivatives with their best yields, used method and conditions of synthesis.

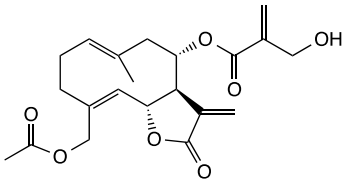
**Table 8. Obtained ester derivatives of onopordopicrin**




| N$^{O}$ | R$^{1}$ | R$^{2}$ | YIELD [%] | METHOD | CONDITIONS[1] |
|---|---|---|---|---|---|
| **1** | acetyl | -OH | **1.8** | anhydride | 5.1 |
| **2** | acetyl | acetyl | **26.0** | anhydride | 5.1 |
| **3** | 4-methylbenzoyl | -OH | **17.8** | acyl chloride | 3.7 |
| **4** | 4-methylbenzoyl | 4-methylbenzoyl | **12.6** | acyl chloride | 3.7 |
| **5** | -OH | 4-methoxybenzoyl | **16.1** | acyl chloride | 3.4 |
| **6** | 4-methoxybenzoyl | 4-methoxybenzoyl | **13.5** | Mukaiyama | 6.3 |
| **7** | 3-chlorobenzoyl | -OH | **7.7** | acyl chloride | 3.9 |
| **8** | 4-chlorobenzoyl | -OH | **8.0** | acyl chloride | 3.8 |
| **9** | 4-chlorobenzoyl | 4-chlorobenzoyl | **20.3** | acyl chloride | 3.8 |
| **10** | 2,5-dichlorobenzoyl | -OH | **2.1** | Mukaiyama | 6.4 |
| **11** | 2,5-dichlorobenzoyl | 2,5-dichlorobenzoyl | **52.0** | Mukaiyama | 6.4 |
| **12** | 2-phenylbutyroyl | 2-phenylbutyroyl | **44.2** | anhydride | 5.2 |
| **13** | -OH | dodecenylsuccinoyl | **21.7** | anhydride | 5.3 |

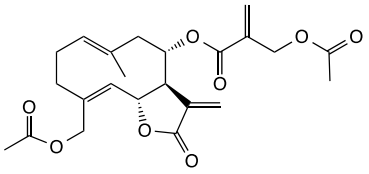
[1] Number of table and item in experimental section.

***Structures of obtained ester derivatives***



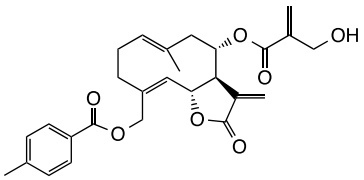
15-acetylonopordopicrin

(1) Yield: 1.8 % (***d*** - table 5.1)



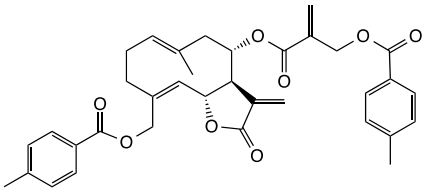
15,3´-diacetylonopordopicrin

(2) Yield: 26.0 % (***d*** - table 5.1)



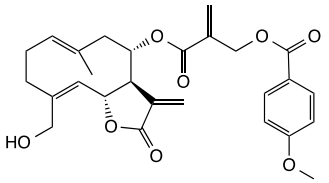
15-(4-methylbenzoyl)onopordopicrin

(3) Yield: 17.8 % (***b*** - table 3.7)



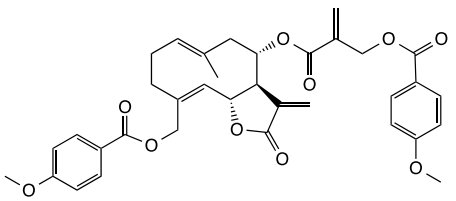
15,3´-di(4-methylbenzoyl)onopordopicrin

(4) Yield: 12.6 % (***b*** - table 3.7)



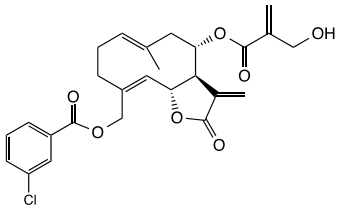
3´-(4-methoxybenzoyl)onopordopicrin

(5) Yield: 16.1 % (***b*** - table 3.4)



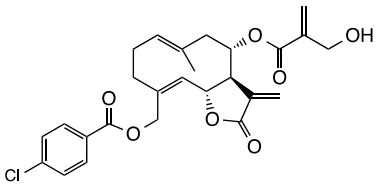
15,3´-di(4-methoxybenzoyl)onopordopicrin

(6) Yield: 13.5 % (***e*** - table 6.3)



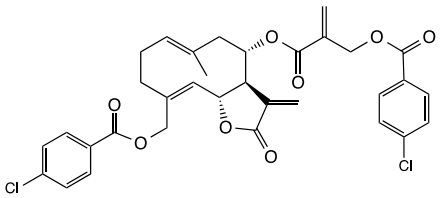
15-(3-chlorobenzoyl)onopordopicrin

(7) Yield: 7.7 % (***b*** - table 3.9)



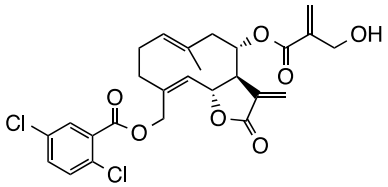
15-(4-chlorobenzoyl)onopordopicrin

(8) Yield: 8.0 % (***b*** - table 3.8)



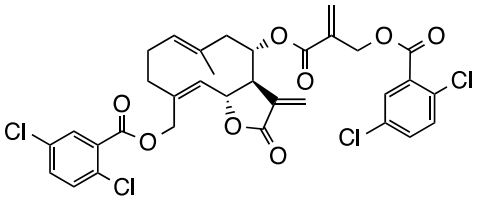
15,3´-di(4-chlorobenzoyl)onopordopicrin

(9) Yield: 20.3 % (***b*** - table 3.8)



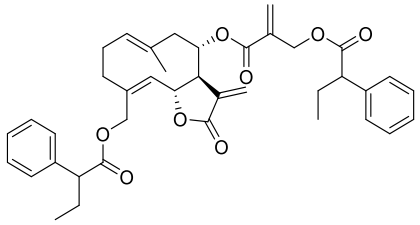
15-(2,5-dichlorobenzoyl)onopordopicrin

(10) Yield: 2.1 % (***e*** - table 6.4)



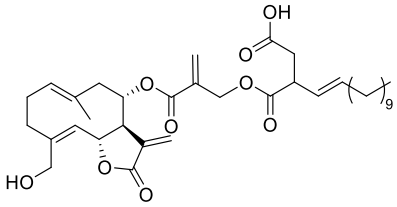
15,3´-bis(2,5-dichlorobenzoyl) onopordopicrin

(11) Yield: 52.0 % (***e*** - table 6.4)



15,3´-di(2-phenylbutyroyl)onopordopicrin

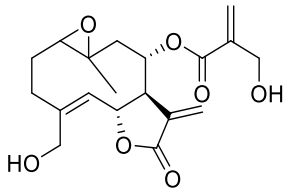
(12) Yield: 44.2 % (***d*** - table 5.2)



3´-dodecenylsuccinoylonopordopicrin

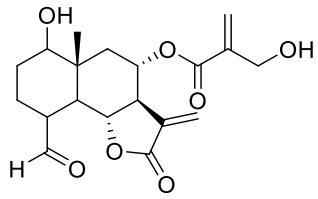
(13) Yield: 21.7 % (***d*** - table 5.3)

## *Structures of other obtained compounds*



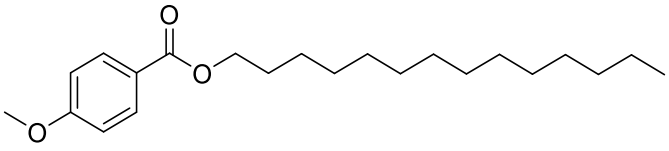
1,10-epoxyonopordopicrin

(14) Yield: 21.4 % (table 7.7)



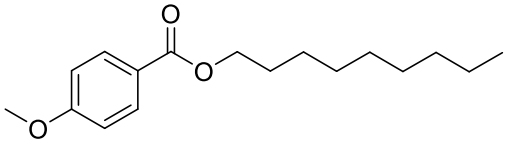
onopordopicrin-15-aldehyde

(15) Yield: 7.7 % (table 7.7)



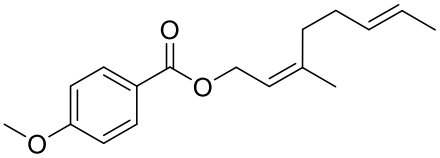
tetradecyl 4-methoxybenzoate

(16) Yield: - % (***a*** - table 1.4)



nonyl 4-methoxybenzoate

(17) Yield: - % (***e*** - table 6.2)



(*Z*)-3,7-dimethylocta-2,6-dien-1-yl 4-methoxybenzoate

(18) Yield: - % (***a*** - table 1.5)

**NMR**

The $^{1}H$ and $^{13}C$ NMR spectra of the crude extract were made (see annex – Figures 34 and 35), where signals corresponding to the sesquiterpenic lactones (STL´s) can be found at 4-6 ppm in $^{1}H$, and 120-170 ppm in $^{13}C$, among others. From the $^{1}H$ and $^{13}C$ NMR spectra of the fractions, it was observed that the STL´s are mainly in the chloroform fraction (Figure 9).

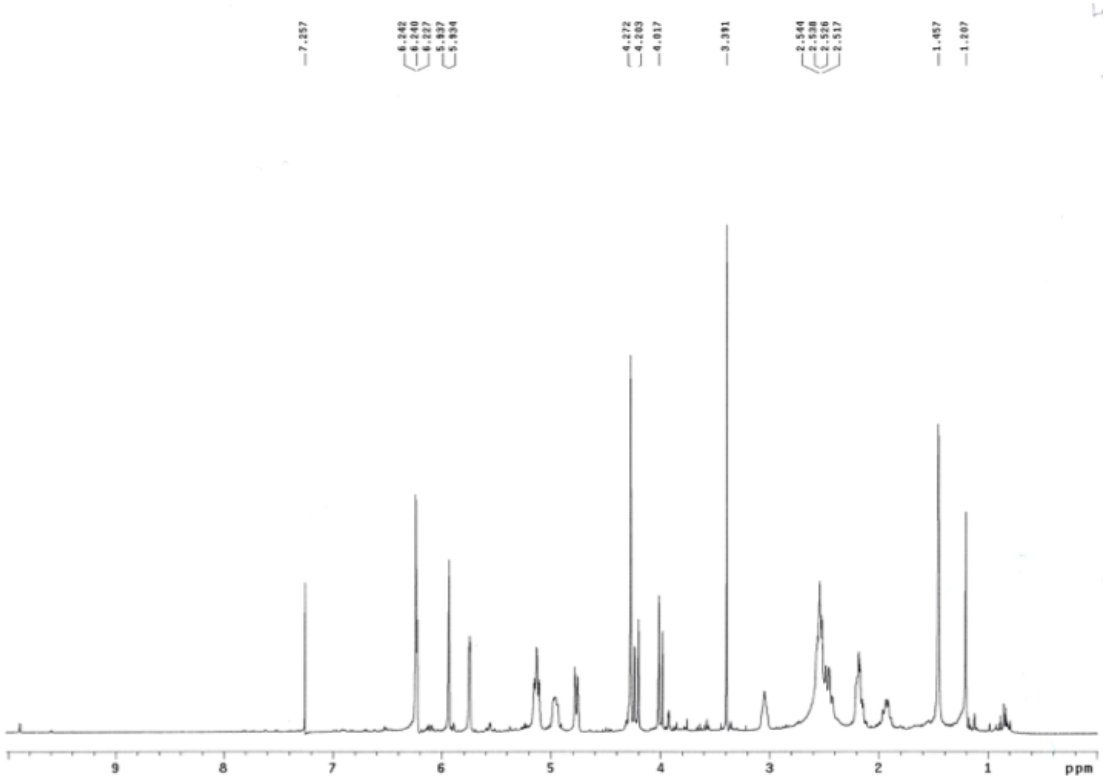
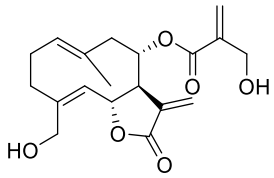



**Figure 9.** $^{1}H$ NMR spectrum of chloroform fraction of *C. ornata*

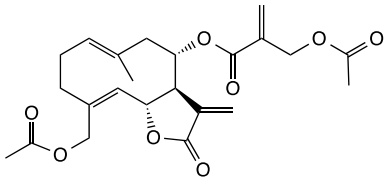
The evolution of reactions was controlled by NMR. For all prepared ONO derivatives both $^{1}H$ and $^{13}C$ NMR spectra were performed. Below are complete spectroscopic data of ONO, compounds (2) and (14) (as a model structures). Unfortunately, complete data for all prepared compounds are not available because of data protection, they were not provided to me by the university. Therefore, there are only interpretations of most relevant NMR data and signal shifts confirming formation of mono-/diester.



Onopordopicrin (**ONO**): light yellow oil; $^{1}$H NMR (400 MHz, $CDCl_3$, d in ppm, *J* in Hz): 6.30 (1H, d, *J* = 3.2 Hz, H-13a), 6.28 (1H, d, *J* = 0.8 Hz, H-4´a), 5.96 (1H, d, *J* = 0.8 Hz, H-4´b), 5.77 (1H, d, *J* = 3.2 Hz, H-13b), 5.12 (1H, dd, $J_1$ = 9.2, $J_2$ = 8.4 Hz, H-6), 5.20 (1H, br t, *J* = 8.0 Hz, H-8), 5.00 (1H, dd, $J_1$ = 11.2, $J_2$ = 5.4 Hz, H-1), 4.83 (1H, d, *J* = 9.2 Hz, H-5), 4.24 (1H, d, *J* = 14.0 Hz, H-15a), 4.28 (2H, s, H-3´), 4.02 (1H, d, *J* = 14.0 Hz, H-15b), 3.09 (1H, m, H-7), 2.61 (1H, m, H-9a), 2.53 (1H, m, H-2a), 2.50 (1H, m, H-2b), 2.22 (1H, m, H-3a), 2.18 (1H, m, H-3b), 1.99 (1H, m, H-9b), 1.51 (3H, s, H-14).

$^{13}$C NMR (100 MHz, $CDCl_3$): 169.94 (C-12), 165.09 (C-1´), 144.13 (C-4), 139.42 (C-2´), 135.30 (C-11), 132.27 (C-10), 129.72 (C-1), 128.33 (C-5), 128.28 (C-4´), 125.47 (C-13), 77.00 (C-8), 73.02 (C-6), 61.99 (C-3´), 61.24 (C-15), 52.88 (C-7), 48.67 (C-2), 39.64 (C-9), 29.91 (C-3), 16.71 (C-14).

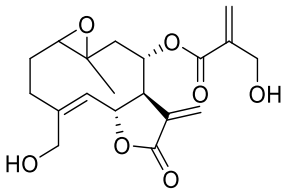
HRMS (ESI) *m/z*, calcd. for $C_{19}H_{25}O_6$ 349.1651, found 349.1733.



15,3´-diacetylonopordopicrin (**2**): light yellow oil; $^{1}$H NMR (400 MHz, $CDCl_3$, d in ppm, *J* in Hz): 6.39 (1H, d, *J* = 0.8 Hz, H-4´a), 6.31 (1H, d, *J* = 3.1 Hz, H-13a), 5.96 (1H, d, *J* = 0.8 Hz, H-4´b), 5.75 (1H, d, *J* = 3.1 Hz, H-13b), 5.25 (1H, br t, *J* = 8.0 Hz, H-8), 5.02 (1H, dd, $J_1$ = 9.1, $J_2$ = 8.4 Hz, H-6), 5.00 (1H, dd, $J_1$ = 11.2, $J_2$ = 5.4 Hz, H-1), 4.98 (1H, d, *J* = 9.1 Hz, H-5), 4.84 (1H, d, *J* = 13.6 Hz, H-15a), 4.76 (1H, d, *J* = 13.6 Hz, H-15b), 4.60 (2H, s, H-3´), 3.11 (1H, m, H-7), 2.58 (1H, m, H-9a), 2.52 (1H, m, H-3a), 2,50 (1H, m, H-9b), 2.21 (1H, m, H-2a), 2.20 (1H, m, H-2b), 2.10 (1H, m, H-3b), 2.10 (6H, s, CO<u>C</u>H$_3$), 1.57 (3H, s, H-14).

$^{13}$C NMR (100 MHz, $CDCl_3$): 170.63 (2 x <u>C</u>OCH$_3$), 169.38 (C-12), 164.21 (C-1´), 139.00 (C-2´), 135.21 (C-11), 135.10 (C-4), 132.18 (C-5), 130.82 (C-1), 130.18 (C-10), 128.79 (C-13 + C-4´), 77.13 (C-8), 77.00 (C-6), 62.30 (C-3´), 61.72 (C-15), 52.76 (C-7), 48.65 (C-9), 34.94 (C-3), 29.10 (C-2), 20.90 (2 x CO<u>C</u>H$_3$), 16.89 (C-14).

HRMS (ESI) *m/z*, calcd. for $C_{23}H_{29}O_8$ 433.1862, found 433.1935.




1,10-epoxyonopordopicrin (**14**): light yellow oil; $^{1}$H NMR (400 MHz, $CDCl_3$, d in ppm, *J* in Hz): 6.26 (1H, d, *J* = 3.2 Hz, H-13a), 6.25 (1H, d, *J* = 0.8 Hz, H-4´a), 5.92 (1H, d, *J* = 0.8 Hz, H-4´b), 5.76 (1H, d, *J* = 3.2 Hz, H-13b), 5.32 (1H, dd, $J_1$ = 9.2, $J_2$ = 8.4 Hz, H-6), 5.14 (1H, br t, *J* = 8.0 Hz, H-8), 2.78 (1H, dd, $J_1$ = 11.2, $J_2$ = 2,5 Hz, H-1), 6.26 (1H, d, *J* = 9.2 Hz, H-5), 4.34 (1H, d, *J* = 14.0 Hz, H-15a), 4.29 (1H, d, *J* = 14.0 Hz, H-15b), 4.33 (2H, s, H-3´), 3.66 (1H, m, H-7), 2.43 (1H, m, H-9a), 2.15 (1H, m, H-2a), 1.41 (1H, m, H-2b), 2.52 (1H, m, H-3a), 2.33 (1H, m, H-3b), 1.60 (1H, m, H-9b), 1.24 (3H, s, H-14).

$^{13}$C NMR (100 MHz, $CDCl_3$): 166.60 (C-12), 164.93 (C-1´), 146.65 (C-4), 139.19 (C-2´), 136.40 (C-11), 127.67 (C-4´), 126.05 (C-13), 125.17 (C-5), 71.36 (C-6), 67.14 (C-8), 63.14 (C-1), 61.97 (C-3´), 61.57 (C-15), 58.88 (C-10), 53.88 (C-7), 47.64 (C-9), 31.90 (C-3), 27.09 (C-2), 17.17 (C-14).

HRMS (ESI) *m/z*, calcd. for $C_{19}H_{25}O_7$ 365.1600, found 365.1803.

The structure of isolated ONO was confirmed after comparison of NMR data with bibliography. The signal shifts in the $^{1}$H NMR spectra (performed in $CDCl_3$) were compared with those indicated by Rustaiyan et al.,[45] being totally coincident. The signal shifts of the $^{13}$C NMR spectra were compared with those indicated by Marco et al.[12] There are some differences in the signal shifts indicated in red (for more than 5 ppm) and green (between 3 and 5 ppm), in general, there is a good coincidence among the signals except for the positions 2, 3, and 9, (table 9). 1D-NMR and 2D-NMR experiments of ONO are attached in the annex (Figures 36-40).

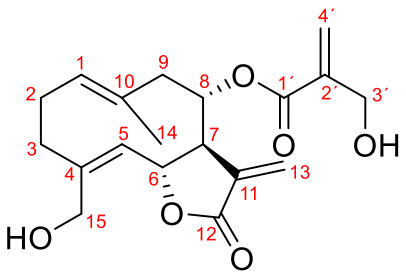



**Figure 10.** Numbering of ONO

**Table 9. Spectral data of onopordopicrin (experimental and bibliography)**

| PROTON | Bibliography[a] ($CD_3OD$; 600 MHz) | Bibliography[b] ($CDCl_3$; 270 MHz) | Experimental ($CDCl_3$; 400 MHz ) | CARBON | Bibliography[c] ($CDCl_3$ + $CD_3OD$; 50 MHz) | Experimental ($CDCl_3$; 100 MHz) |
|---|---|---|---|---|---|---|
| **H-1** | 5.08 (*dd*, *J* = 12.6, 3.0 Hz, 1H) | 5.02 (*dd*) | 5.00 (*dd*, *J* = 11.2, 5.4 Hz, 1H) | **C-1** | 129.8 | 129.72 |
| **H-2** | 2.31 (*m*, 1H)<br>2.20 (*m*, 1H) | 2.24 (*m*) | 2.50 (*m*)<br>2.53 (*m*) | **C-2** | 26.3 | 48.67 |
| **H-3** | 2.02 (*m*, 1H)<br>2.63 (*ddd*, *J* = 12.0, 5.3, 2.1 Hz, 1H) | 2.24 (*m*)<br>2.60 (*m*) | 2.22 (*m*)<br>2.18 (*m*) | **C-3** | 34.7 | 29.91 |
| **H-4** | - | - | - | **C-4** | 144.2 | 144.13 |
| **H-5** | 4.95 (*d*, *J* = 10.0 Hz, 1H) | 4.83 (*d*) | 4.83 (*d*, *J* = 9.2 Hz, 1H) | **C-5** | 128.4 | 128.33 |
| **H-6** | 5.20 (*dd*, *J* = 8.5, 8.7 Hz, 1H) | 5.13 (*dd*) | 5.12 (*dd*, *J* = 9.2, 8.4 Hz, 1H) | **C-6** | 76.6 | 73.02 |
| **H-7** | 3.28 (*m*, 1H) | 3.09 (*m*) | 3.09 (*m*) | **C-7** | 53.0 | 52.88 |
| **H-8** | 5.12 (*m*, 1H) | 5.20 (*dd*) | 5.20 (*br t*, *J* = 8.0 Hz, 1H) | **C-8** | 73.1 | 77.00 |
| **H-9** | 2.58 (*br d*, *J* = 11.0 Hz, 1H)<br>2.53 (*br d*, *J* = 12.4 Hz, 1H) | 2.6 (*m*)<br>2.01 (*m*) | 2.61 (*m*)<br>1.99 (*m*) | **C-9** | 48.7 | 39.64 |
| **H-10** | - | - | - | **C-10** | 132.4 | 132.27 |
| **H-11** | - | - | - | **C-11** | 135.4 | 135.30 |
| **H-12** | - | - | - | **C-12** | 169.9 | 169.94 |
| **H-13** | 6.18 (*d*, *J* = 3.5 Hz, 1H)<br>5.81 (*d*, *J* = 3.1 Hz, 1H) | 6.30 (*d*)<br>5.78 (*d*) | 6.30 (*d*, *J* = 3.2 Hz, 1H)<br>5.77 (*d*, *J* = 3.2 Hz, 1H) | **C-13** | 125.5 | 125.47 |
| **H-14** | 1.54 (*br s*, 3H) | 1.52 (*s*) | 1.51(*s*) | **C-14** | 16.8 | 16.71 |
| **H-15** | 4.02 (*d*, *J* = 13.6 Hz, 1H)<br>4.26 (*d*, *J* = 13.6 Hz, 1H) | 4.32 (*d*)<br>4.11 (*d*) | 4.02 (*d*, *J* = 14.0 Hz, 1H)<br>4.24 (*d*, *J* = 14.0 Hz, 1H) | **C-15** | 61.3 | 61.24 |
| **H-1´** | - | - | - | **C-1´** | 165.1 | 165.09 |
| **H-2´** | - | - | - | **C-2´** | 139.4 | 139.42 |
| **H-3´** | 4.27 (*m*, 2H) | 4.36 (*s*) | 4.28 (*s*) | **C-3´** | 62.1 | 61.99 |
| **H-4´** | 6.29 (*m*, 1H)<br>5.97 (*m*, 1H) | 6.29 (*s*)<br>5.97 (*s*) | 6.28 (*d*, *J* = 0.8 Hz, 1H)<br>5.96 (*d*, *J* = 0.8 Hz, 1H) | **C-4´** | 126.3 | 126.28 |

[a] *J. Chromatogr. A*, 2017, **1524**, 266-272; in $CD_3OD$[46]. [b] *Phytochemistry* 1979, **18**, 883-884; in $CDCl_3$[45]. [c] *Phytochemistry* 2005, **66**, 1644–1650; in $CDCl_3$ + $CD_3OD$[12]. (in red differences in δ of more than 5 ppm, in green differences between 3 and 5 ppm)

The $^1H$ NMR and $^{13}C$ NMR spectra of ester derivatives (1-13) were compared with the spectra of ONO. The main difference between them was observed at signals of $^1H$ and $^{13}C$ at positions C-15 and C-3´, while other signals of ONO-structure were coincident with ONO (Figure 11). Also, new signals belonging to new substituents appeared.

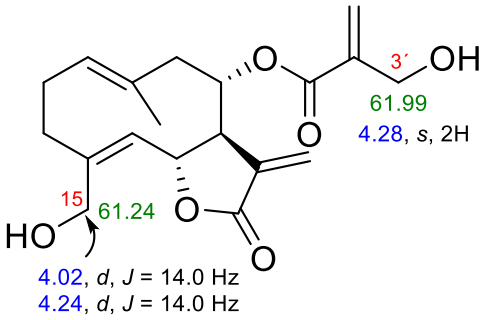



**Figure 11.** Spectroscopic data of ONO at positions (15, 3´) of the main interest (δ in ppm).

Compounds **(1)** and **(2)**

The $^{1}$H NMR and $^{13}$C NMR spectra were performed for the two isolated acetyl esters. For the more polar compound (1) in $^{1}$H NMR spectrum, signals at 4.75 and 4.86 ppm with coupling constant *J*=13.6 Hz were observed instead of signals 4.02 and 4.24 ppm in ONO spectrum. New signals correspond to the methylene at C-15. The less polar compound (2) has similar signal at 4.76 and 4.84 ppm (*J*=13.6 Hz) and new signal at 4.60 ppm instead of 4.28 ppm in ONO, that correspond to ester in the side chain. In both spectra signal of protons on acetyl group at 2.10 ppm, respectively 2.11 ppm were observed. In the $^{13}$C NMR new signals around 170 ppm corresponding to carbonyls and signal at 20.90 ppm of methyl groups were observed (annex – Figures 41 and 42).

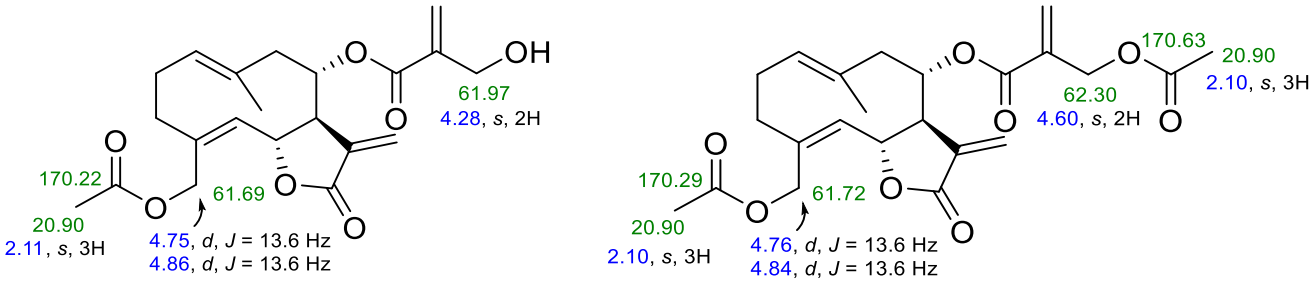
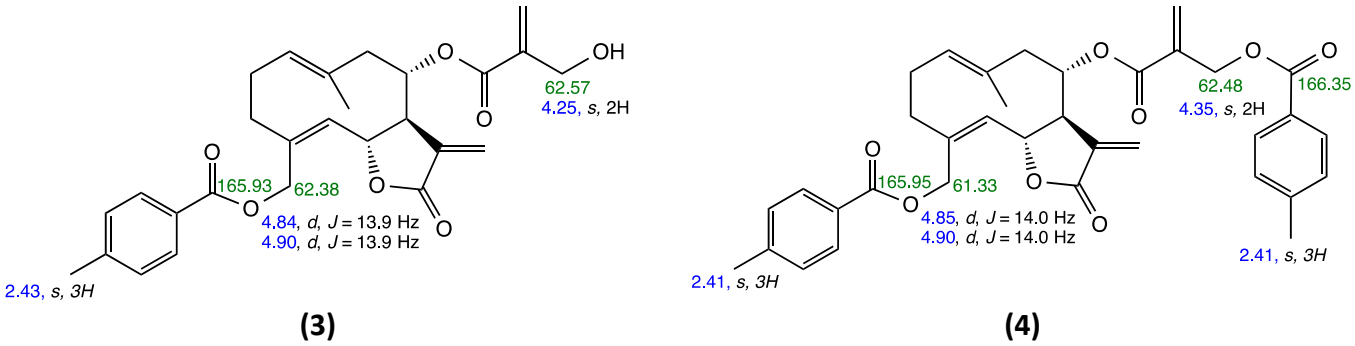



**Figure 12.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compounds (1) and (2) (δ in ppm).

Compounds **(3)** and **(4)**

The $^{1}$H NMR and $^{13}$C NMR spectra of 4-methylbenzoyl derivatives new signals corresponding to the aromatic substituent appeared (annex – Figures 43 and 44). In $^{1}$H NMR, signals at 4.84 and 4.90 ppm (*J*=13.9 Hz) (compound 3) or at 4.85 and

4.90 ppm (*J*=14.0 Hz) (compound 4) were observed instead of signals at 4.02 and 4.24 ppm (*J*=14.0 Hz) of ONO spectrum corresponding to C-15. In the $^{1}$H NMR spectrum of diester (4) signal shift of the ester on C-3´ at 4.35 ppm appeared. In $^{13}$C NMR carbonyls around 165 ppm corresponding to esters were in both compounds observed.

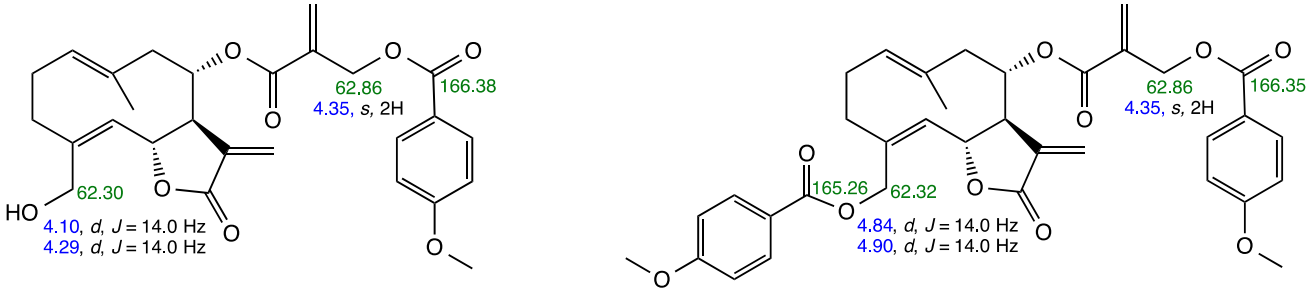



**(3)** **(4)**

**Figure 13.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compounds (3) and (4) (δ in ppm).

Compounds **(5)** and **(6)**

In the $^{1}$H NMR spectra of 4-methoxybenzoyl monoester (5) new signal shift at 4.35 ppm instead of 4.28 ppm of C-3´ in ONO spectrum was observed. The $^{1}$H NMR spectrum of diester (6) differs in signals at 4.84 and 4.90 ppm (*J*=14.0 Hz) corresponding to protons next to the ester group on C-15. Also, in $^{1}$H NMR and $^{13}$C NMR spectra signals corresponding to aromatic substituent were observed (annex – Figures 45 and 46).

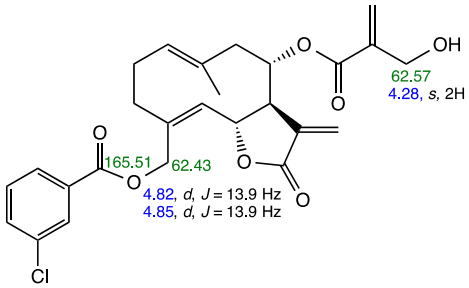



**(5)** **(6)**

**Figure 14.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compounds (5) and (6) (δ in ppm).

Compound **(7)**

For 3-chlorobenzoyl derivative only monoester (7) was synthesized. In the $^{1}$H NMR spectrum signals at 4.82 and 4.85 (*J*=13.9 Hz) indicating presence of ester group on C-15 were observed. In the $^{13}$C NMR spectrum signal at 165.51 ppm corresponding to carbonyl of aromatic substituent appeared.

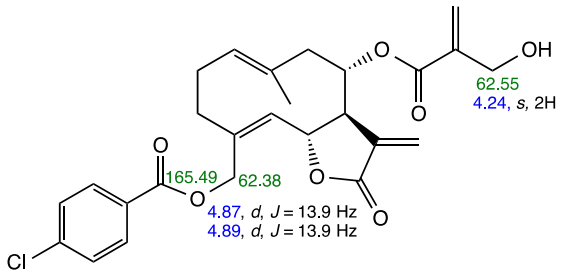



**(7)**

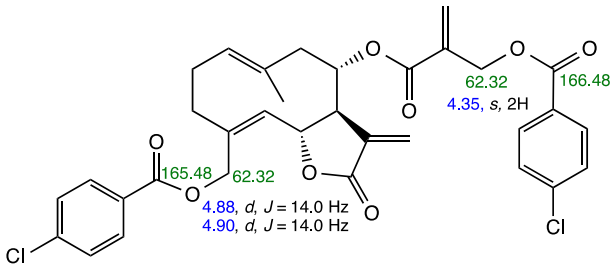
**Figure 15.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compounds (7) (δ in ppm).

Compounds **(8)** and **(9)**

The $^{1}$H NMR and $^{13}$C NMR spectra of 4-chlorobenzoyl derivatives were performed. In spectra of both compounds, signals corresponding to ester at C-15 were observed: for compound 8 - $^{1}$H: 4.87, 4.89 ppm (*J*=13.9 Hz), $^{13}$C: 62.38 ppm; for compound 9 - $^{1}$H: 4.88, 4.90 ppm (*J*=14.0 Hz), $^{13}$C: 62.32 ppm. In the $^{1}$H NMR spectrum of diester (9) signal shift at 4.35 ppm corresponding to ester at C-3´ appeared. In the $^{13}$C NMR spectra of both compounds signals around 165 ppm corresponding to carbonyls of aromatic substituents were observed.




**(8)**

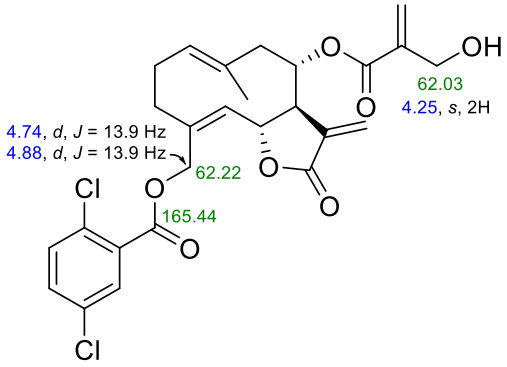



**(9)**

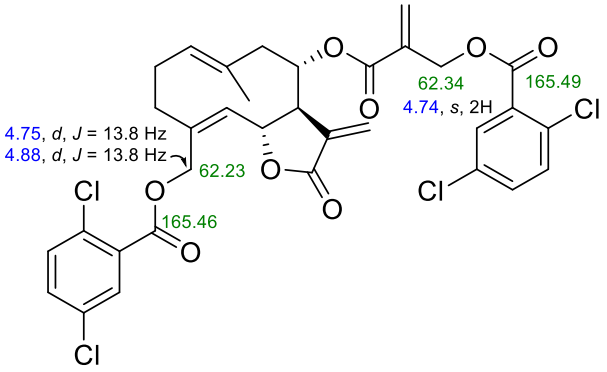
**Figure 16.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compounds (8) and (9) (δ in ppm).

Compounds **(10)** and **(11)**

In the $^{1}$H NMR spectra of both compounds new signals corresponding to ester at C-15 were observed: for compound 10 - $^{1}$H: 4.74, 4.88 ppm (*J*=13.9 Hz); for compound 11 - $^{1}$H: 4.75, 4.88 ppm (*J*=13.8 Hz). In the $^{1}$H NMR of diester (11) signal shift appears at 4.74 ppm corresponding to two protons at C-3´, (annex – Figure 47). In the $^{13}$C NMR spectra of both compounds signals around 165 ppm referring to carbonyls of aromatic substituents were observed.




**(10)**

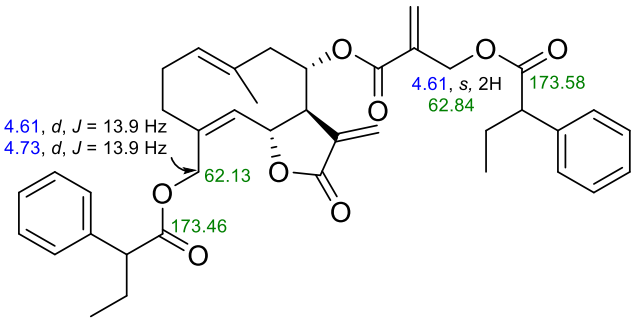



**(11)**

**Figure 17.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compounds (10) and (11) (δ in ppm).

Compound **(12)**

The $^{1}$H NMR and $^{13}$C NMR spectra of obtained diester (12) were performed. In the $^{1}$H NMR signals shift at 4.61 and 4.73 ppm instead of 4.02 and 4.24 ppm (*J*=14.0 Hz), and 4.61 ppm instead of 4.28 ppm in ONO spectrum were observed. These signals correspond to esters in positions C-15 and C-3´. In $^{13}$C NMR signals at 173.46 and 173.58 ppm correspond to carbonyls of 2-phenylbutyroyl substituents.

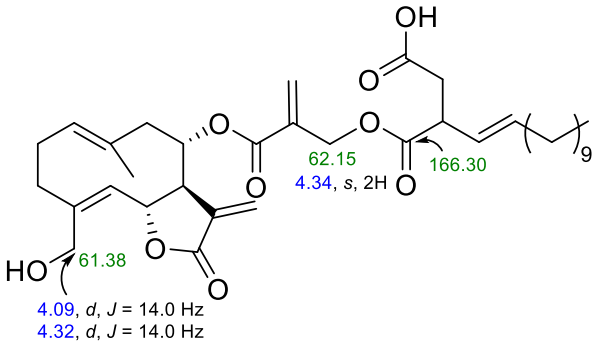



**(12)**

**Figure 18.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compound (12) (δ in ppm).

Compound **(13)**

In the $^{1}$H NMR new signal appear at 4.34 ppm instead of 4.28 ppm in ONO spectrum. That signal corresponds to ester at C-3´. The signals at C-15 (4.09 and 4.32 ppm, *J*=14.0 Hz) corresponds to those in ONO (4.02 and 4.24 ppm, *J*=14.0 Hz), so the compound forms only monoester at C-3´. In $^{13}$C NMR, the signal at 166.30 ppm refers to carbonyl of ester.



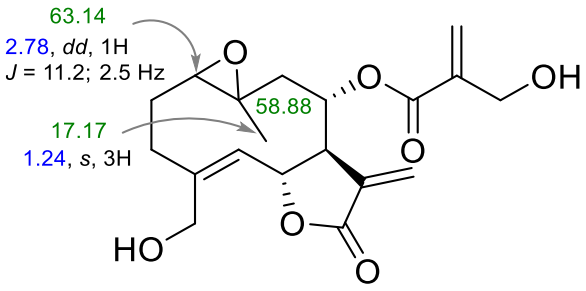

**(13)**

**Figure 19.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compound (13) (δ in ppm).

Compound **(14)**

The spectral data of epoxy-derivative were compared with those indicated by Roselli et al.[47], (table 10). The structure of 1,10-epoxide was confirmed from 1D-NMR and 2D-NMR experiments.

The formation of epoxide resulted in significant signal shifts at positions C-1, C-10, and C-14. In the $^{1}$H NMR spectra signal at C-1: 2.78 ppm (1H, *dd*, $J_1$=11.2, $J_2$=2.5 Hz) appeared instead of C-1: 5.00 ppm (1H, *dd*, $J_1$=11.2, $J_2$=5.4 Hz) in ONO spectrum. The signal of protons of C-14 representing methyl group shifted from 1.51 ppm of ONO to 1.24 ppm of epoxide derivative. In $^{13}$C NMR, the signal of methyl group changed from 16.71 ppm of ONO to 17.17 ppm of its epoxide. The signals of olefinic carbons at positions C-1 (129.72 ppm) and C-10 (132.27 ppm) of ONO disappeared and new signals were observed at 63.14 ppm (C-1) and 58.88 (C-10).




**(14)**

**Figure 20.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compound (14) (δ in ppm).

**Table 10. Spectral data of 1,10-epoxyonopordopicrin (experimental and bibliography[47])**

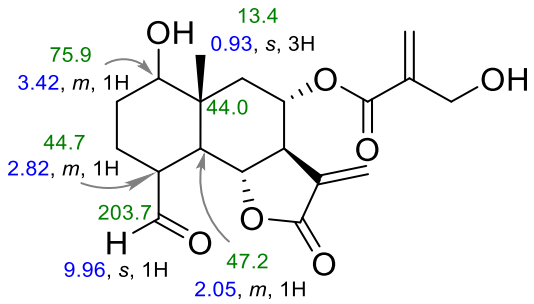
| PROTON | Bibliography[a] ($CDCl_3$; 300 MHz) | Experimental ($CDCl_3$; 400 MHz) | CARBON | Bibliography[a] ($CDCl_3$; 150 MHz) | Experimental ($CDCl_3$; 100 MHz) |
|---|---|---|---|---|---|
| **H-1** | 2.76 (*dd*, *J* = 11.2, 2.5 Hz) | 2.78 | **C-1** | 67.3 | 63.14 |
| **H-2** | 2.32 (*m*)<br>2.14 (*m*) | 2.15<br>1.41 | **C-2** | 24.9 | 27.09 |
| **H-3** | 2.54 (m)<br>2.30 (*m*) | 2.52<br>2.33 | **C-3** | 31.8 | 31.90 |
| **H-4** | - | - | **C-4** | 146.8 | 146.65 |
| **H-5** | 5.32 (*d*, *J* = 9.8 Hz) | 6.26 | **C-5** | 125.4 | 125.17 |
| **H-6** | 5.29 (*dd*, *J* = 9.8, 8.1 Hz) | 5.32 | **C-6** | 75.9 | 71.38 |
| **H-7** | 3.07 (*m*) | 3.66 | **C-7** | 53.8 | 53.88 |
| **H-8** | 5.12 (*bdd*, *J* = 10.7, 10.5 Hz) | 5.14 | **C-8** | 71.4 | 67.14 |
| **H-9** | 2.44 (*bd*, *J* = 13.8 Hz)<br>1.66 (*dd*, *J* = 13.8, 10.7 Hz) | 2.43<br>1.60 | **C-9** | 47.5 | 47.64 |
| **H-10** | - | - | **C-10** | 58.5 | 58.88 |
| **H-11** | - | - | **C-11** | 135.2 | 136.40 |
| **H-12** | - | - | **C-12** | 169.6 | 166.60 |
| **H-13** | 6.25 (d, *J* = 3.6 Hz)<br>5.73 (*d*, *J* = 3.1 Hz) | 6.26<br>5.76 | **C-13** | 124.8 | 126.05 |
| **H-14** | 1.28 (*s*) | 1.24 | **C-14** | 17.2 | 17.17 |
| **H-15** | 4.40 (*d*, *J* = 14.5 Hz) | 4.33 | **C-15** | 61.5 | 61.57 |
| **H-1´** | - | - | **C-1´** | 164.3 | 164.93 |
| **H-2´** | - | - | **C-2´** | 139.1 | 139.19 |
| **H-3´** | 1.80 (*bdd*, *J* = 6.9, 6.9 Hz) | 4.33 | **C-3´** | 62.1 | 61.97 |
| **H-4´** | 6.28 (*bs*)<br>6.17 (*bs*) | 6.25<br>5.92 | **C-4´** | 125.0 | 127.67 |

[a] *Natural Product Communications*, 2010, 5 (5), 675-680; in $CDCl_3$.

Compound **(15)**

The $^1H$ NMR and $^{13}C$ NMR spectra of obtained aldehyde were performed. The data of the aldehyde were compared with those indicated by Rustaiyan et al.[48] In $^1H$ NMR new signal for aldehyde appears at 9.96 ppm, the signal of methyl group changes from 1.51 ppm of ONO to 0.93 ppm, new signals for protons appear at C-4 (2.82 ppm) and C-5 (2.05 ppm), and signal at C-1 shifted from 5.00 ppm to 3.42 ppm. In $^{13}C$ NMR new signal for aldehyde at 203.7 ppm was observed. The signals of olefinic carbons at positions 1,10 and 4,5 shifted from around 130 ppm to approximately 45 ppm except signal

belonging to C-1 (75.9 ppm), where the shift is affected by presence of hydroxy group on carbon.



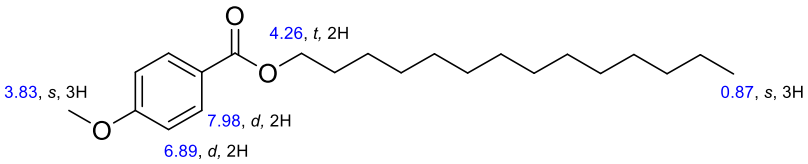

**(15)**

**Figure 21.** Some $^{1}$H-NMR and $^{13}$C-NMR shifts of compound (15) (δ in ppm).

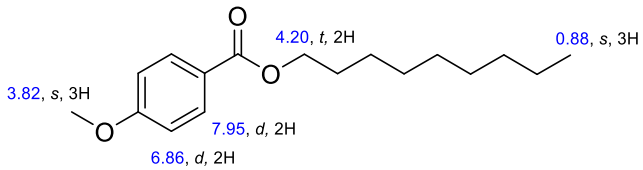
Compounds **(16)**, **(17)** and **(18)**

The $^{1}$H NMR spectra of model esters in the process of esterification-conditions´ optimalization were performed. The spectra of the obtained esters showed signal at 4.26 ppm (16), 4.20 ppm (17), and 4.38 ppm (18) corresponding to two protons of the methylene $\underline{CH_2}OCO$. The aromatic part of esters was represented by three signals: a singlet at 3.83 ppm (16) / 3.82 ppm (17) / 3.79 ppm (18) that corresponded to three protons of the methoxy group, and two signals at 6.86 and 7.89 ppm (16) / 6.86 and 7.95 ppm (17) / 6.90 and 7.98 ppm (18) corresponding to aromatic protons. Also, the aliphatic part of the molecules was observed, (annex – Figure 48).

**(16)**




**(17)**




**(18)**

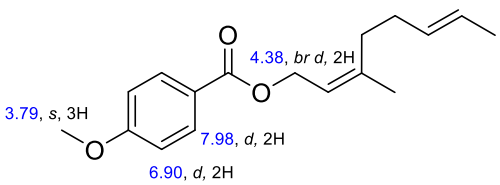



**Figure 22.** Some $^{1}$H-NMR shifts of compounds (16), (17), and (18) (δ in ppm).

## DISCUSSION

### Preparation of the extract. Fractionation. Purification of the major compound.

The flowering aerial part of *C. ornata* was collected in the Zamora province, Spain. From 3.36 kg of plant, 8.89 g (0.26 %) of the crude extract were obtained.

The crude extract was fractionated with hexane (0.87 g, 9.80 %), chloroform (5.70 g, 64.1 %), ethyl acetate (1.45 g, 16.3 %) and n-butanol (0.87 g, 9.80 %).

The chloroform fraction contained a high proportion of a STL´s, however, it was purified by column chromatography to give 4.87 g of a pure compound, which represents 54.8 % of the crude extract from the plant. $^{1}H$ and $^{13}C$ NMR, 2D experiments (HSQC, HMBC, COSY) and MS spectra were performed (Figures 36-40, annex). The high-resolution mass spectrum (HRMS) showed an ion at m/z = 348.1795 corresponding to the formula $C_{19}H_{25}O_6$ $[M + H]^+$.

### Synthesis of esters

#### *a. Steglich esterification*

As ONO is a highly functionalized molecule, it was decided to obtain the ester-type derivatives under mild Steglich conditions. First, the reaction conditions were optimized with the aliphatic alcohol, 1-tetradecanol, using 4-methoxybenzoic acid (MOBA), which is easy to visualize under UV and by $^{1}H$ NMR, to monitor the progress of the reaction.

In the first place, CDI was used as a coupling agent (table 1, *item 1*) however, without the reaction progress; then, it was repeated with addition of DMAP (table 1, *item 2*), but without any improvement (the progress in the reaction). Therefore, modifications of reaction conditions including changing of the solvent (a mixture of DCM/DMF instead of THF), the coupling agent (EDC and DMAP), and increasing the number of equivalents of MOBA were performed resulted in achieving the evolution of the reaction (table 1, *item 3*). Finally, the use of 3 equivalents of MOBA, 5 equivalents of EDC and DMAP and a new variation in the proportion of solvents and increasing the reaction time to 24 hours (table 1, *item 4*), resulted in formation of the desired ester (16), (Figure 23). The product of reaction was not purified.

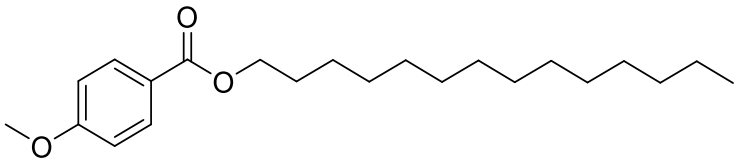


**Figure 23.** Structure of tetradecyl 4-methoxybenzoate (16)

The last conditions were applied to the nerol, an allyl alcohol more similar to ONO (table 1, *item 5*) than the aliphatic tetradecanol. (*Z*)-3,7-Dimethylocta-2,6-dien-1-yl 4-methoxybenzoate was obtained, but not purified, (Figure 24).

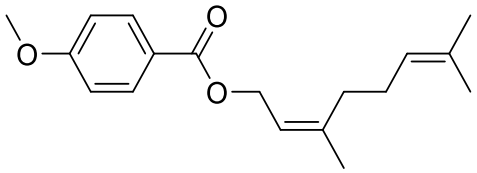


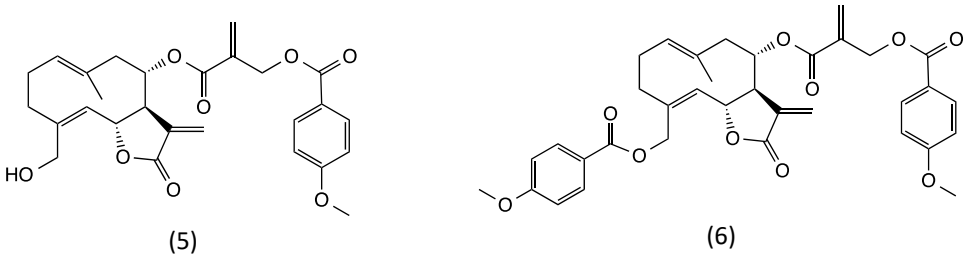
**Figure 24.** Structure of (*Z*)-3,7-dimethylocta-2,6-dien-1-yl 4-methoxybenzoate (18).

Finally, these conditions were applied to ONO (table 2, *item 1*). The $^{1}$H NMR spectrum of the reaction mixture did not show any signal corresponding to the methylenes of an ester. Only the signals corresponding to ONO, MOBA and 4-methoxybenzoic anhydride were detected. Therefore, it was decided to change the solvent to THF, as well as the proportions of MOBA and coupling agent/catalyst, and to increase the reaction time to 72 hours (table 2, *item 2*). It allowed to obtain the ONO monoester (5) in 5.1 % yield and traces of the diester (6) were observed, according to its $^{1}$H NMR spectrum.

In a further attempt, CDI as a coupling agent (table 2, *item 3*) was used, but only traces of monoester (5) were detected according to the $^{1}$H NMR spectrum. Lastly, an aliphatic acid (the 3-bromopropionic) was used in the same conditions of *item 2.1* without achieving any progress (table 2, *item 4*). The esterification of ONO under Steglich conditions led to low reaction yields of the monoesters (5); 4-methoxybenzoic anhydride (identified by $^{1}$H NMR) was obtained as a main product. Therefore, other reaction conditions were applied, using more reactive acid derivatives, such as acyl chlorides and anhydrides.

***b. Reactions with acyl chlorides***

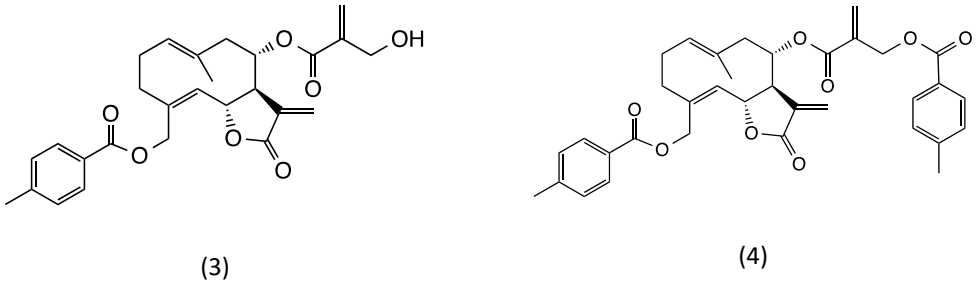
Reaction of ONO with acyl chloride in the presence of TEA was performed in THF or the mixture of DCM-DMF because of low solubility of ONO. ONO was treated with 4-methoxybenzoic chloride under these conditions (table 3, *items 1* and *2*). However, the reactions did not lead to formation of desired esters (5) and (6). Replacement of TEA with pyridine (table 3, *item 3*), together with addition of DMAP (table 3, *item 4*) allowed to obtain the diester (6) in 6.0 % and monoester (5) in 16.1 % yields, (Figure 25).



(5) (6)

**Figure 25.** Structures of 3´-(4-methoxybenzoyl)onopordopicrin (5) and 15,3´-di(4-methoxybenzoyl)onopordopicrin (6)

The reaction of ONO and 4-methylbenzoic chloride was performed in pyridine (table 3, *item 5*), in presence of DMAP (table 3, *item 6*), and in THF in presence of DMAP and TEA (table 3, *item 7*). Under first condition it was observed formation of monoester (3) in 2.2 % yield. The second led to formation of monoester (3) in 3.6 % yield and diester (4) in 3.0 % yield. The last one allowed to obtain diester (4) in 12.6 % yield and monoester (3) in 17.8 % yield, (Figure 26).

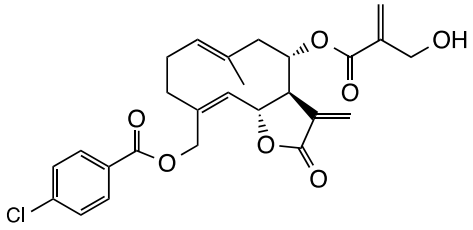


(3) (4)

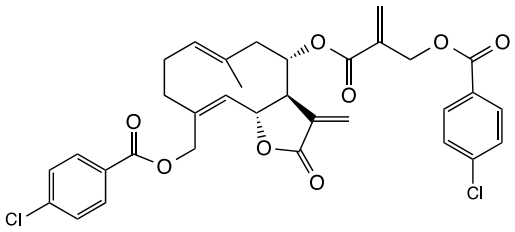
**Figure 26.** Structures of 15-(4-methylbenzoyl)onopordopicrin (3) and 15,3´-di(4-methylbenzoyl)onopordopicrin (4)

The use of chlorine derivatives, 4-chlorobenzoic and 3-chlorobenzoic chloride in pyridine, (table 3*, items 8* and *9*) provided moderate results. The reaction with 4-chlorobenzoic chloride resulted in 20.3 % yield in diester (9) and 8.0 % yield for monoester (8), (Figure 27). The reaction with 3-chlorobenzoic chloride provided only

monoester (7) in 7.7 % yield, (Figure 28). The reaction of ONO with 3,5-dinitrobenzoic chloride did not proceed (table 3, *item 10*).

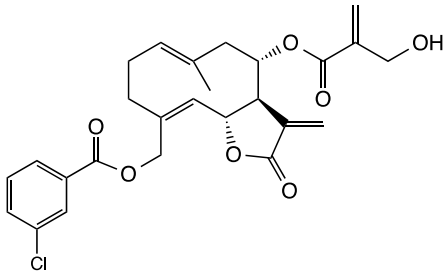



(8)




(9)

**Figure 27.** Structures of 15-(4-chlorobenzoyl)onopordopicrin (8) and 15,3´-di(4-chlorobenzoyl)onopordopicrin (9)




(7)

**Figure 28.** Structure of 15-(3-chlorobenzoyl)onopordopicrin (7)

The treatment of ONO with different acyl chlorides gave mono and/or diester in low yields (7.7 - 20.3 %), in general the monoester was formed on OH group in position 15, however, in case of the MOBCl took place on OH group of the side chain.

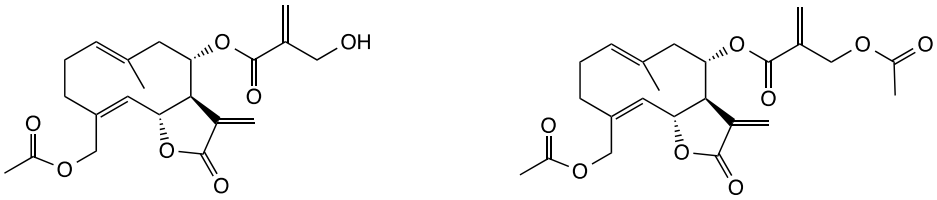
### *c. Yamaguchi esterification*

The next method for the esterification of natural products, that was described by Yamaguchi et al.[37], was applied. It is described that by this method esters could be prepared in high yields using an alcohol, together with an aliphatic acid and aromatic acid chloride.

The method was carried out with ONO and two different aliphatic acids. In the first reaction, 3-bromopropionic acid and 4-methylbenzoic chloride (table 4, *item 1*) were used, and in the second, hexanoic acid and 4-methylbenzoic chloride (table 4, *item 2*) were tried. In both cases only moderate evolution was observed. The isolated compounds were mixtures of aliphatic and aromatic esters (annex - Figures 49 and 50).

### *d. Reactions with anhydrides*

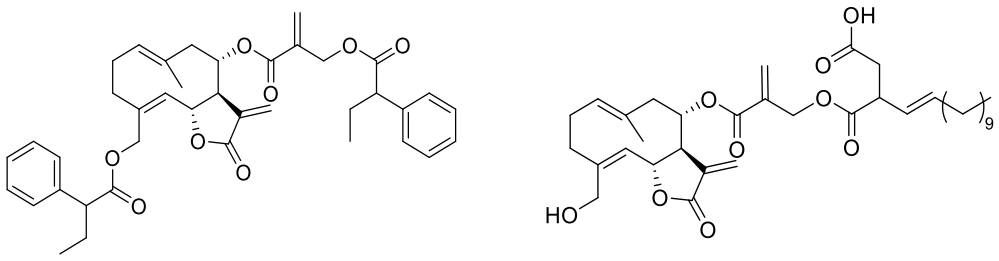
The ONO was treated under standard acetylation conditions: reaction of ONO with $Ac_2O$ in pyridine for 24 hours at rt led to formation of the diester (2) in moderate yield (26.0 %) and the monoester (1) in very low yields of (1.8 %) (table 5, *item 1*), (Figure 29).



(1) (2)

**Figure 29.** Structures of 15-acetylonopordopicrin (1) and 15,3´-diacetylonopordopicrin (2)

ONO was treated under the same conditions with phenyl-butyric anhydride (table 5, *item 2*) and dodecenyl-succinic anhydride (table 5, *item 3*), obtaining the diester (12) in the first case with a 44.2 % yield and the monoester (13) in the second reaction in 21.7 % yield, (Figure 30). The fact that the diester with dodecenyl-succinic anhydride was not observed could be attributed to the large size of the aliphatic chain, which could exert some type of stereo hindrance.



(12) (13)

**Figure 30.** Structures of 15,3´-di(2-phenylbutyroyl)onopordopicrin (12) and 3´-dodecenylsuccinoylonopordopicrin (13)

### *e. Mukaiyama esterification*

The treatment of 1-nonanol with MOBA, Mukaiyama's reagent in DMC and DMAP catalysis (table 6, *item 2*), led to obtaining the expected ester (17), that was not purified, (Figure 31).

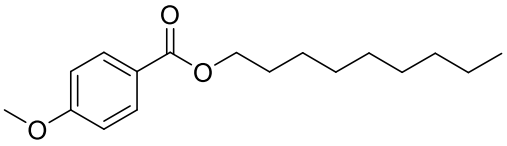
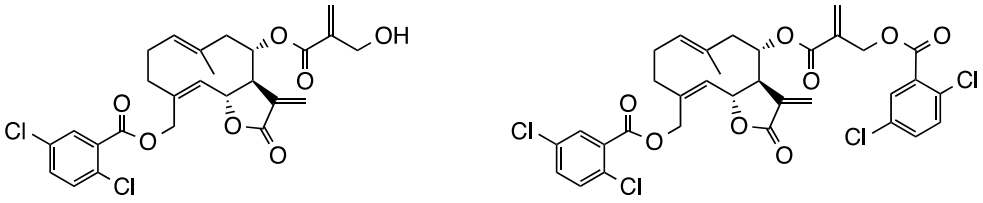


**Figure 31.** Structure of nonyl 4-methoxybenzoate (17)

Reaction of ONO with MOBA under the same condition led to formation of the corresponding diester (6) with a yield of 13.5 % and equivalent proportion of MOB-anhydride (table 6, *item 3*). The same reaction conditions were also applied to ONO and 2,5-dichlorobenzoic acid giving the diester (11) in 52.0 % yield and traces of the monoester (10) (table 6, *item 4*), (Figure 32).



(10) (11)

**Figure 32.** Structures of 15-(2,5-dichlorobenzoyl)onopordopicrin (10) and 15,3´-bis(2,5-dichlorobenzoyl)onopordopicrin (11)

**Synthesis of 1,10-epoxyonopordopicrin**

The most widely used procedure to synthesize epoxides is a reaction of the compound with *m*CPBA in DCM. It has been previously indicated that ONO is slightly soluble in DCM, so co-solvents must be added, or the solvent in the reaction must be changed. The different reaction conditions (different solvents, *m*CPBA equivalents, *m*CPBA addition order, temperature, and reaction time) have been applied. In general, difficult-to-separate reaction mixtures were obtained. In their $^{1}H$ NMR and $^{13}C$ NMR spectra, signals corresponding to esters of *m*-chlorobenzoic acid and an aldehyde among others, can be seen.

The first reaction was carried out with 1.5 equivalents of *m*CPBA, without evolution (table 7, *item 1*). Then the amount of *m*CPBA was risen to 3.6 equivalents (table 7, *item 2*), without success, and then to final 4.0 equivalents of *m*CPBA, that were considered to be needed. Also, the change of solvent system from pyridine/DCM to pyridine/THF was advantageous and led to obtention of epoxide (14) in 15.5 % yield (table 7, *item 3*). In order to optimize the reaction conditions to gain better yields, other

conditions were applied. The treatment of the reaction at 0 °C led to better formation of epoxide, but the formed epoxide reacted, with excessive amounts of *meta*-chlorobenzoic acid resulting in a formation of its ester as a side product (table 7, *item 4*). Then, the addition order was changed (table 7, *item 5*), without significant progress, the pyridine equivalents were risen to 2.0 equivalents (table 7, *item 8*), but only traces of some aldehyde were observed. Joining these two changes (table 7, *item 6*) led to obtention of some aldehydes, which were difficult to separate. Finally, the usage of 3.2 equivalents of pyridine as a main difference to previous conditions (table 7, *item 7*) led to formation of 1,10-epoxide (14) in 21.4 % yield and aldehyde (15) in 7.7 % yield, (Figure 33).

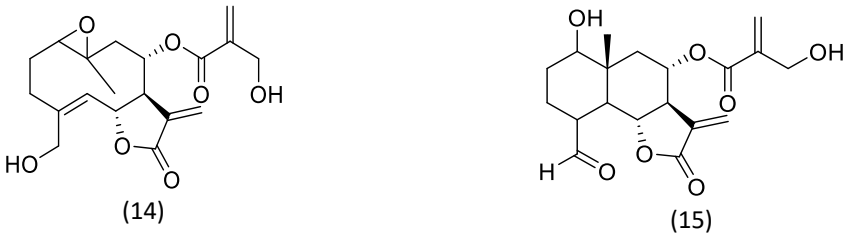



**Figure 33.** Structures of 1,10-epoxyonopordopicrin (14) and onopordopicrin-15-aldehyde (15)

The chosen method should provide epoxides on double bonds 1,10 and 4,5 with sterical preference to 1,10-double bond. From the NMR spectra of all reactions was observed, that the terminal methylenes (C-13, C-4´) do not change. The epoxidation of 1,10-double bond was confirmed after interpretation of 1D-NMR and 2D-NMR experiments and comparison with spectroscopic data in literature.[47] The 1,10-epoxide was treated with different Lewis acids under Barrero et al. conditions,[41] in order to study the possible closure of the germacran ring, however, only complex mixtures of difficult resolution were obtained.

## BIOASSAYS AND SAR

The extract, the fractions of *C. ornata* and some pure synthesised compounds were sent to be tested *in vitro* against *Leishmania donovani*, *Trypanosoma cruzi* and *Plasmodium falciparum*. Unfortunately, the results are not available at the time of writing this diploma work, therefore SAR cannot be established.

## CONCLUSIONS

*1)* The ether extract obtained from the aerial part of *Centaurea ornata* represented 0.26 % of the dried plant. The extract was fractionated by solvents of increasing polarity. The chloroform fraction containing ONO represented 64.1 % of the crude extract, which after purification by column chromatography gave pure onopordopicrin in 54.8 % of the crude extract.

*2)* Onopordopicrin was used to obtain ester derivatives and the epoxide on the double bond at 1,10. To obtain the esters, five different methods were applied: a) Steglich esterification, b) reaction with acyl chlorides, c) Yamaguchi esterification, d) reaction with anhydrides, and e) Mukaiyama esterification, of which the best results were obtained by methods b), d) and e). Thus, 7 monoesters and 6 diesters were synthesized and characterized according to their physicochemical properties. The Steglich and Yamaguchi methods provided very low yields (up to 5.1 %) with difficult purification of product. For the reaction with acyl chlorides low yields (up to 20.3 %) were obtained. The disadvantage of this method is the formation of an anhydride of chosen acid as a side product in excessive amounts. In the case of treatment with anhydrides low to moderate yields (from 21.7 to 44.2 %) were obtained in the three reactions carried out, either in the mono or diester. The Mukaiyama method seemed to be the best method for preparation of diesters with moderate yields (up to 52.0 %), and easy separation and purification of product. Unfortunately, the method was applied only in two reactions with ONO due to time restrictions, and its suitability for preparation of other esters needs to be tested.

*3)* Onopordopicrin 1,10-epoxide was difficult to obtain. The reactions ran in low yields due to the complexity of the molecule, isolating among other derivatives an aldehyde.

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## ANNEXES

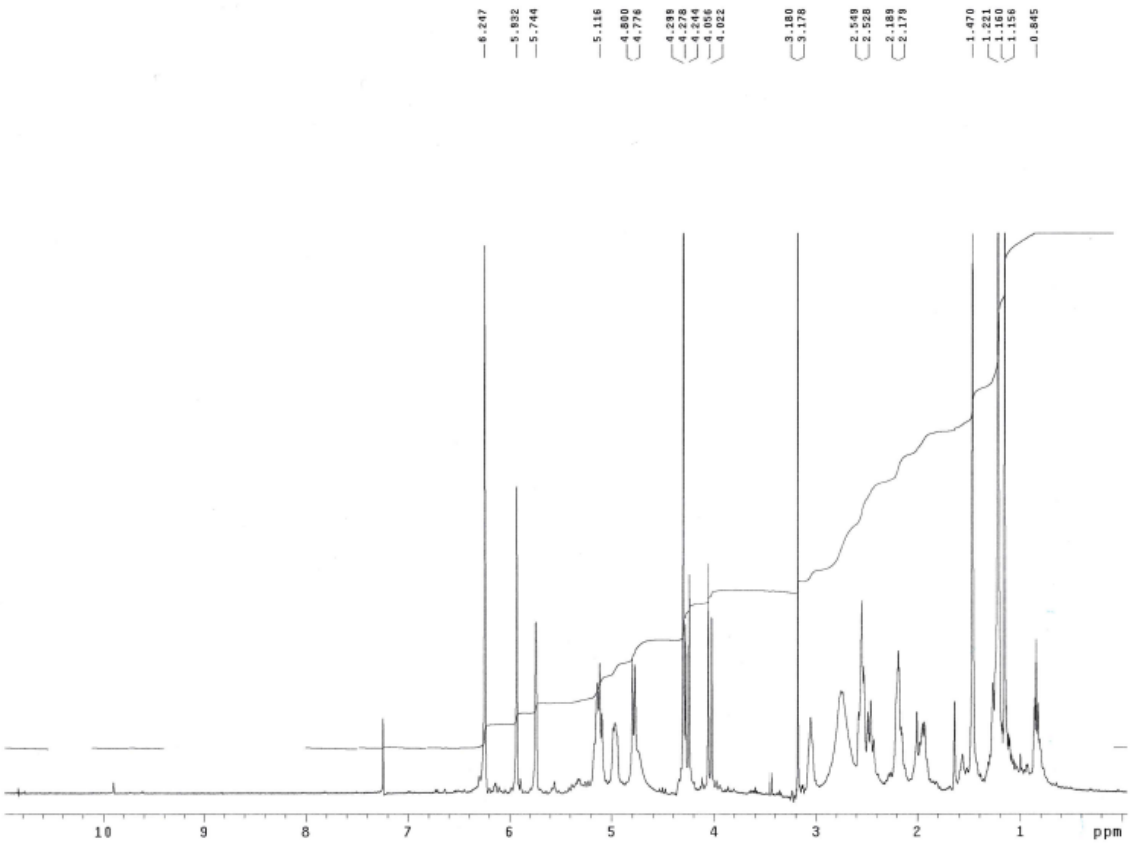


**Figure 34.** $^{1}$H NMR spectrum of ether extract of *C. ornata*

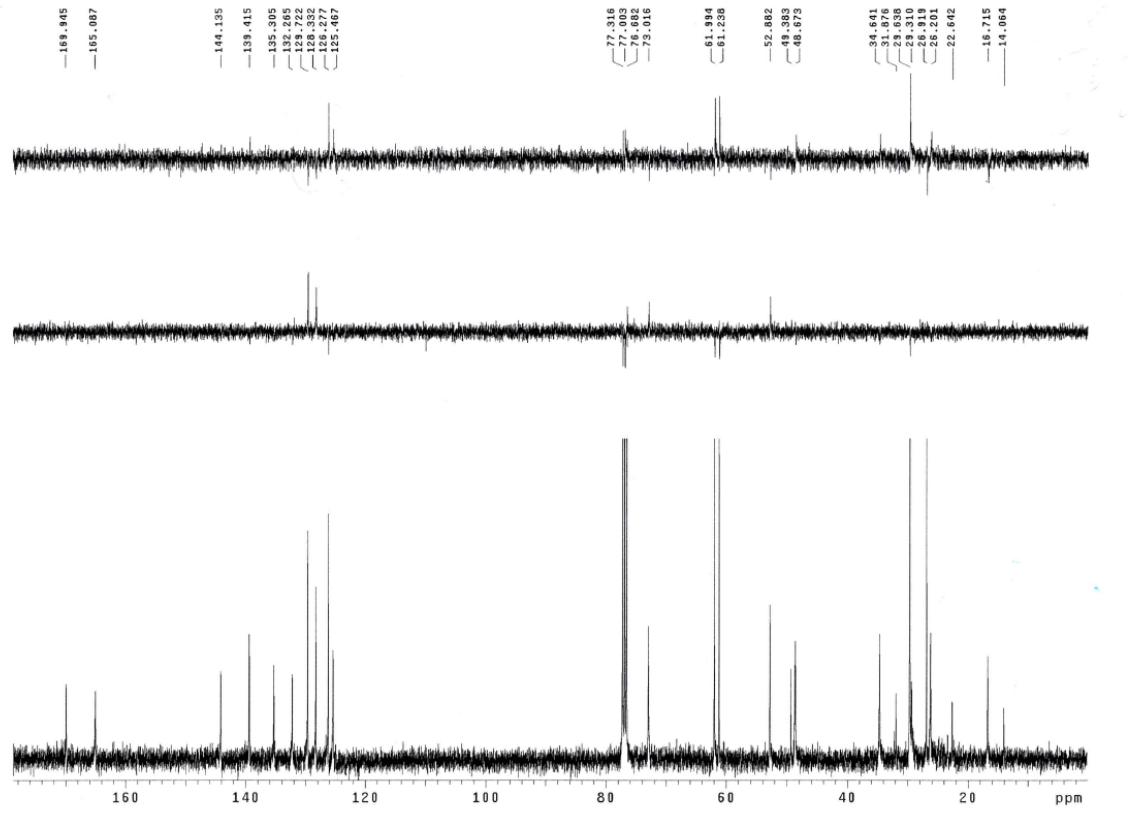


**Figure 35**. $^{13}$C NMR spectrum of ether extract of *C. ornata*

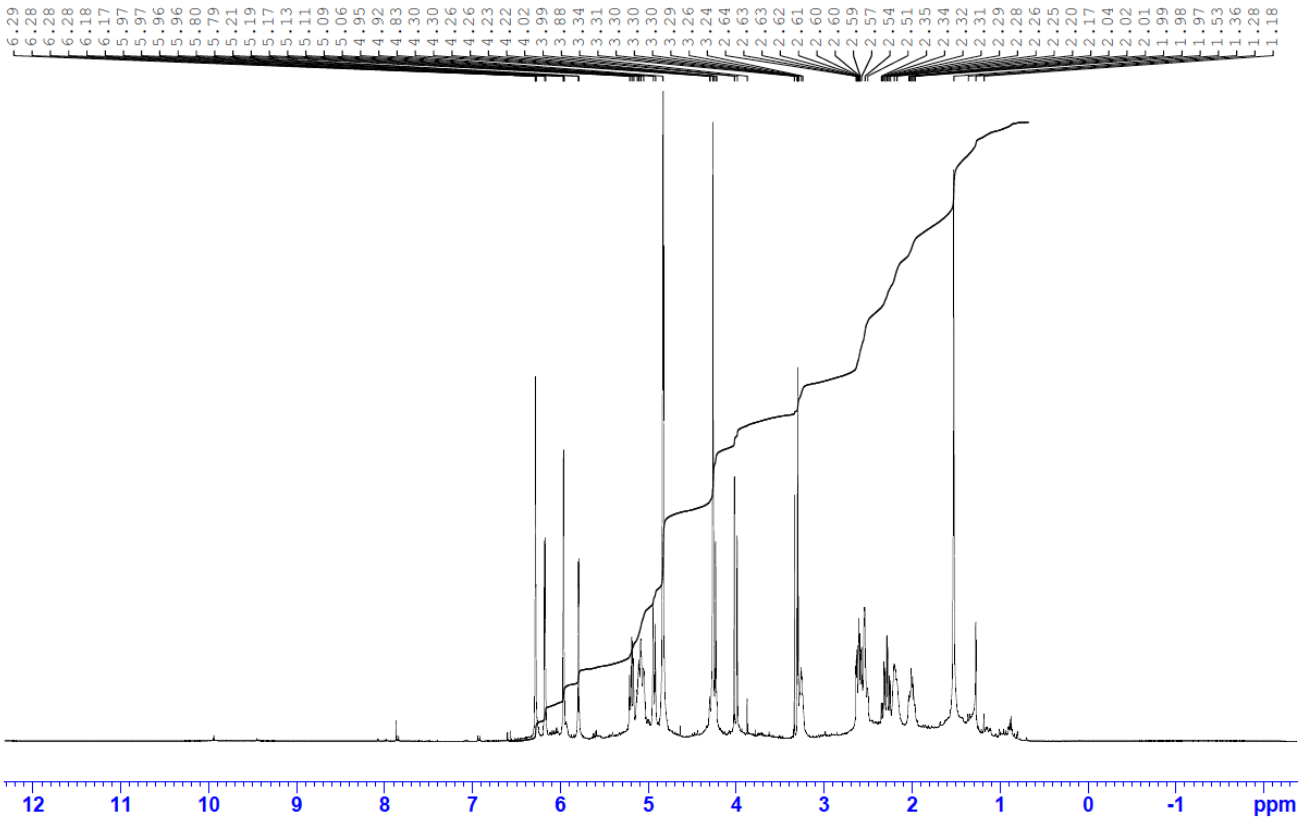


**Figure 36.** $^{1}$H NMR spectrum of onopordopicrin

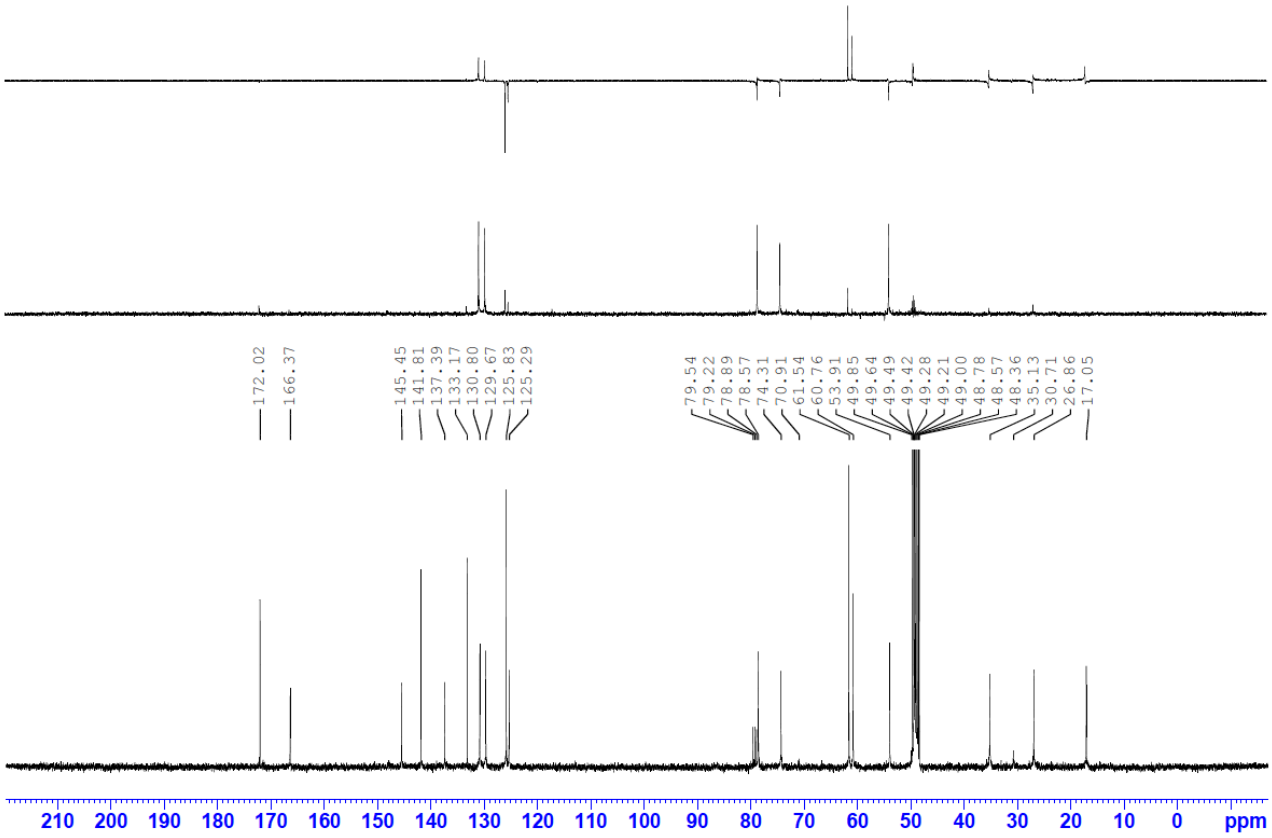


**Figure 37.** $^{13}$C NMR spectrum of onopordopicrin

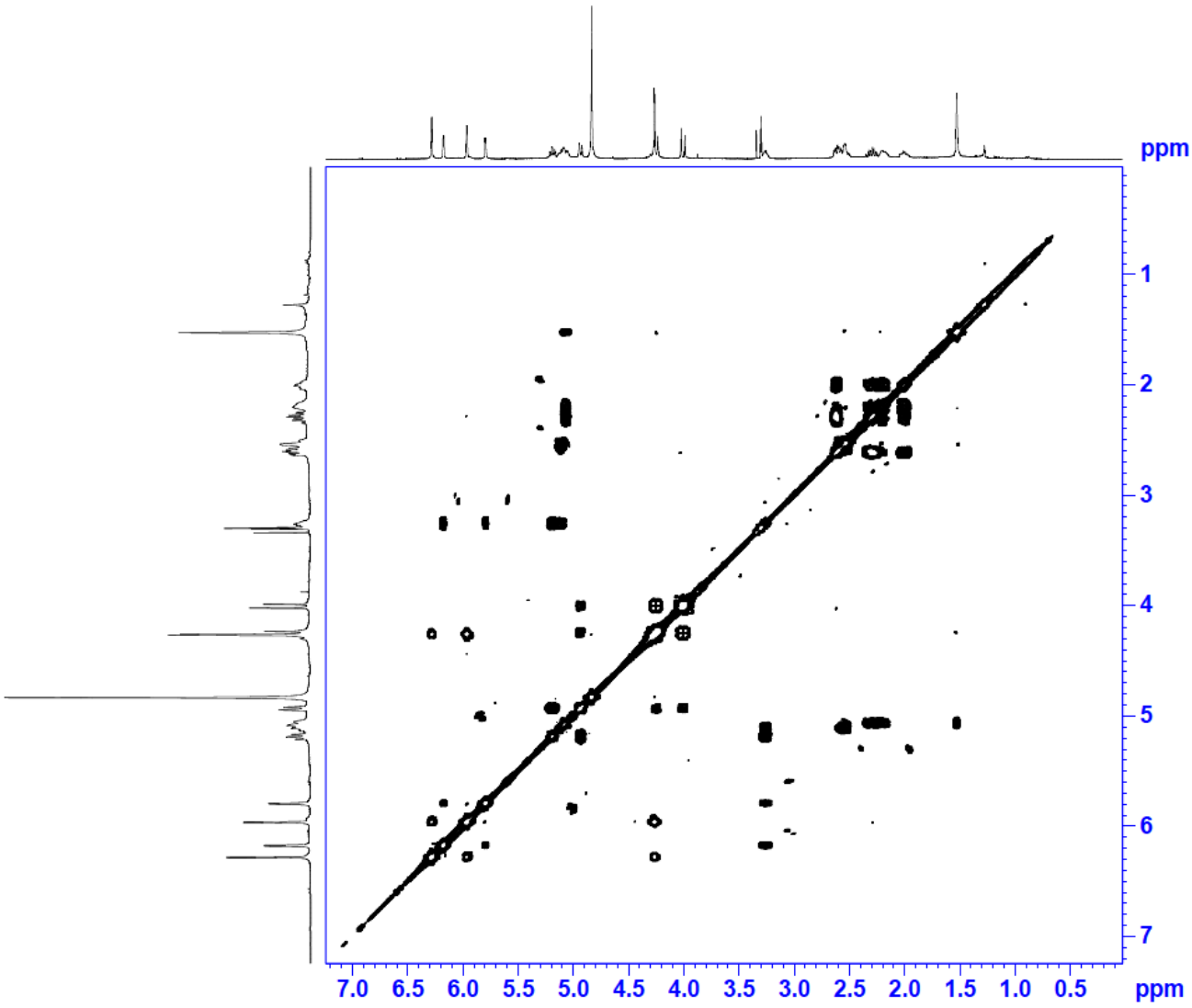


**Figure 38.** COSY spectrum of onopordopicrin

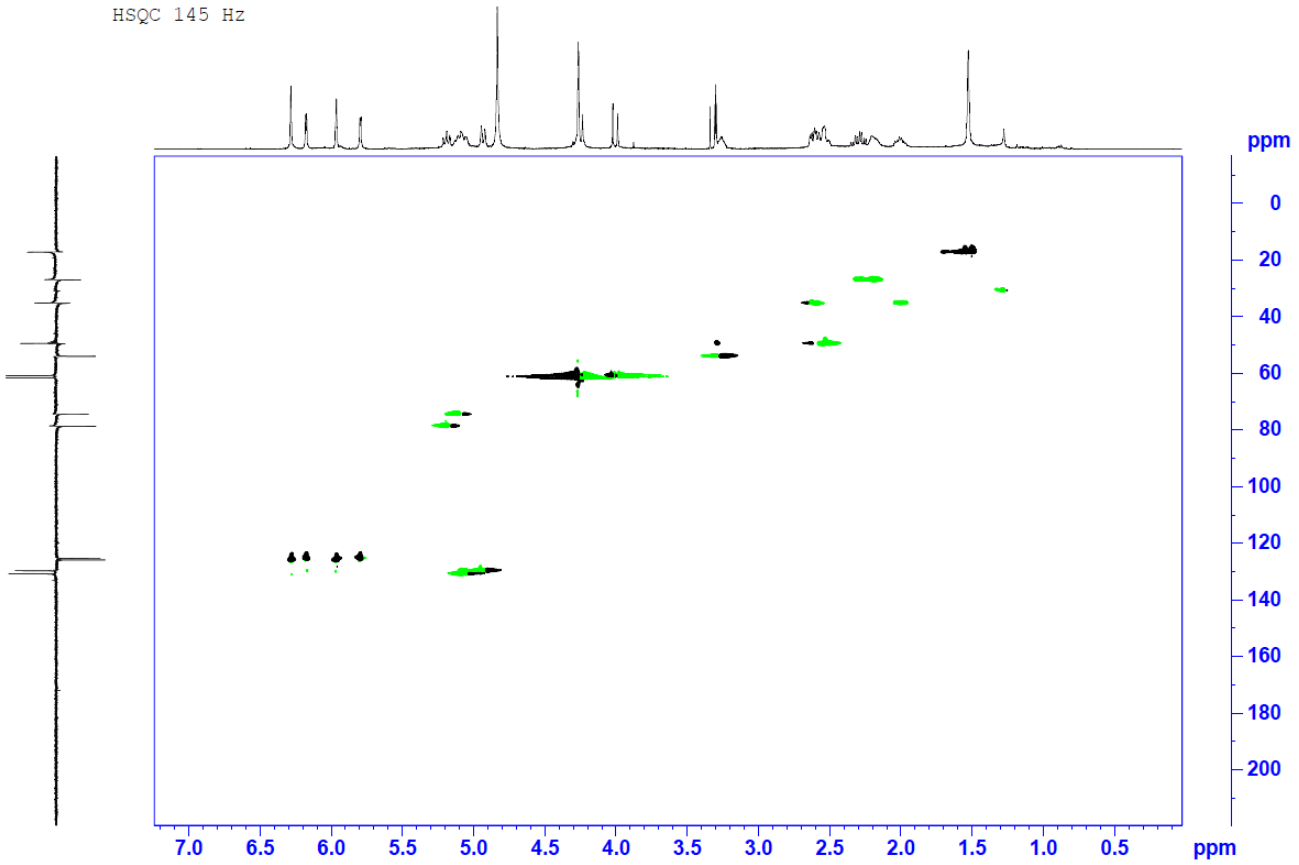


**Figure 39.** HSQC spectrum of onopordopicrin

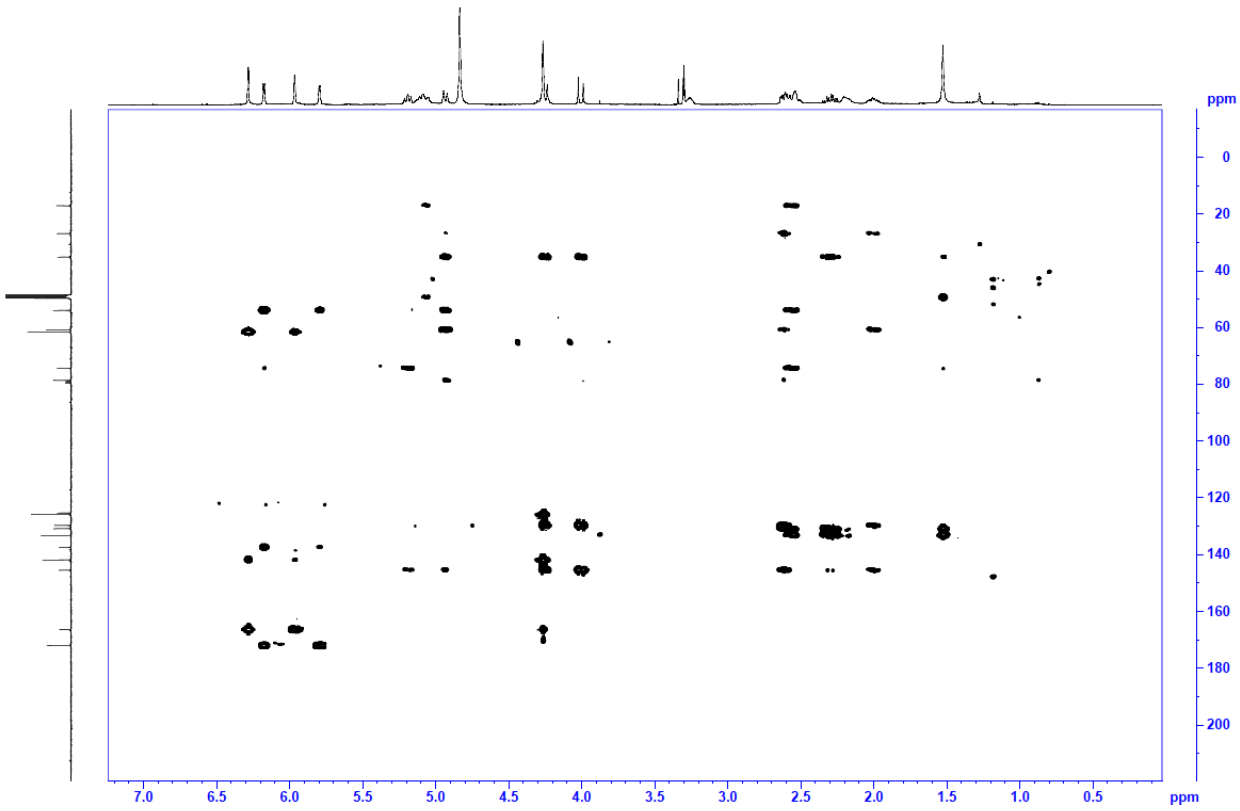


**Figure 40.** HMBC spectrum of onopordopicrin

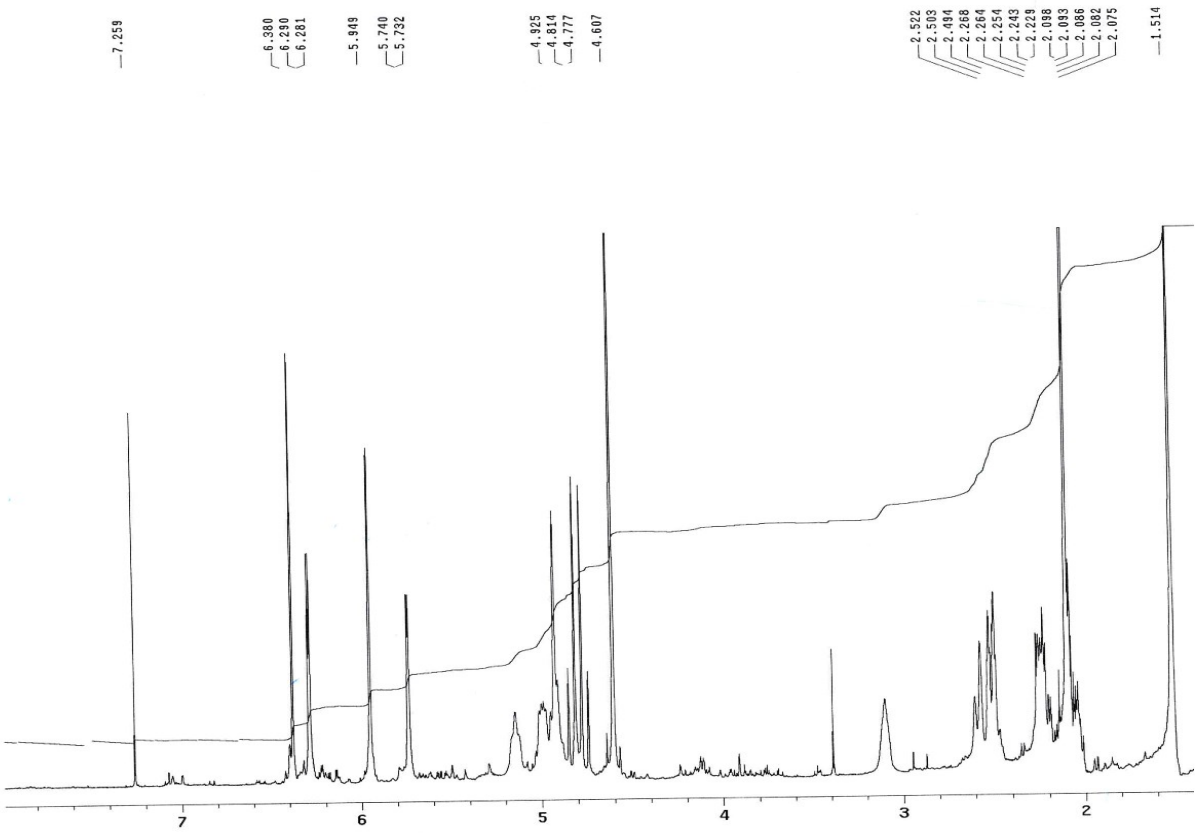


**Figure 41.** $^{1}$H NMR spectrum of compound (2)

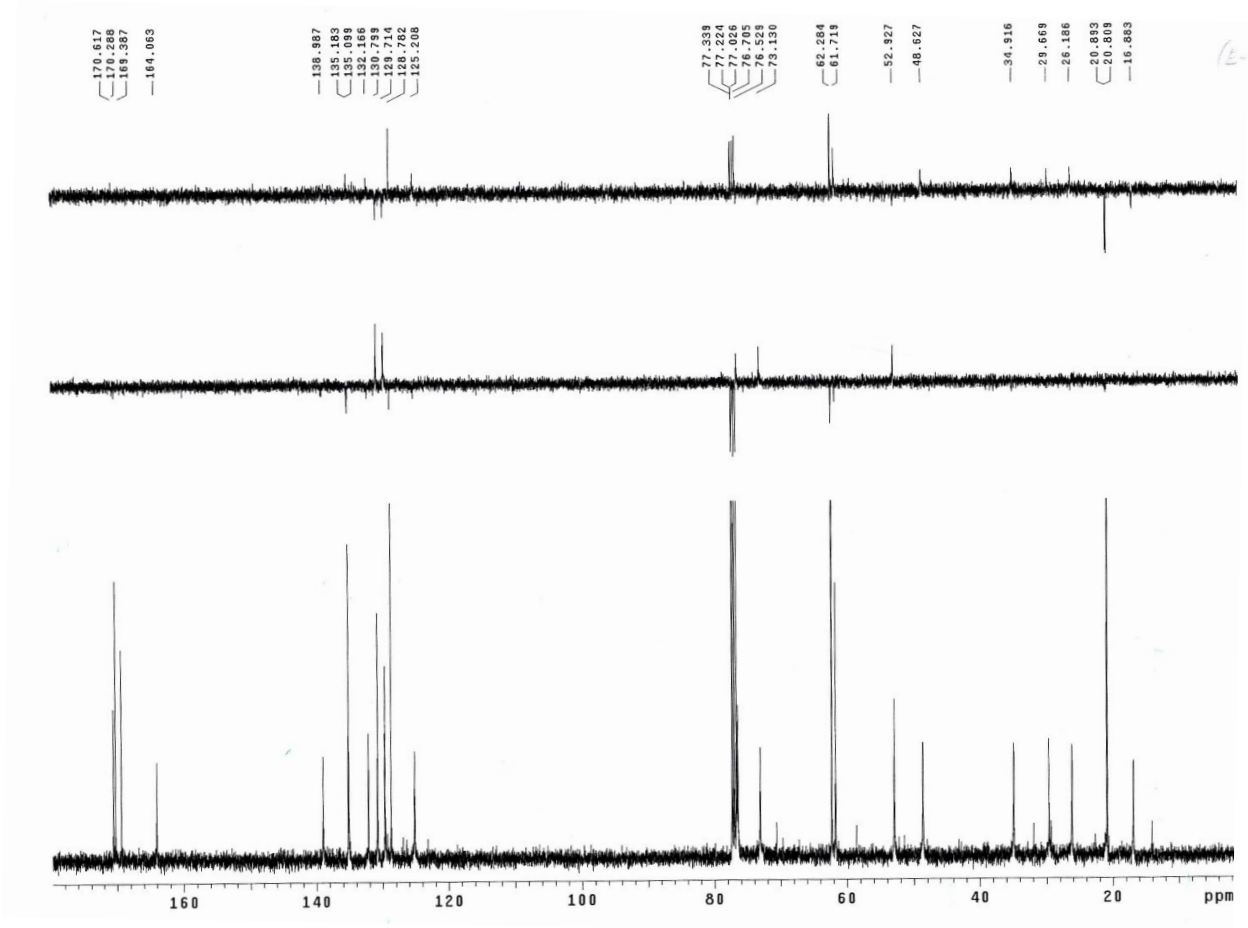


**Figure 42.** $^{13}$C NMR spectrum of compound (2)

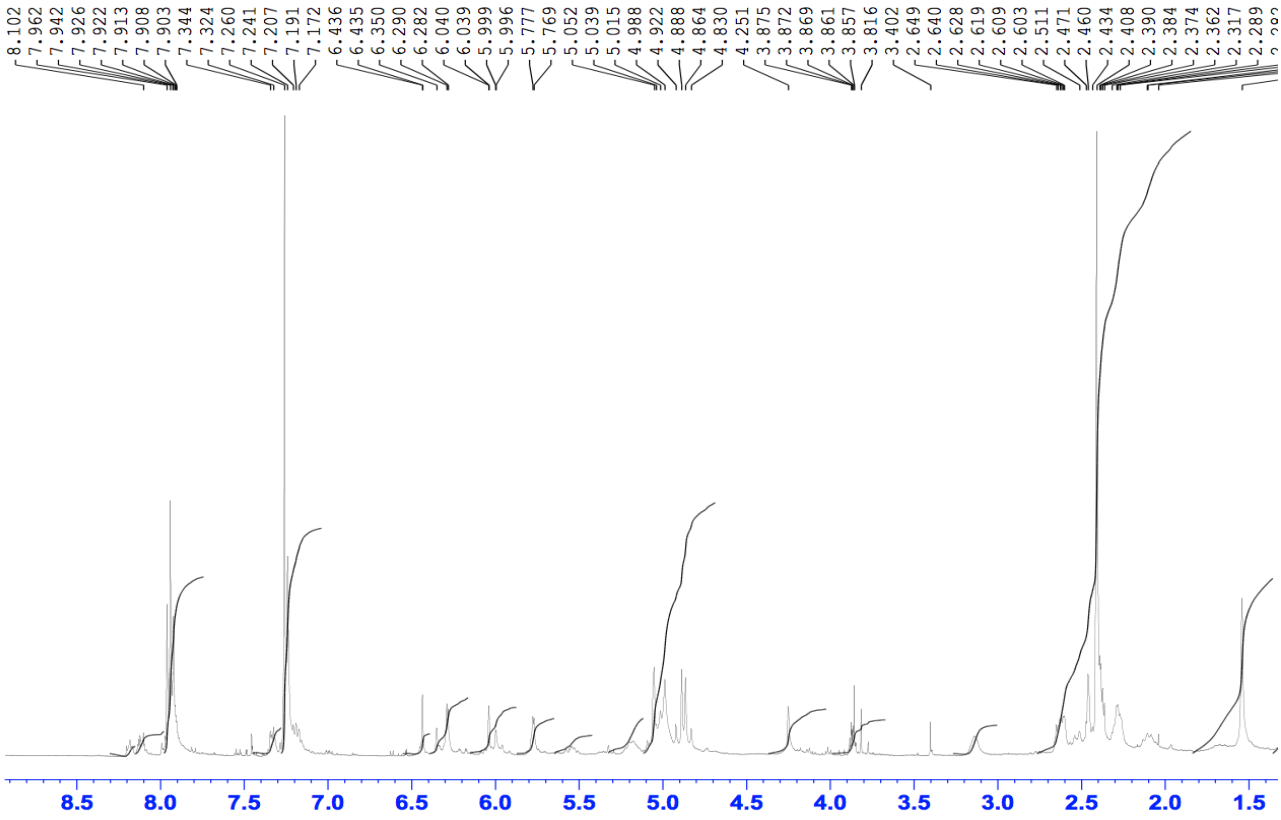


**Figure 43.** $^{1}$H NMR spectrum of compound (3)

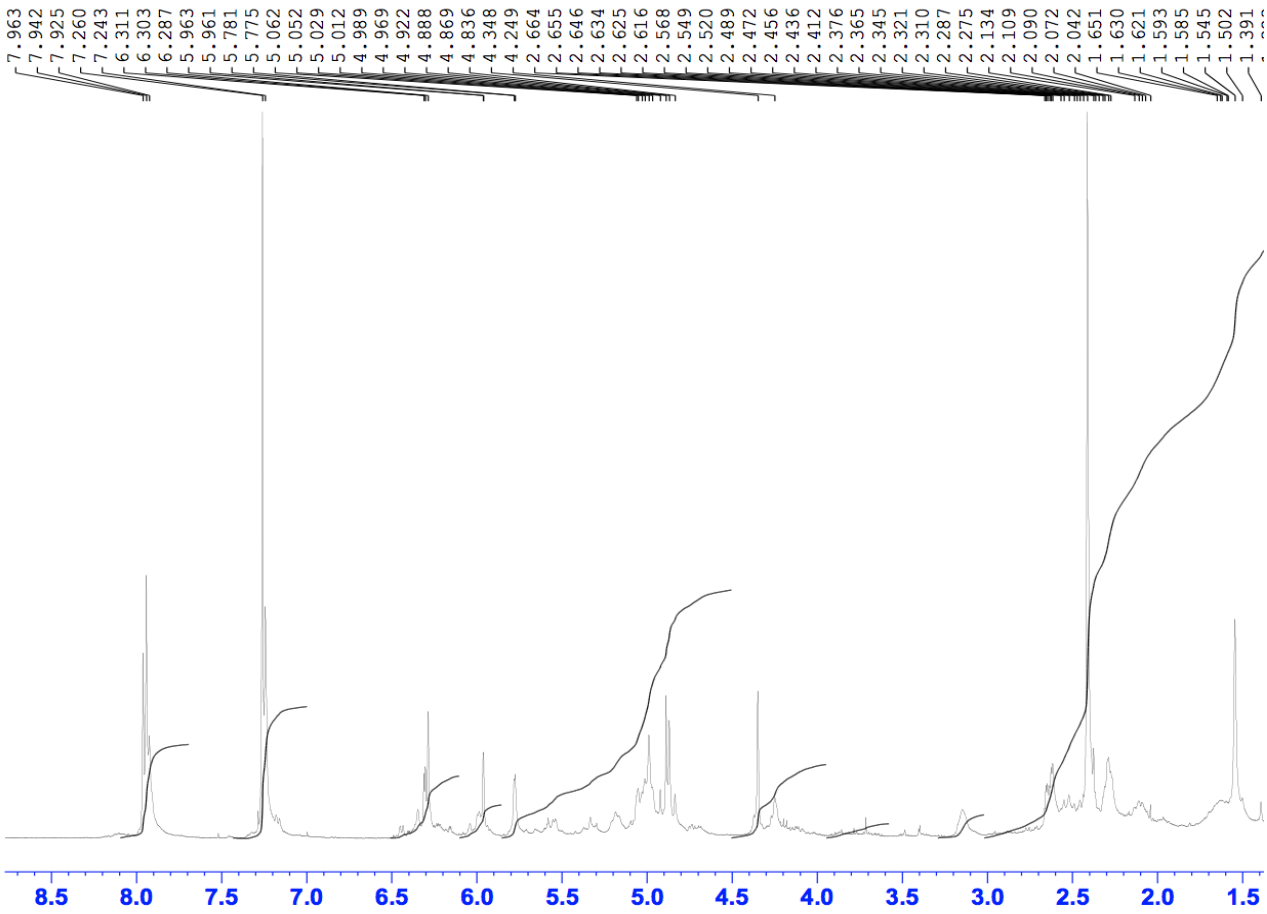


**Figure 44.** $^{1}$H NMR spectrum of compound (4)

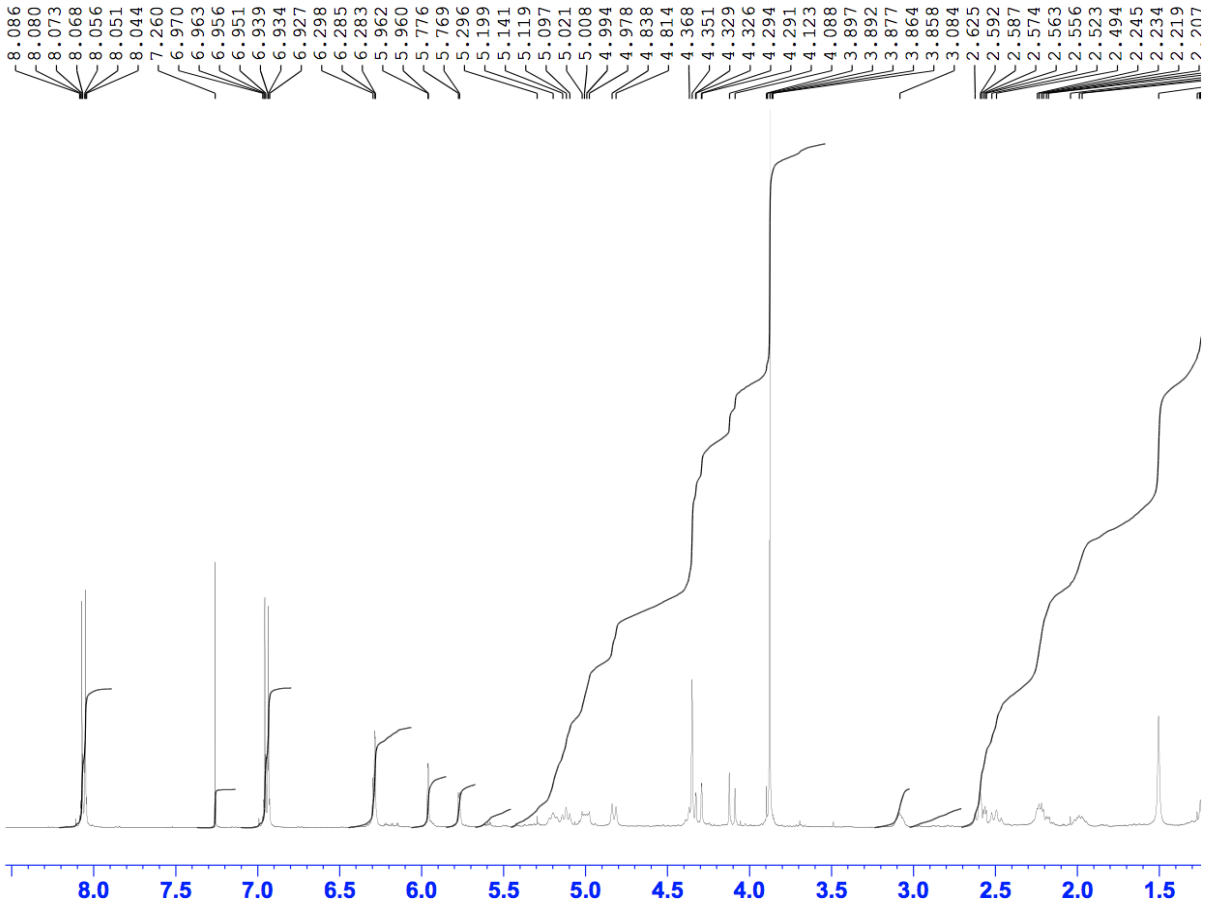


**Figure 45.** $^{1}$H NMR spectrum of compound (5)

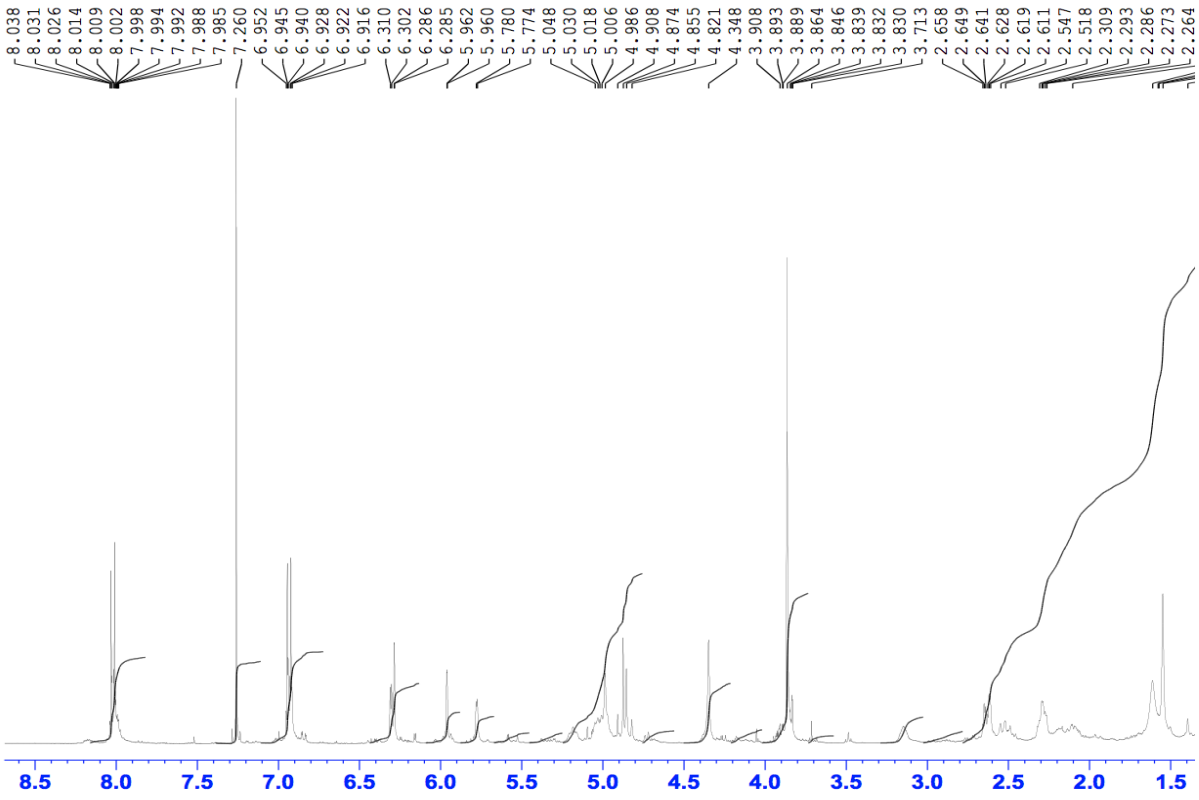


**Figure 46.** $^{1}$H NMR spectrum of compound (6)

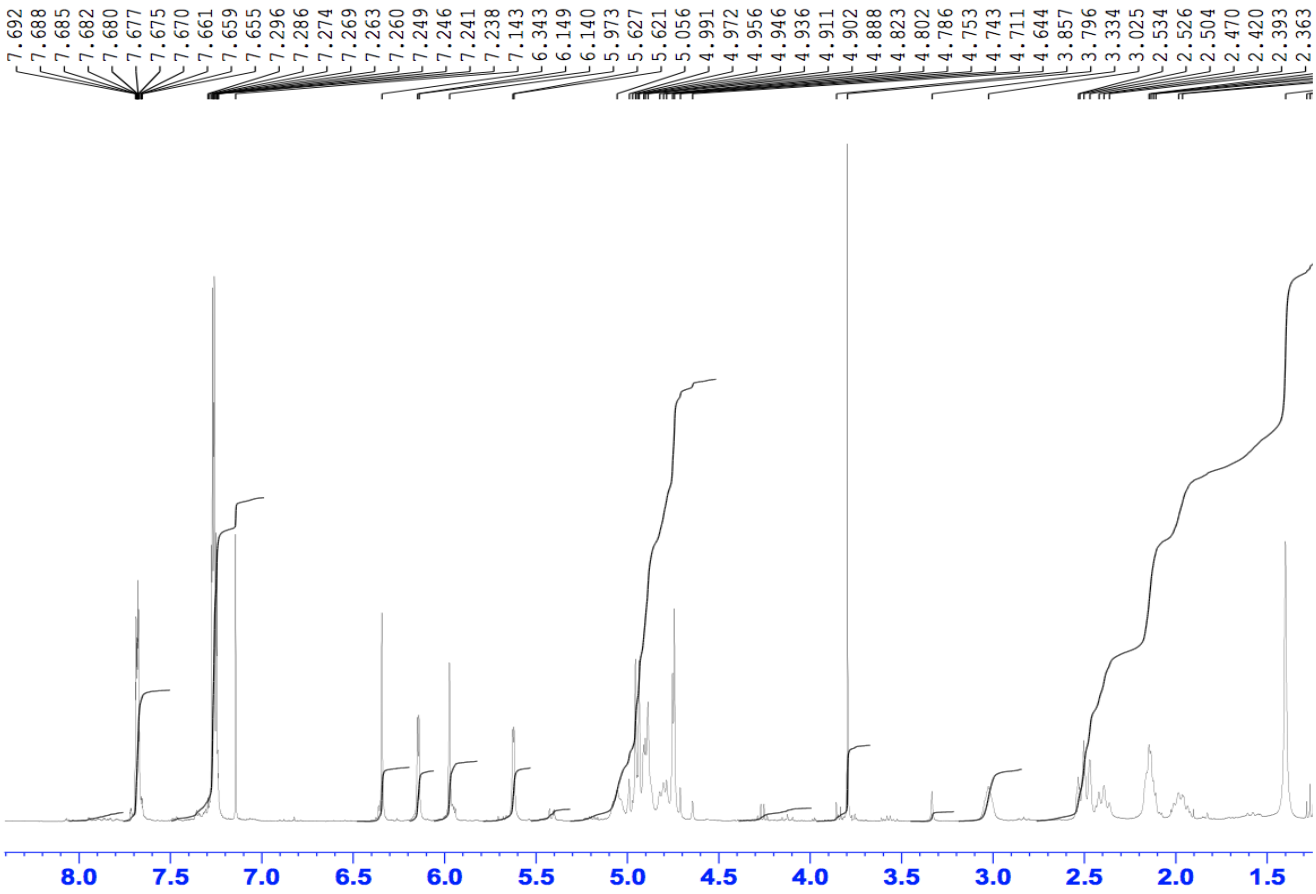


**Figure 47.** $^{1}$H NMR spectrum of compound (11)

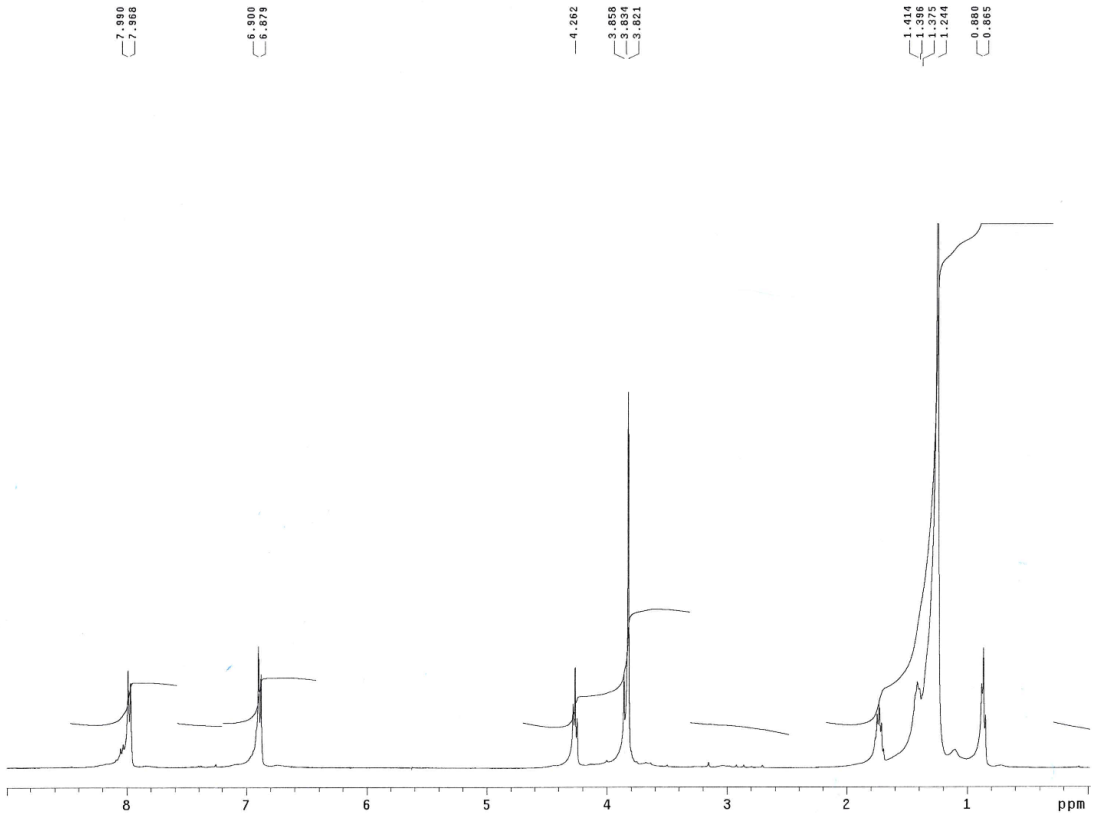


**Figure 48.** $^{1}$H NMR spectrum of the tetradecyl 4-methoxybenzoate (16)

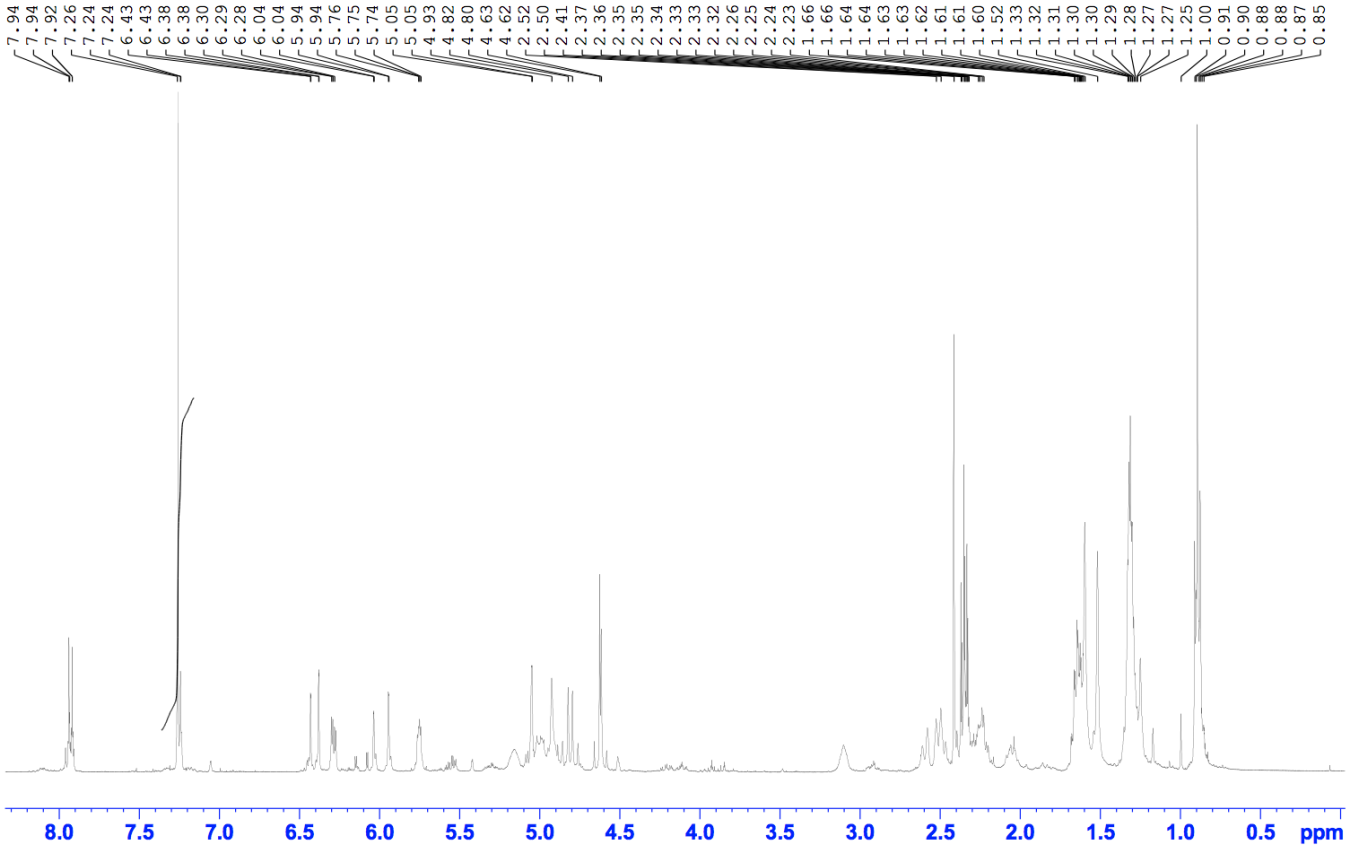


**Figure 49.** $^{1}$H NMR spectrum of ONO-aromatic/ONO-aliphatic diester obtained by Yamaguchi method

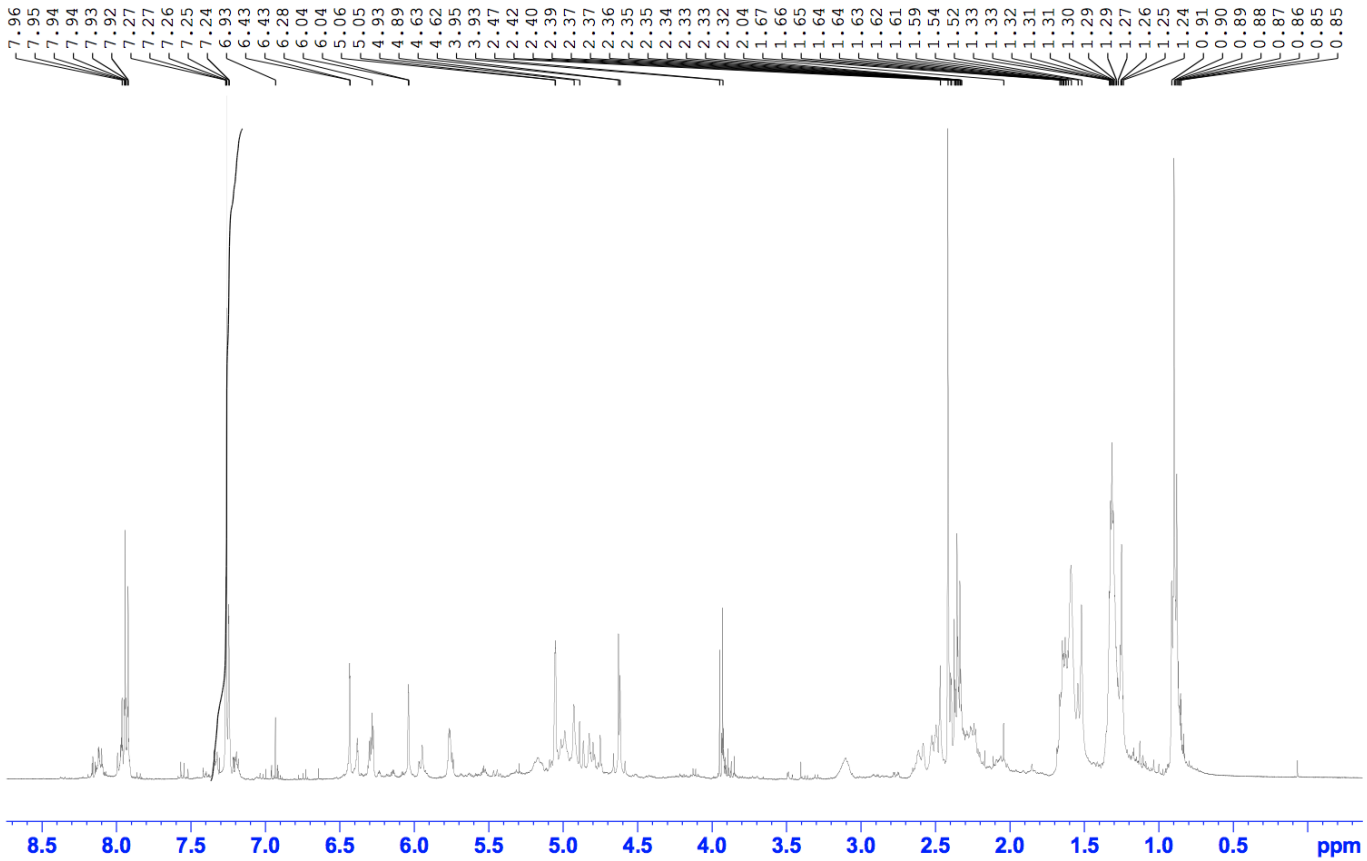


**Figure 50.** $^{1}$H NMR spectrum of ONO-aromatic/ONO-aliphatic monoester obtained by Yamaguchi method