Universidade de Lisboa

Faculdade de Farmácia

Electrochemical oxidation of abietanes

Inês Alexandra de Sá Martins

Trabalho de Campo orientado pelo Doutor Jaime Alfredo da Silva Coelho, Investigador Auxiliar da Faculdade de Ciências da Universidade de Lisboa e coorientado pelo Professor Doutor Carlos Alberto Mateus Afonso, Professor Catedrático da Faculdade de Farmácia da Universidade de Lisboa.

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Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

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Resumo

Os pinheiros são coníferos, dos quais se pode adquirir a madeira e extrair o "tall oil" bruto, a resina e a goma da madeira. Através da destilação da resina podemos adquirir a colofónia e terebintina. A colofónia consiste numa mistura complexa de terpenos neutros e diterpenos ácidos, onde se incluem os abietanos, que são diterpenoides tricíclicos aos quais o ácido abiético (AA) e dehidroabiético (DHA) pertencem.

A eletroquímica combina a transferência de eletrões no elétrodo com uma reação química, sendo que podem ser controladas utilizando corrente ou potencial constante. Acabam por ser reações "mais verdes", seguras, eficientes e económicas. A eletrólise pode ser direta, onde se oxida/reduz diretamente a molécula, ou indireta, onde um se utiliza mediadores, reagentes capazes de oxidar/reduzir seletivamente a molécula.

A eletroquímica em fluxo é uma técnica que permite o *scale up* industrial da eletroquímica, tendo vantagens sobre esta como o oferecer uma maior proximidade entre elétrodos e uma maior área de superfície do elétrodo, o que diminui o tempo de reação e permite a dispensa do uso de eletrólitos de suporte.

Neste contexto, o principal objetivo desta investigação assentou em acrescentar valor biossintético à colofónia e aos seus abietanos, utilizando reações orgânicas clássicas, eletroquímica e química em fluxo para sintetizar novos derivados e estudar os potenciais de oxidação destes compostos.

Relativamente ao trabalho experimental, começou se por preparar os derivados metil éster do AA e do DHA, pois seriam compostos mais estáveis. Estudamos os potenciais de oxidação destes compostos. Desenvolvemos e otimizamos protocolos para a oxidação eletroquímica, onde conseguimos alcançar a oxidação benzílica direta tanto do DHA como do MDHA com bons rendimentos, a oxidação indireta do MDHA (utilizando Cl_4NHPI como mediador) e também conseguimos alcançar a oxidação de AA e MAA com rendimentos moderados. Por fim estudou-se o uso de eletroquímica em fluxo para realizar estas oxidações, usando-se o DHA como material de partida.

Palavras-chave: Colofónia, Abietanos, Eletroquímica, Mediadores, Fluxo contínuo.

Abstract

Pine trees are coniferous, from which we can extract wood, raw tall oil, wood gum, and gum. Through the distillation of gum, we can acquire turpentine and colophony.

Colophony is a complex mixture of neutral terpenes and acidic diterpenes, including abietanes, which are tricyclic diterpenoids to which abietic acid (AA) and dehydroabietic acid (DHA) both belong.

Electrochemical reactions combine the electron transfer at an electrode with a chemical reaction and can be controlled using constant current or constant potential. Overall, they are very environmentally friendly, safer, efficient, and economical. The electrolysis process can be direct, where we are able to directly oxidize/reduce the molecule, or indirect, where we use a mediator – a selective oxidizing/reducing reagent.

Flow electrochemistry is a technique that allows the industrial scale up of electrochemistry, having advantages over it such as offering greater proximity between electrodes and a higher surface area of the electrode, which decreases the reaction time and allows the dispensation of the use of supporting electrolytes.

In this regard, the main objective of this research was to add biosynthetic value to colophony and its abietanes, using classic organic reactions and electrochemistry to synthesize new derivates and then study the oxidation potentials of these compounds.

Regarding the experimental work, we started by preparing the methyl ester derivatives of AA and DHA, as they would be more stable compounds. We studied the oxidation potentials of these compounds. We developed and optimized protocols for electrochemical oxidation, where we achieved the direct benzylic oxidation of both DHA and MDHA with good yields, the indirect oxidation of MDHA (using Cl_4NHPI as a mediator) and we also achieved the oxidation of AA and MAA with moderate yields. Finally, we studied the use of flow electrochemistry to achieve these oxidations using DHA as starting material.

Keywords: Colophony, Abietanes, Electrochemistry, Mediators, Continuous flow.

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Acronyms

δ	Chemical shift
AA	Abietic Acid
d	Duplet
CE	Counter electrode
COSY	Correlation spectroscopy
DCM	Dichloromethane
DHA	Dehydroabietic Acid
DMF	Dimethylformamide
ET	Electron transfer mediator
HSQC	Heteronuclear single quantum correlation
HMQC	Heteronuclear Multiple Quantum Coherence
H−T	Hydride transfer mediator
HAT	Hydrogen atom transfer mediator
J	Coupling constant
M	Multiplet
MAA	Methyl Ester of Abietic Acid
MDHA	Methyl Ester of Dehydroabietic Acid
MTBE	Methyl tert-butyl ether
NMR	Nuclear magnetic resonance
Rpm	rotations per minute
r.t	Room temperature
s	Singlet
sept	Septet
t	Triplet
TBATFB	Tetrabutylammonium tetrafluoroborate

THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV	Ultraviolet
WE	Working electrode

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1 Introduction

1.1 Pinus Resin

Pine trees, which belong to the genus *Pinus*, are evergreen coniferous that are broadly distributed in the northern hemisphere, from tropical areas to northern areas in America and Eurasia. From pine, we can attain either wood or non-wood products, where non-wood products can be divided into three groups (1–4):

- **Gum** – A commodity that can have other names like pine resin or oleoresin, and it is the exudate extracted from living trees. It is a thick and sticky material, opaque and grayish white. Through distillation of gum, two other products can be acquired, rosin (also known as colophony or resin fraction) and turpentine;
- **Wood Gum** – Obtained through solvent extraction of trunks, stumps, and roots of dead trees. Can also be distilled, giving rosin and turpentine;
- **Raw tall oil** – A byproduct from the paper industry, pinewood chips are acid digested to extract cellulose and the crude tall oil is obtained by fractional distillation of the residue.

Colophony can also be obtained from other conifers besides pine, such as trees from *Araucariaceae*, *Cupressaceae*, *Phyllocladaceae*, and *Podocarpaceae* families. The substance can also be obtained from other non-conifer trees, such as trees from *Asteraceae*, *Celastraceae*, *Hydrocharitaceae*, and *Lamiaceae* species and even some known fungi species that can also produce abietane diterpenoids (5).

Chemically, gum has two fractions:

- **Turpentine fraction** – Essential oil, transparent and volatile, a mixture of a wide variety mono- and sesquiterpenes, with insecticidal and fungicidal properties (1,6,7).
- **Resin fraction or Colophony** – Complex mixture of the neutral part (10%) and acidic diterpenes (abietic and pimaric acids) (90%), solid at room temperature. Regarding the neutral part, it consists of terpenes, sesquiterpenes, and diterpenes, (such as α -pinene, β-pinene, 3-carene, α-terpineol, 4-allylanisole, camphene, and longifolene). And the abietic, pimaric, isopimaric, and labdanic

acids are part of the acidic diterpenes. Overall, the major component is the abietic acid, usually more than 50% (1,3,4,7).

1.2 Colophony and Turpentine

The main product acquired from the gum is colophony, a transparent solid, whose color can vary from pale yellow to brown. It is also glassy and brittle but softens with the increase in temperature. These physical-chemical parameters, the ratio between terpene and resin fractions, and even the ratio between each terpene in the pine resin are tightly related to the pine species. Other factors that also influence the colophony composition are the extraction process, handling and storage, which part of the tree is used to extract, and the environment/geographical location where the tree grows (1,3,4,7–9).

In the pine tree, the gum works towards protecting the tree from external damage, where turpentine function is to fluidify the colophony, so when it evaporates with contact with the atmosphere, the colophony solidifies and seals the wound, trapping insects or covering the wounds, and with the flow of the gum, it can also push out the insect (2,6,10).

In the industry, the gum itself has no application, but its products, colophony, and turpentine, have a wide range of industrial applications, either in their original form or after suffering modifications, in order to either provide a product that meets the needs of the consumer or to enhance the desired property, like colour, solubility or oxidation resistance (1,7). Besides the reactions in **table 1**, other reactions that colophony can be subject to are Diels-Alder addition, Friedel-Crafts type reactions, isomerization, dimerization, oxidation, and the ring can be cleaved (7,11–16).

Regarding turpentine, it is first necessary to separate its components by distillation, and then each suffers different transformation reactions. These products are applied industrially in various areas, similar to the ones where colophony and its derivatives are applied, like in solvents, paints, varnishes, in the production of paper glues, flavourings and fragrances, perfumes, food products (chewing gum or fruit coatings), disinfectants, adhesives, synthetic rubbers, coatings, printing inks, and others (1).

Table 1. The different modifications that unmodified rosin may undergo and their practical uses (1,4).

Modification process	Uses
Hydrogenation	Perfumes, cosmetics, plasticizers, tackifiers, and lacquers
Disproportionation (hydrogenation then dehydrogenation)	Printing ink, adhesives, tackifiers, synthetic rubber, paper sizing, insulators
Pentaerythritol esterification	Adhesives
Glycerol esterification	Adhesives, printing ink, paper, fabric finish, varnishes, chewing gum
Maleic anhydride modified	Paper, glue, paint, printing ink, lacquers
Fumaric acid modified	Lacquers
Formaldehyde modified	Paper sizing, printing ink
Polymerization (dimers formed on heating with acids)	Adhesives, inks, varnishes
Salt formation (Na, K, NH_4, Ca, Co, Pb, Mg, Zn resonates)	Soaps, detergents, printing ink, paper sizing

1.3 Colophony and Turpentine

Terpenes are one of the most important, complex, and diverse group of secondary metabolites, a group of organic compounds that are not essential for the plant's survival, but with essential character in their survival and interaction with the environment. Terpenes are simple hydrocarbons whereas terpenoids consist of modified terpenes with different functional groups or rearrangement of the carbon skeleton. Both derive from five-carbon isoprene units, being classified according to the structural organization of carbons or the number of rings (acyclic, monocyclic, bicyclic, tricyclic, tetracyclic, macrocyclic, and miscellaneous structures), and the number of isoprene units in their structure, being divided in monoterpenes (2 units, 10C), sesquiterpenes (3 units, 15C), diterpenes (4 units, 20C), triterpenes (6 units, 30C), and carotenoids (8 units, 40C) (6,17–21).

Diterpenes have two types of functions in the organism, either as a defense mechanism (abietane and pimaric diterpenes have insecticide properties), or as phytohormones in plants (gibberellins are a group of compounds that participate in the general metabolism, germination, growth, development of plants) (17).

Volatile oils are complex mixtures of volatile substances, generally odorous, lipophilic, and liquid. They consist mainly of terpenoids with low molecular weight, such as monoterpenes and sesquiterpenes (6,22).

Conifers produce resin acids, which are mostly a group of tricyclic monocarboxylic diterpenoids, being divided in abietane-type, including the abietic, dehydroabietic, neoabietic, palustric, and levopimaric acid, and pimaric-type, including pimaric, sandaracopimaric, and isopimaric acid. Some can also produce bicyclic diterpenes. Their structures are shown in **figure 1** (7,10,19,20).

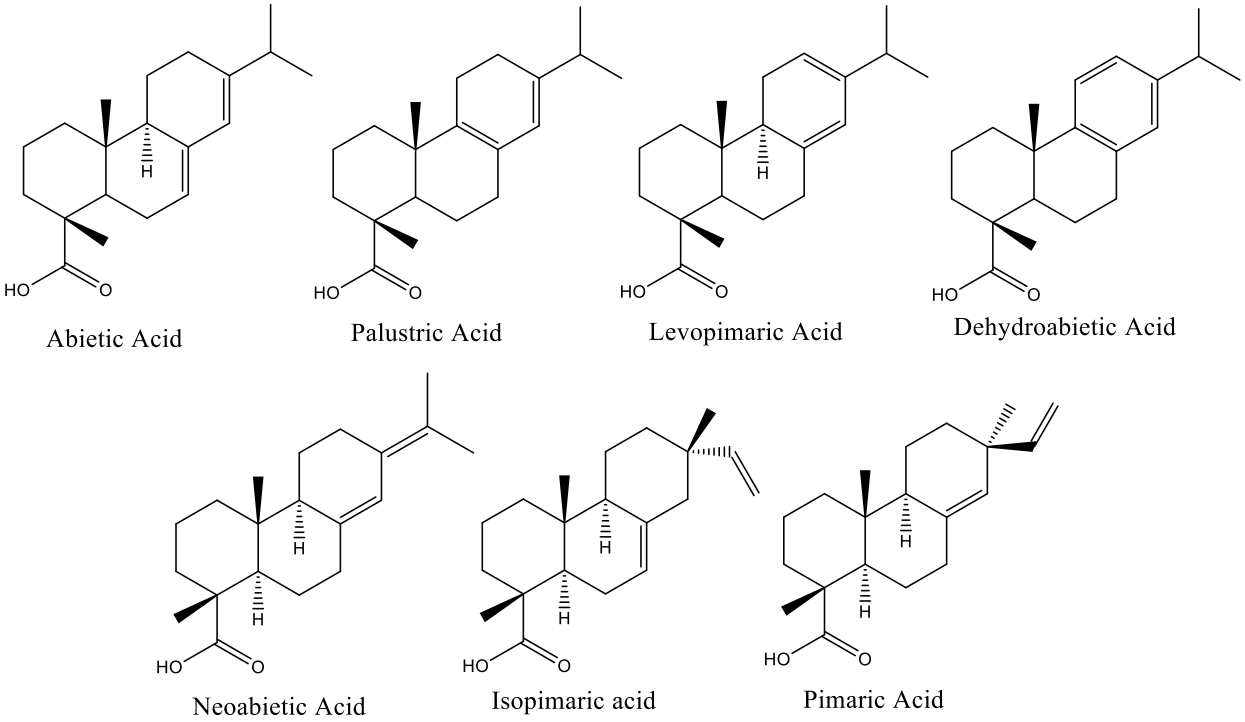


Figure 1. Abietic acids and Pimaric acids (10).

1.4 Abietanes

As stated above, abietanes are tricyclic diterpenoids to which abietic acid and dehydroabietic acid both belong to and are known to possess a wide range of biological activities as seen in **table 2**.

Table 2. Biological Activities of Abietanes.

Biological Activity
Antimicrobial – Mostly against gram-positive bacteria (2,5,17–20)
Antiviral – Inhibits the reproduction of multiple virus, like herpes simplex virus type 1, cytomegalovirus, varicella-zoster virus, and Epstein-Barr virus (17,22,23)
Antimalaria (17)
Antileishmaniasis – A study has shown that a mixture of abietane is more active and less toxic than the reference drug, glucantime (2,17)
Antitumoral and antimutagenic (2,17,19,20)
Antioxidant (17)
Cardiovascular protector – Improves microcirculation, inhibits platelet adhesion and aggregation, protects against myocardial ischemia, and antiarrhythmic activities) (2,5,19)
Antiallergic and anti-inflammatory – It has been used in skin allergies induced by IgE, in the treatment of chronic diseases such as rheumatism and gout (2,5,19,20,24)
Antiulcer and gastroprotective – Ecabet® is a drug that is sold in Japan that is used to treat peptic ulcer disease and reflux esophagitis, and the active substance of this drug is a derivative from dehydroabietic acid that had shown high antiulcer activity (2,5,17,19,25)
Regarding cutaneous wounds, these compounds both protect from infections and parasites and also promote and accelerate the healing process (possess angiogenic properties and promote the migration of endothelial cells (24,26)

1.5 Electrochemistry Reactions

Electrochemical reactions combine the electron transfer at an electrode, which converts the substrate to a reactive intermediate (ion radical, radical, anion, and cation) or produces a reagent (electrophile, nucleophile, acid, and base), with a chemical reaction (27,28).

For the electrolysis, a few materials are needed, such as:

- Power source and electrolysis cell (undivided or divided, with the second being used when the product generated at the counter electrode interferes with the main reaction or reacts with the substrate) (29,30);
- Electrodes – There are three types of electrodes, the reference, the working (WE), and the counter electrode (CE). The electron transfer occurs on the surface of the WE, and the choice of its material can influence the

reactivity/selectivity (27), being some examples graphite, glassy carbon, zinc, nickel, and copper;

- Supporting electrolyte and the solvent – Electrolyte offers a source of positive and negative ions which carry the charge through the circuit (counterions for the reactive intermediaries), improves conductivity, and reduces resistance (27,29,30).

The power source pushes electrons from the anode into the cathode, causing two separated processes, at the anode, it is formed an oxidative environment, where molecules are oxidized to a carbocation/radical intermediate (electron abstraction) and the electrons from the reaction mixture are transferred to the electrode. While at the cathode it is formed a reductive environment, where the transference of electrons from the electrode into the reaction mixture reduces the molecules to form carbanion/radical (electron addiction) (28–30).

Electrochemical reactions can be controlled in one of two ways (29,30):

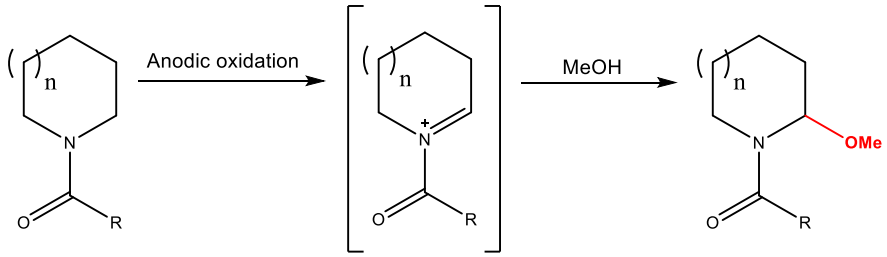
- Constant current – The advantage of its use is that it is easier to set up the reaction. The disadvantage is that it has low selectivity since the potential climbs as the substrate is consumed, so due to over-oxidation/reduction;
- Constant potential – The advantage of its use is that is more selective since the potential stays the same throughout the course of the reaction. The disadvantage is that requires a reference electrode and as the substrate is consumed, the current drops, taking longer for the reaction to be over.

The use of electrochemistry in reactions ends up applying many of the principles of green chemistry, such as the reduction of waste since the reagent is not bound to a chemical compound, uses safer chemicals (toxic oxidants and reductants can be replaced by electricity; unstable and dangerous reagents can be synthesized *in situ* ready to be used) and greener solvents (in most case are easily removed and recycled by distillation), the reactions are energy efficient since they are conducted at room temperature and pressure and the driving force of the reaction is electricity (27,31–33).

Other advantages of the use of electrochemistry are that reactions usually have higher yields, are more selective and the resulting products have higher purity. The selectivity of the reactions can be tuned by the type of electrodes/electrolyte used and the potential

used. The degree of conversion of the molecule can also be managed by regulating charge consumption. And the reactions conditions of electrochemistry are considered to be mild since these reactions can be done at atmospheric pressure and room temperature (28,31).

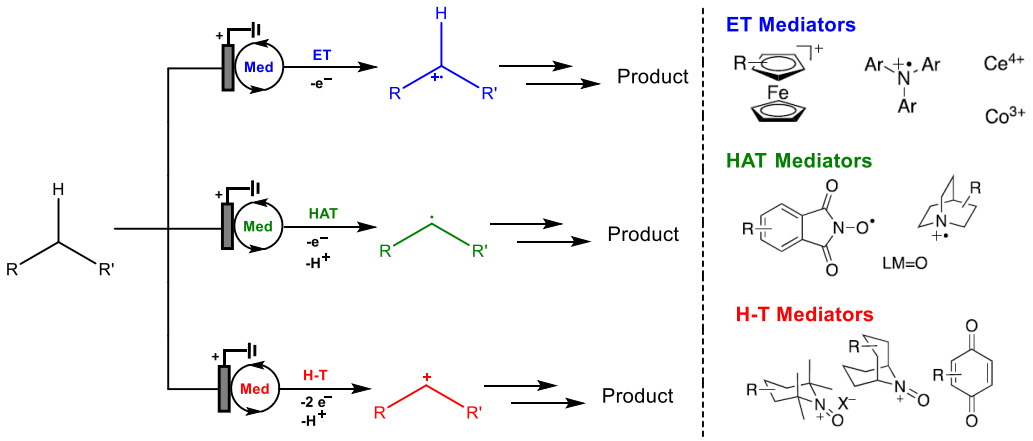
Regarding electrochemical reactions, direct electrolysis is possible for most molecules, thus we can directly oxidize/reduce the molecule at the working electrode, but this is not always achievable, because some molecules have higher oxidation/reduction potentials, running the risk of working outside the optimal window of the solvent used (values at which the solvent can be oxidized/reduced) (27). For example, regarding direct anodic oxidation of C-H bonds, these are reactions already described, such as Shono oxidation, but they are limited to weak C-H bonds or to simpler substrates (34).



Scheme 1. Shono oxidation.

As an alternative we have indirect electrolysis, where we use mediators, a selective oxidizing or reducing reagent, that can be used substoichiometric or catalytic amounts, since the mediator first undergoes electron transfer at the WE, then promotes the oxidation/reduction of the subtract, being then regenerated at the CE (27,31).

The mediators allow the reaction to occur at lower potentials than in direct electrolysis and can also make the reaction more selective since the interaction between the mediator and the substrate is more specific. There are several different types of mediators that can be divided into three groups, electron transfer (ET) mediators, hydrogen atom transfer (HAT) mediators, and hydride transfer (H−T) mediators, as seen in **scheme 2** (27,35).



Scheme 2. Examples of the three groups of mediators, ET, HAT, and H-T, and the reactions that they mediate.

1.6 Flow Electrochemistry

Flow electrochemistry is a technique that allows the academic and industrial scale up of batch electrosynthesis. Besides offering a higher electrode surface area to reaction volume ratio, which reduces reaction times, it also aids to overcome some of the limitations seen in macro batch electrochemistry, such as ohmic drop, mass transfer, and selectivity (28). Likewise, it helps to avoid other limitations seen in electrochemistry in batch, such as electrode surface area is limited, a supporting electrolyte is needed, makes the reaction more time-consuming, less cost-efficient, and requires workup (31,33).

Other advantages provided are that the process is safer (for example, it allows the controlled generation and consumption in situ of radicals and highly reactive intermediates), faster, and more reproducible way to synthesize products with high selectivity and purity, while being more "environment friendly" and reducing costs, energy, and solvent consumption (28,31,36).

Nevertheless, flow electrochemistry faces some problems, such as clogging, either because of electrode fouling or of solids that do not dissolve, that can come from side reactions, starting material, or reaction products. In addition, the presence of gases (H_2 or N_2), which are by-products of electrochemical reactions, that create gas-liquid segments since they do not have space to escape. These segments increase local current densities, affecting the performance of the flow cell since the gas does not conduct electricity (28).

The flow electrochemistry consists of five components, as seen in **figure 2**, which are pumps that work as the delivery system of the reaction mixture, mixing unit, flow cell/reactor (can vary given the application, some examples are chip reactors, coil reactors, tube-in-tube, column reactors, and others), collection unit, power source. To this basic setline, other components can be added, for example, a back-pressure regulator, an analytical instrumentation, such as UV-Vis or IR, to monitor the reaction in real-time, or multiple reactors can be added in series to allow multiple reactions in sequence. The residence time is how much time the reactants spend in the flow cell, and it's influenced by flow rate and reactor volume (28).

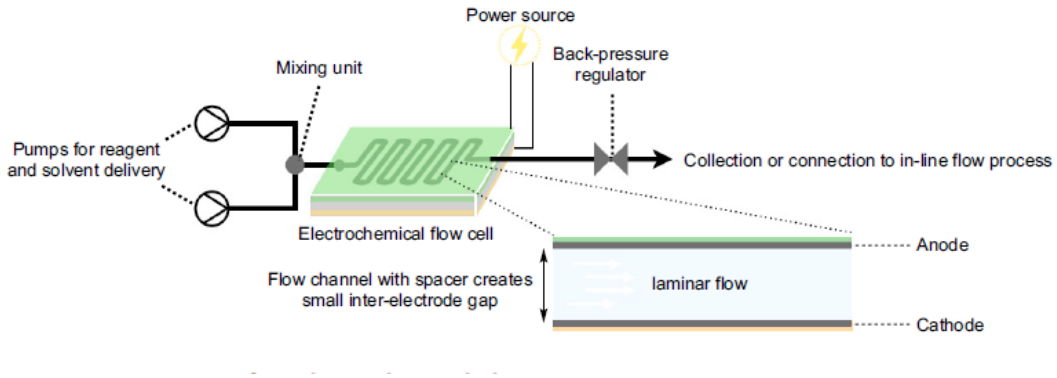


Figure 2. Basic Components of an Electrochemical Flow Setup (28).

The basic principle of flow electrochemistry is that the injectors continuously pump the reaction solution into a flow reactor. Here, allied with being usually a laminar flow, the progress of the reaction changes the concentration gradient, which creates diffusion, allowing mass transfer along the electrode (28,32,33).

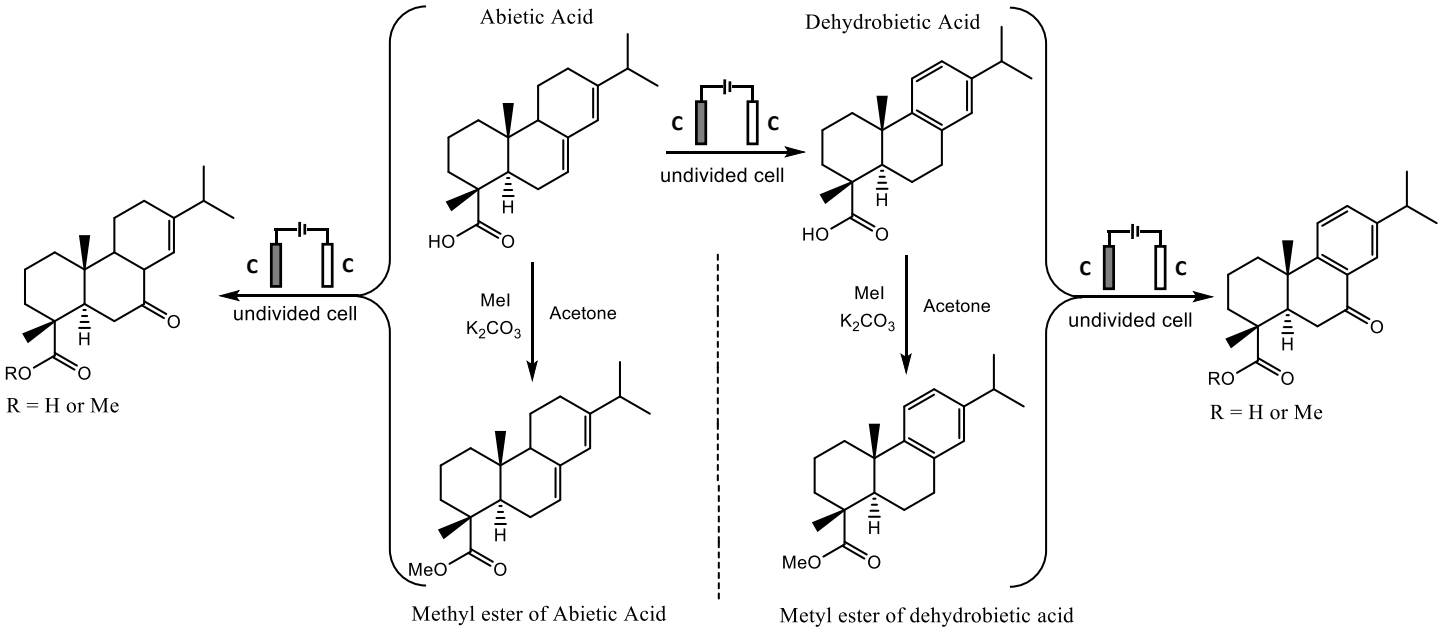
The most simple and common flow cell consists of two plate electrodes facing one another, separated by isolating spacers, creating an in-between space where the reaction mixture flows through. This type of cell has a uniform current and potential distribution. So, since the electrodes are separated by spacers, the tight gap between them enables the reaction to be quicker and reduces resistance, allowing to reach higher currents with lower concentrations of supporting electrolyte or even without its presence, making the reaction cleaner, more economic and reduces waste (28,31–33).

2 Objectives

Colophony, a natural resin obtained from conifer species (14), is constituted by a group of diterpenes known as abietanes, which, along with its derivatives, have been found to have a wide variety of interesting biological activities, including the antimicrobial (2,5,17–20), antiviral (17,22,23), antimalaria (17), antitumoral (2,17,19,20), antiulcer (2,5,17,19,25), antiallergic and anti-inflammatory activities (2,5,19,20,24).

The objective of this project was to add biosynthetic value to colophony and its abietanes (**scheme 3**), with the following goals:

- Synthesize derivates of abietic and dehydroabietic acid with the use of classic organic reactions;
- Develop an electrochemical process to convert abietic acid into dehydroabietic acid;
- Develop electrochemical reactions for the oxidation of activated and non-activated C-H bonds of the abietanes;
- Apply the developed electrochemical processes to colophony as starting material;
- Determine the oxidation potentials of the abietic and dehydroabietic acid and their derivatives.



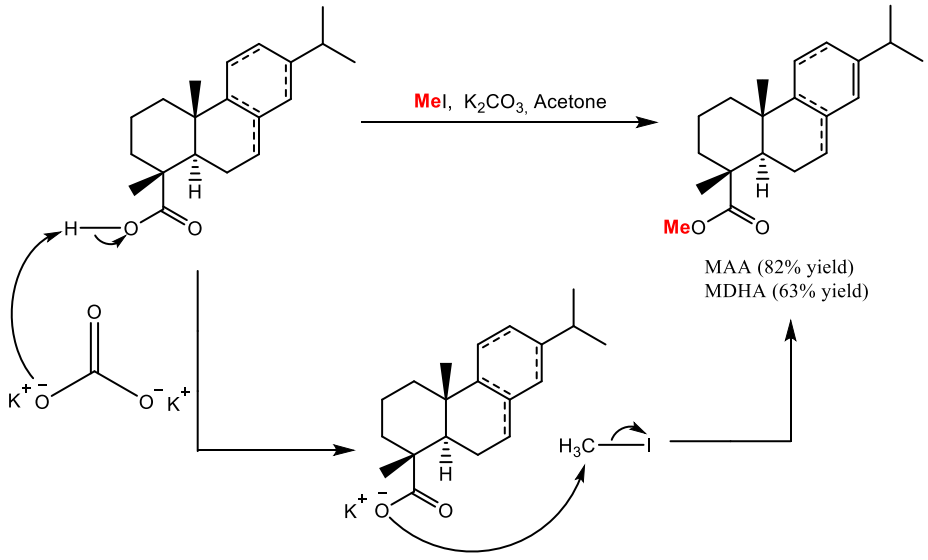
Scheme 3. Abietanes reaction scheme.

3 Results and Discussion

3.1 Esterification of abietanes

In order to synthesize the methyl ester derivative of both abietic acid (AA) (37–39) and dehydroabietic acid (DHA) (40), we followed a reported protocol, where the potassium carbonate-promoted S_N2 reaction with iodomethane is described in **scheme 4** (41).

Both methyl ester of abietic acid (MAA) and of dehydroabietic acid (MDHA) were prepared in good yields, 82%, and 63%, respectively. These compounds were characterized by ^{1}H-NMR and ^{13}C-NMR, which are in agreement with the previously reported data (37–41).



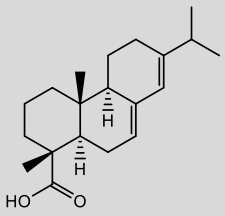
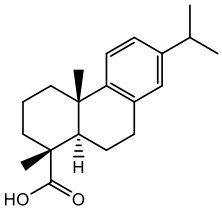
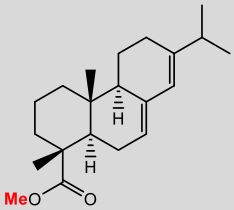
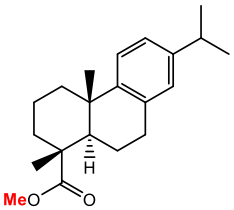
Scheme 4. Synthesis of the methyl ester derivative of both AA and DHA, via nucleophilic substitution of the corresponding carboxylates.

3.2 Cyclic Voltammetry studies of Abietic and Dehydroabietic Acid and of their Methyl Ester derivatives

Cyclic voltammetry experiments showed that each of the four molecules exhibits two irreversible oxidation potentials (**table 3**).

We did multiple runs for all four molecules during the cyclic voltammetry experiments, varying the concentration of the compound and the maximum potential of the experience.

Table 3. Cyclic Voltammetry studies of the abietanes.

Molecule				
Oxidation ($Ep_{/2}$) V	1.5 - 1.7 V 2.2 - 2.3 V	2.1 - 2.3 V 2.5 - 2.7 V	1.6 - 1.7 V 2.4 - 2.5 V	2.3 - 2.4 V 2.7 - 2.9 V

Finally, comparing both abietic acid and dehydroabietic acid with their methyl ester derivatives (**figures 3 and 4**), we can observe that they have similar behaviors and that their oxidation points are similar.

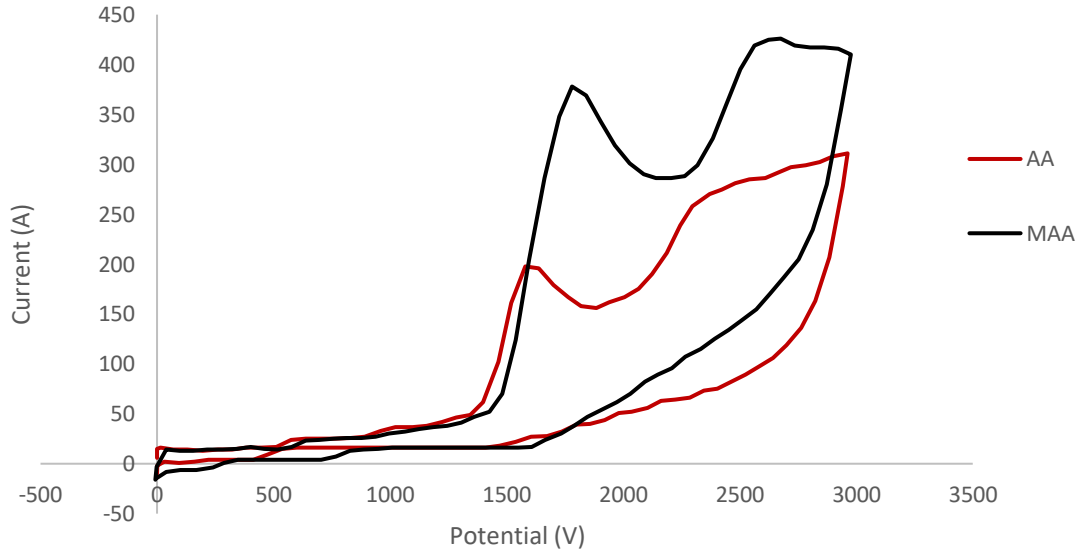


Figure 3. Comparison of Cyclic Voltammetry of AA (6.8 mmol/mL) with MAA (10.8 mmol/mL) up to 3.0 V.

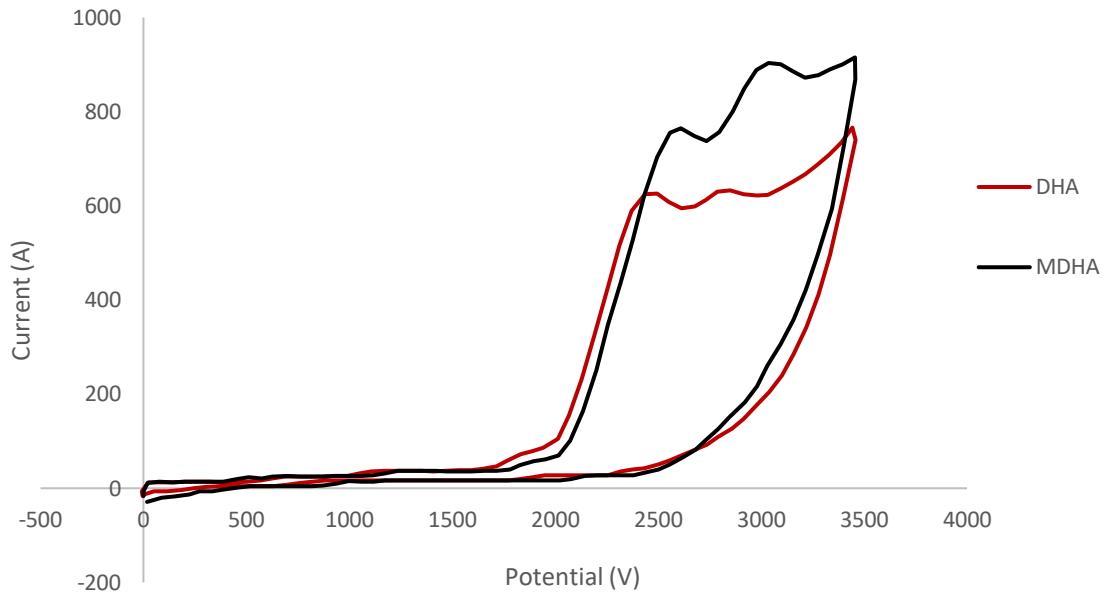


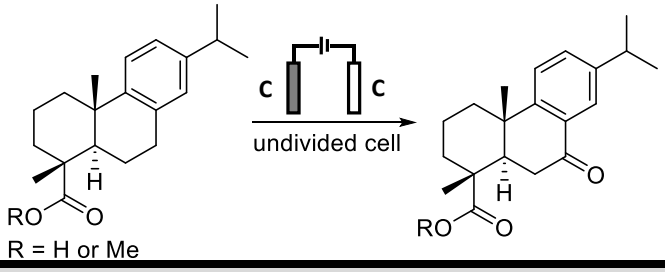
Figure 4. Comparison of Cyclic Voltammetry of DHA (9.3 mmol/mL) with MDHA (11.7 mmol/mL) up to 3.0 V.

3.3 Oxidation of Abietanes AA, MAA, DHA and MDHA

Regarding the benzylic oxidation of Dehydroabietic Acid (DHA) and Methyl Ester of Dehydroabietic Acid (MDHA), as can be seen in **table 4**, multiple experiments were performed in which the reaction conditions were changed to achieve oxidation of the starting material. All these reactions were performed at room temperature without care to remove air or moisture, unless stated otherwise. The internal standard used in the NMR's analysis was 1,3,5-trimethylcyclohexane.

Based on reported protocols for the oxidation of the benzylic position (42–44), we started by adapting them and then optimizing them to our compounds in order to achieve the benzylic oxidation.

Table 4. Reaction conditions and results of the oxidation reactions of DHA and MDHA.



Entry	MDHA or DHA Quantity (mg)/ Concentration (mol/L)	Supporting electrolyte	Solvent	Current (mA)	Potential (V)	Time (h)	Electricity (F/mol)	Yield (%)
1	MDHA, 50.2/0.03	$LiClO_4$ 0.094 M	CH_3CN/H_2O 94:6	5.0	-------	17.3	20.23	18[b]
2	MDHA, 201.4/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	5.0	-------	15.75	4.59	63.9
3	MDHA, 199.9/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	5.0	-------	18.75	5.47	80.3
4	MDHA, 50.9/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	10.0	-------	2	4.92	66
5	MDHA, 49.9/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	20.0	-------	1	5.47	85
6[a]	MDHA, 50.9/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	10.0	-------	1	4.66	68.8
7	MDHA, 200.9/0.06	TBATFB 0.15 M	CH_3CN/H_2O 94:6	20.0	-------	8.5	10.0	16[b]
8	DHA, 50.7/0.02	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	5.0	-------	16	17.56	traces
9	DHA, 50.8/0.02	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	2.5	-------	16.2	8.97	traces
10	DHA, 49.6/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	1.3-1.4	24	4.77	n.d.
11	DHA, 50.4/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3	5.46	traces
12	DHA, 200.4/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3.2	2.97	traces
13	DHA, 200.3/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	8	6.69	65.4

[a]Reaction was performed under an inert atmosphere. [b]Yield was calculated by comparison with an internal standard in NMR.

Based on the data depicted in **table 4**, some conclusions can be taken regarding the oxidation of MDHA, such as the concentration of the supporting electrolyte can greatly affect the yield of the reaction, so with higher concentrations, we observed higher yields, while with a low concentration we observed a lower yield (**entry 1 vs 3**). Also, the supporting electrolyte used affected greatly the yield of the reaction, when we used lithium perchlorate we had higher yields, while with TBATFB, the reaction had a lower yield and was not complete (**entry 3 vs 7**).

When we increase the current of the reaction, the reaction is faster with a low formation of impurities and a similar yield to those of the reactions with lower current (**entry 3 vs 4 and 5**).

The reaction is observed to be reproducible since when it is done under the same conditions, we observe similar results (**entry 2 vs 3**).

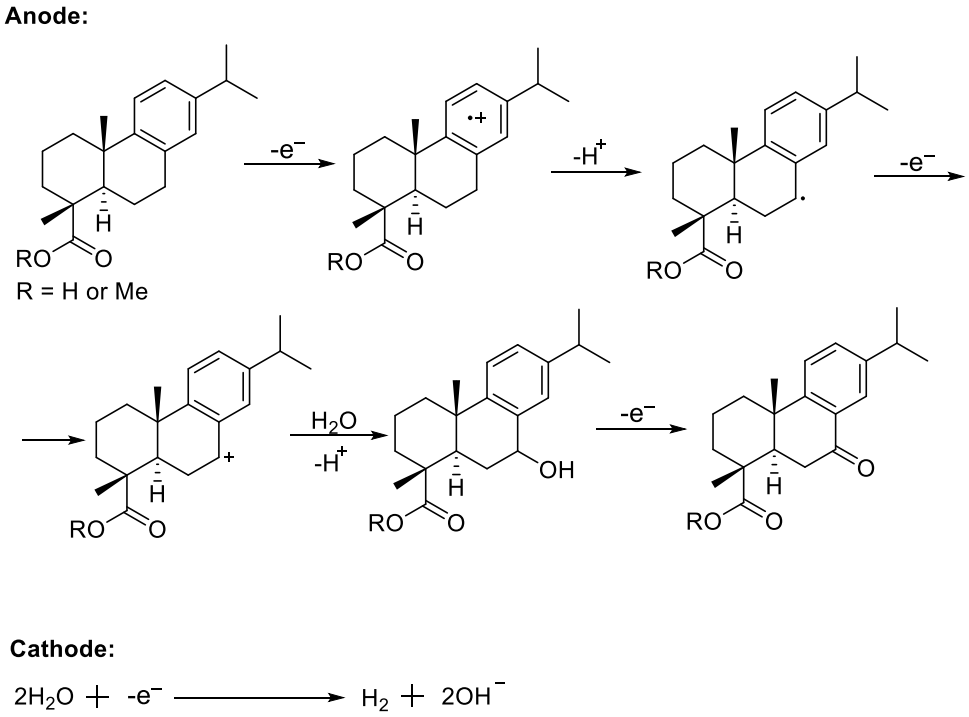
When the reaction was performed under an inert atmosphere (argon) (**entry 6**), although the reaction had a good yield, it was not complete, there was still a large amount of MDHA present, and through analysis of the ^{1}H NMR, we observe that the reaction was less specific.

Based on the data in **table 4**, some conclusions can be taken regarding the oxidation of DHA, such as, that if a constant current of 5.0 mA is used, complete conversion is achieved but the reaction is not selective, and if lower currents are used, complete conversion is no longer achieved. However, using a constant potential, complete conversion is achieved with greater selectivity for the desired product (**entry 8 vs 9 vs 13**). Regarding the potential, the reaction did not occur at lower potentials (**entry 10**), and by NMR analysis, neither traces of the product nor of the starting material were observed.

The duration of the reaction also proved to be an important factor for complete conversion of the starting material into the product (**entry 12 vs 13**), being that 8h was the time with the highest yield.

Based on the literature (42), the proposed mechanism of benzylic oxidation (**scheme 5**) starts with the aromatic ring being oxidized at the surface of the anode to give the radical cation, which then deprotonates into the benzyl radical, suffering subsequent oxidation, forming the benzyl cation. This intermediate is then attacked by water, subsequent

deprotonation generates benzylic alcohol, which is oxidized to give the aimed product. Due to cathodic reduction, water is electrolyzed to release hydrogen gas.



Scheme 5. Proposed mechanism for the oxidation of DHA and MDHA.

In conclusion, the oxidation of the benzylic position is an extensively described reaction in literature, and some examples of conditions used for the oxidation of DHA or MDHA are shown in **table 5**. The great advantage of our work is the use of electrochemistry, allowing us to perform this reaction under simpler and more environmentally friendly conditions, with the use of fewer and less toxic reagents. Moreover, the oxidations were generally faster and with total conversion, with greater selectivity, having, in the end, good yields.

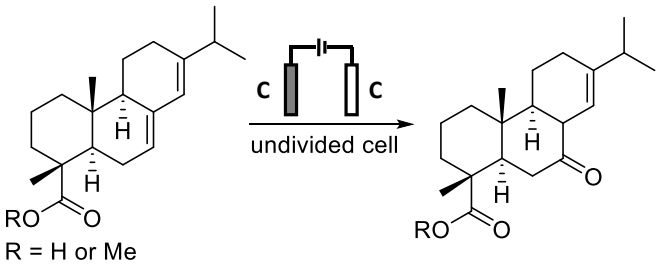
Table 5. Oxidation of the benzylic position.

Literature	Reaction conditions	Time (h)	Yield (%)
Synthesis and structural characterisation of ring B oxidized derivatives of dehydroabietic acid (45)	CrO_3 (stoichiometric)	12	67
Synthesis and Antibacterial Activity of Benzenesulfonylhydrazone Derivatives of Methyl Dehydroabietate (46)	CrO_3 (catalytic)	25h	70.6
Regioselective routes towards 14-hydroxyabietane diterpenes. A formal synthesis of immunosuppressant (L)-triptolidefrom (D)-abietic acid (38)	Jones reagent (CrO_3 in aq. H_2SO_4) (stoichiometric)	0.5	11
Synthesis of Complex and Diverse Compounds through Ring Distortion of Abietic Acid (41)	Swern oxidation	--------	52
Oxidation Products of Abietic Acid and Its Methyl Ester (14)	H_2SO_4	3	0.09

Regarding the oxidation of Abietic Acid (AA) and of Methyl Ester of Abietic Acid (MAA), as it can be seen in **table 6**, multiple experiments were performed in which the reaction conditions were changed to achieve oxidation of the starting material. All these reactions were done at room temperature without care to remove air or moisture, unless stated otherwise.

At first, our aim was to use electrochemistry for the oxidation of the allylic position of the starting material, being there some works that report this oxidation. There are works of Shono's group relating the direct allylic oxidation of α-pinene (although with low yields) (47) and of Baran's group that achieved the same oxidation with the use of mediators (34). Plus, considering the work developed regarding benzylic oxidation, we started by using and adapting those conditions to achieve allylic oxidation of the starting material. With the reactions result, we observed that it was not the product of the allylic oxidation that was being obtained, but rather the product of the olefinic oxidation.

Table 6. Reaction conditions and results of the oxidation reactions of AA and of MAA.



Entry	MAA or AA Quantity (mg)/ Concentration (mol/L)	Supporting electrolyte	Solvent	Current (mA)	Potential (V)	Time (h)	Electricity (F/mol)	Yield (%)
1	MAA, 50.9/0.03	TBATFB 0.092 M	MeOH	5.0	-------	14.83	16.23	n.d.
2	MAA, 49.9/0.05	TBATFB 0.091 M	CH_3CN	5.0	-------	15.77	18.38	n.d.[a]
3	MAA, 50.2/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3	3.10	5 [a]
4	MAA, 50.1/0.03	$LiClO_4$ 0.12 M	CH_3CN/H_2O 94:6	-------	1.2 – 1.4	6	2.04	3 [a]
5	MAA, 204.5/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	1.6	9.43	1.46	6
6	MAA, 201.7/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.0	9.48	1.65	22
7	MAA, 200.5/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3.50	2.03	29.5
8	MAA, 200.4/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3.13	2.65	25
9	MAA, 200.1/0.06	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	2.13	2.10	47.9
10	AA, 50.4/0.03	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3	4	n.d.
11	AA, 201.6/0.07	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	4	1.72	12
12	AA, 204.6/0.07	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	3.63	2.21	9
13	AA, 202.6/0.07	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.0	13.78	-------	10
14	AA, 201.6/0.07	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.0	5	2	4
15	AA, 208.5/0.07	$LiClO_4$ 0.15 M	CH_3CN/H_2O 94:6	-------	2.4	5.3	2.5	48.4

[a]Yield was calculated by comparison with an internal standard in NMR.

Based on the data in **table 6**, some conclusions can be taken in regard to the oxidation of MAA, for instance, the presence of water in the solvent mixture is important for the reaction to occur since in reactions where water was not added, the oxidation product was not observed (**entries 1 and 2 vs 9**), and by NMR analysis, neither traces of the product nor of the starting material were observed.

We observe that with the use of constant potential, we were able to observe the formation of the product of oxidation since reactions with constant potential are more selective than those with a constant current. We studied the use of different potentials to obtain the desired product, noting that with lower potentials (**entry 4**) the reaction had a negligible yield, and at potentials other than 2.4 V, the reaction occurs but takes longer to complete and had lower yields (**entries 5 and 6 vs 9**).

The reaction is observed to be reproducible since when it is done under the same conditions, we observe similar results (**entries 7 to 9**).

Based on the data in **table 6**, some conclusions can be taken regarding the oxidation of AA, such as, the product of this reaction is unstable and degrades. And the reaction is shown to be reproducible since when it is done under the same conditions, we observe similar results (**entries 11 and 12**).

Regarding **entry 10**, by NMR analysis, neither traces of the product nor of the starting material were observed.

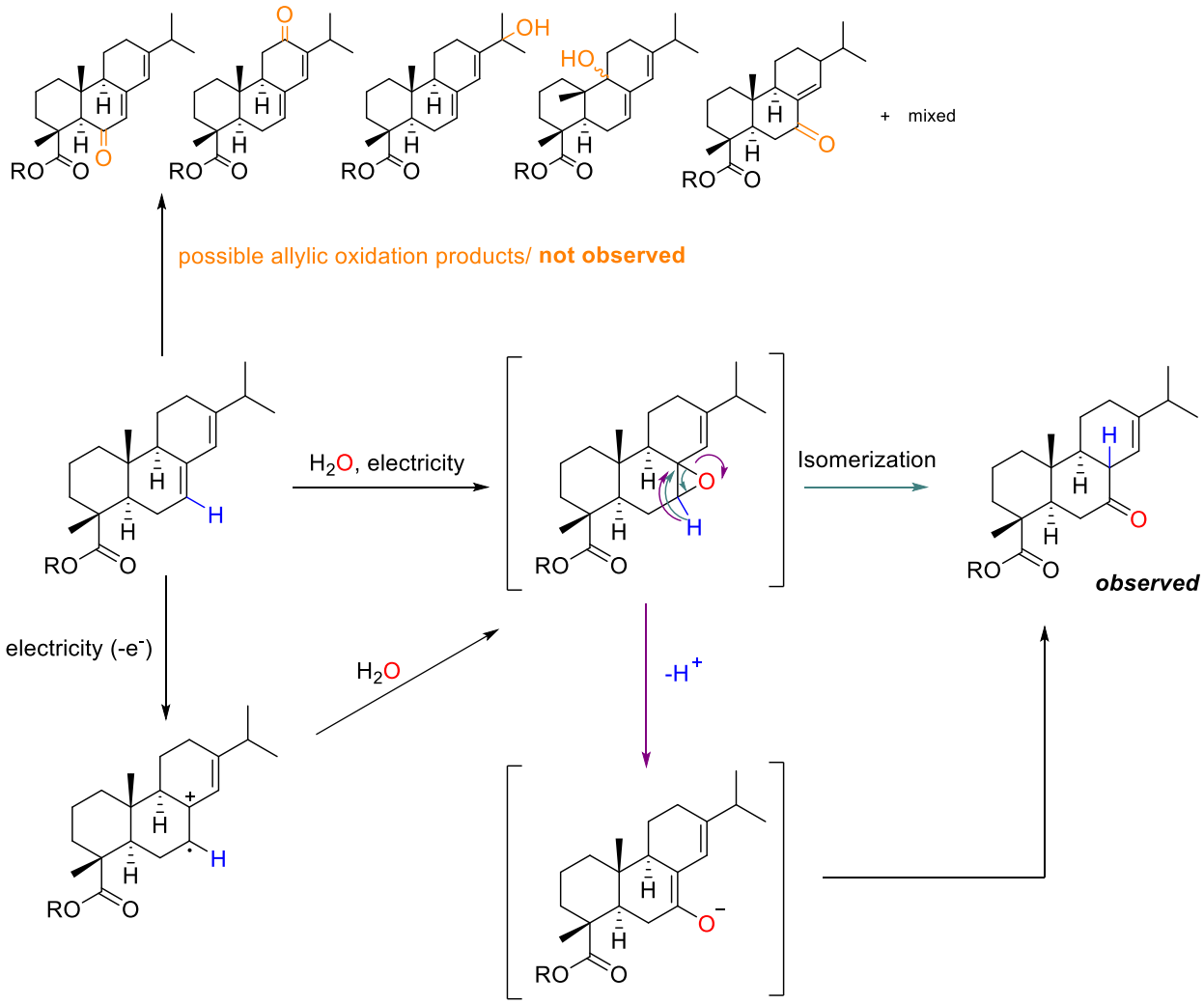
As to the potential used in the reaction, at a lower potential, the reaction still occurs but takes more time (**entries 13 and 14 vs 15**).

The first obstacle we encounter about the oxidation of AA and MAA, was knowing which product was being formed because both molecules have multiple activated allylic positions, so different products can arise. Based on some oxidation protocols of these compounds, we were able to foresee some of the products we could expect from this reaction, being some examples in **scheme 6** (14,48–50).

Based on the literature, the proposed mechanism of the oxidation of AA and MAA (**scheme 6**) starts with either the vinylic position being oxidized at the surface of the anode to give a radical cation and the intermediate formed is attacked by water, forming the epoxide or there is the formation of hydrogen peroxide in situ by the oxidation of water, achieving the epoxide by electrophilic oxygen transfer to the double bond (51,52). Afterwards, through ring-opening, we observe the formation of the ketone.

This can happen by two possible ways, either we have deprotonation, which forms an oxyl, that suffers reorganization to form the ketone (purple arrows). Or we have direct reorganization of the epoxide which forms the ketone (turquoise arrows) (53,54).

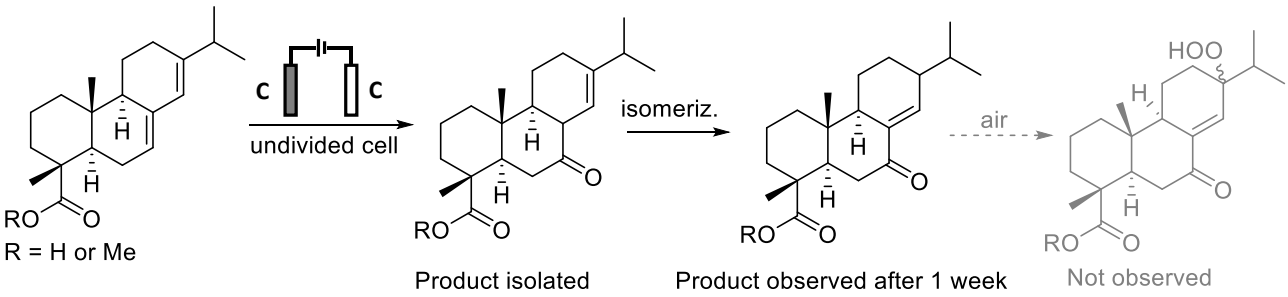
To further understand this mechanism and see if the epoxide is an intermediate of this oxidation of AA and MAA, we have to synthesize the epoxide and submit it to the same reaction conditions as the starting material to verify if the expected product is formed (50,52).



Scheme 6. Examples of enones we could obtain from allylic oxidation and the proposed mechanism.

The second obstacle we encountered was that the reaction product would undergo decomposition (**scheme 7**), as it oxidizes with exposure to air. Such reaction is described in the literature, that under aerial exposure, abietic acid suffers self-oxidation and enones, including resin acid derivatives, oxidize to form peroxides (50).

We also observed so, after isolating the reaction product, two NMRs were performed, with a one-week gap between the two, and it was observed conversion of about 50% of the reaction product into an isomer (not isolated, diagnostic signals only observed) but, despite being described in the literature, we did not observe the formation of peroxide (41). It is important to note that the compound isolated from the reaction was not exposed to air for a very long time, it was mostly kept in vacuum.

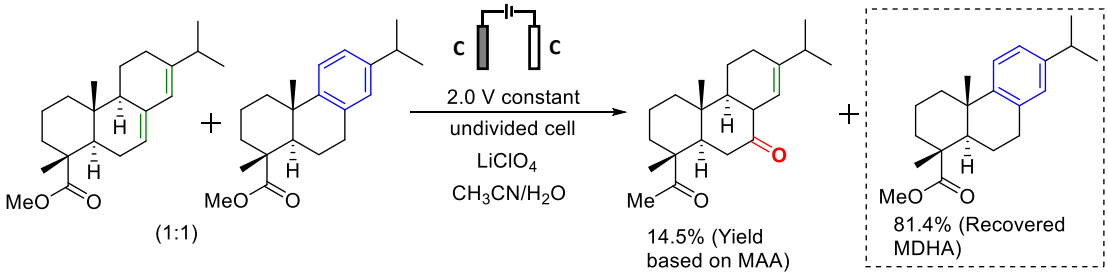


Scheme 7. Oxidation of AA and degradation of the reaction product.

One of our specific objectives with the oxidation of the abietanes was to subsequently oxidize the colophony, a complex mixture of several abietanes, including DHA and AA. Considering the oxidation potentials of these molecules and their methyl derivatives, we studied the selectivity of the oxidation of abietanes when to a mixture of MDHA and MAA, we apply such reaction conditions that we only oxidize the compound with a lower oxidation potential, the MAA (**scheme 8**). This reaction was done at room temperature without care to remove air or moisture.

To achieve this reaction selectively in which only MAA was oxidized, a lower potential was used, which included the first oxidation potential of MAA but not the oxidation potentials of MDHA and limited the number of electrons that could be supplied to the reaction medium.

By the ^{1}H NMR analysis, it is concluded that the reaction was selective since it was only observed the oxidation of MAA and not of MDHA, and yield was calculated by comparison with an internal standard in NMR and regarding the amount of starting material of each molecule.



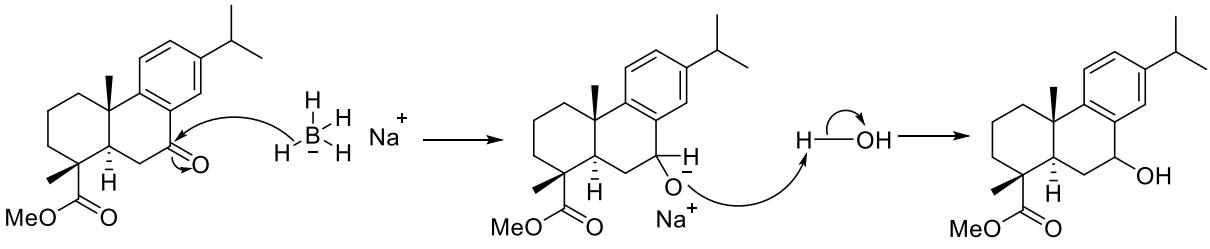
Scheme 8. Reaction conditions and results of the oxidation reaction of MAA.

3.4 Reduction of the ketone to the secondary alcohol

To elucidate more about the mechanism behind the oxidation of abietanes, we started by reducing the ketone to the secondary alcohol, and afterwards, we would submit it to the same oxidation reaction conditions to see if the ketone was formed. If the reaction occurred, we could say that it could be a possible intermediate of the oxidation reaction (**scheme 5**). This test remains to be done.

So, to proceed to this reduction, we followed a reported protocol (55), where to the solution of the product of the oxidation of MDHA in methanol we add sodium borohydride. At the end of the reaction, we observed the total conversion to the secondary alcohol with a 92% yield.

Regarding the mechanism behind this reaction (**scheme 9**), we have two steps, first addition and then protonation. So, in the first step, the H atom detaches from the borohydride and adds to the carbonyl carbon, forming a C-H bond and breaking the C-O bond, thus forming an alkoxide. In the second step, a proton from water or an acid is added to the alkoxide to make the alcohol (56,57).



Scheme 9. Reduction with sodium borohydride of the ketone to the secondary alcohol.

3.5 Cyclic Voltammetry studies of the secondary alcohol derivative

The cyclic voltammetry experiments indicate that the secondary alcohol derivative exhibit two irreversible oxidation potentials (**figure 5**).

We did multiple runs for this molecule during the cyclic voltammetry experiments, varying the concentration of the compound and the maximum potential of the experience. By analyzing the graphics of the multiple runs for this compound during the cyclic voltammetry experiments, we can draw the conclusions stated below.

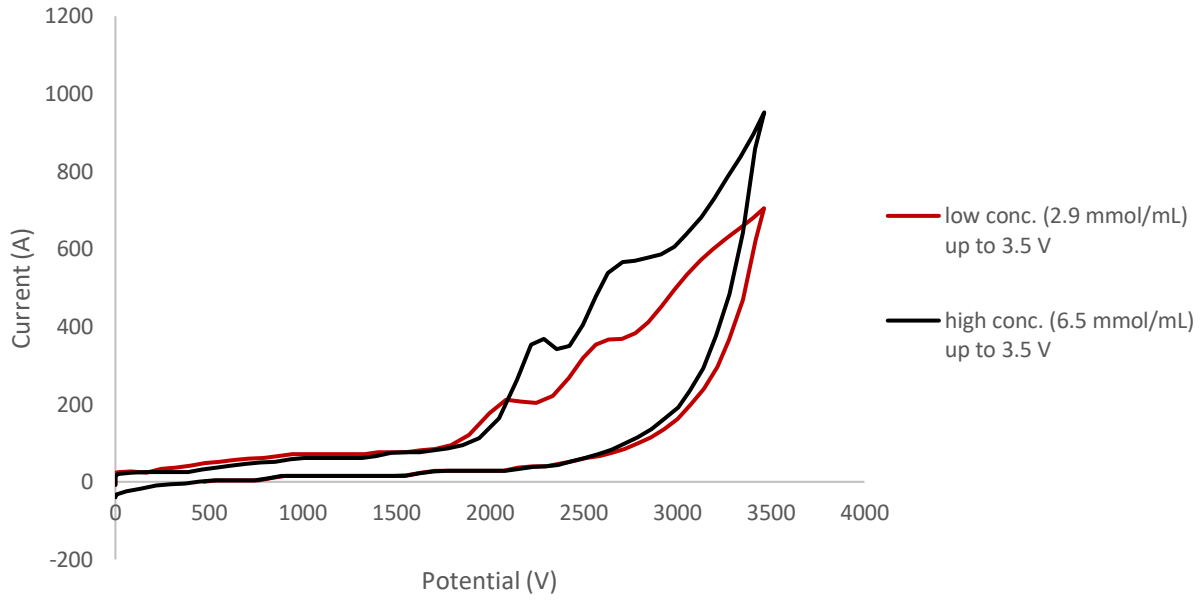
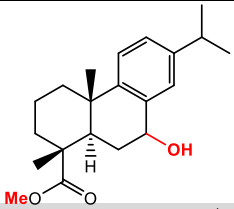
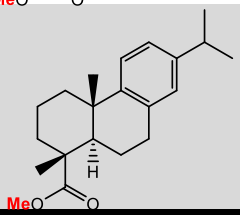


Figure 5. Cyclic Voltammetry of secondary alcohol derivative.

Finally, comparing both the oxidation potentials of the secondary alcohol derivative with MDHA (**table 7**), we can observe that the first has lower oxidation potentials.

Table 7. Cyclic Voltammetry studies of the secondary alcohol derivative.

Molecule	Oxidation ($Ep_{/2}$) V	Low concentration tested mmol/mL	High concentration tested mmol/mL
	1.9 – 2.1 V 2.4 - 2.5 V	2.9	6.5
	2.3 - 2.4 V 2.7 - 2.9 V	5.6	11.7

3.6 Electrochemical Oxidation with Mediators

3.6.1 Allylic and Benzylic Oxidation

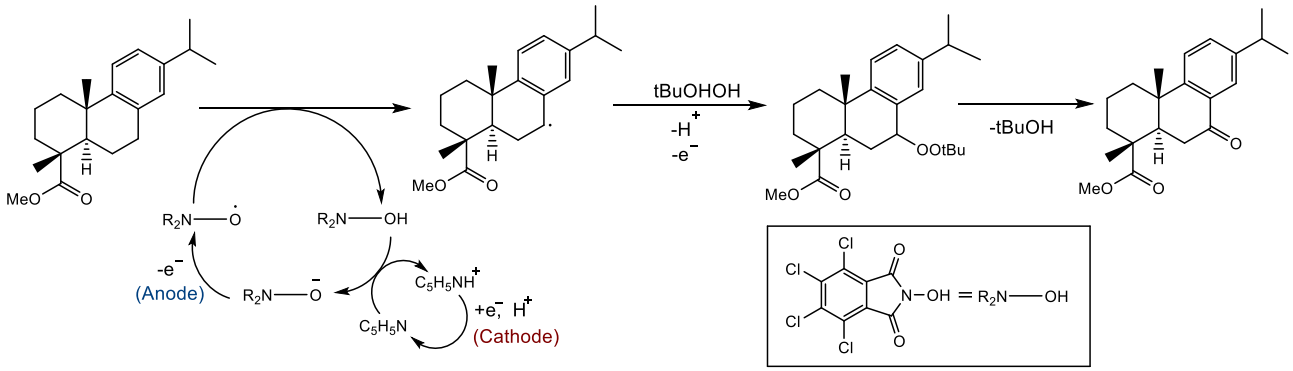
Similar to benzylic oxidations, allylic oxidations are also generally based on the use of toxic reagents, such as chromium or selenium, or on the use of transition metal catalysts, like Pd and Rh, which causes difficulties in industrial settings (58).

Regarding the use of electrochemistry for indirect benzylic oxidation, as it is well studied, there are some protocols reported. While the direct oxidation of allylic positions is less described, mostly since the C-H bonds of these positions have very high oxidation potentials (59). To circumvent this problem, indirect electrolysis reactions can be performed, where mediators are used, which allow the reaction to occur at lower potentials (27,35). As an example, we have the work developed by Baran's group, where using tetrachloro-*N*-hydroxyphthalimide (Cl_4NHPI) as a mediator, were able to achieve the allylic oxidation of multiple substrates with good yields (34).

Having as base the protocol developed by Baran's group (34), we proceeded with the allylic oxidation of Methyl Ester of Abietic Acid (MAA) and we also adapted the protocol to proceed with the benzylic oxidation of Methyl Ester of Dehydroabietic Acid (MDHA).

Regarding the mechanistic aspects of this reaction (**scheme 10**), we adapted the proposed mechanism to MDHA as starting material, thus first we had the deprotonation

of Cl_4NHPI by pyridine, followed by anodic oxidation, giving tetrachlorophthalimido-N-oxyl, which subsequently mediates the abstraction of a hydrogen atom from the starting material, regenerating Cl_4NHPI and generating the benzylic radical of the starting material which then reacts with ^tBuOOH to from a benzylic peroxide, which upon elimination of ^tBuOOH, affords the oxidation of the benzylic position.



Scheme 10. Proposed mechanism for electrochemical benzylic oxidation.

Table 8. Reaction conditions and results of the oxidation reactions of MDHA and of MAA.

Entry	MDHA or MAA Quantity (mg)/ Concentration (mol/L)	Supporting electrolyte	Solvent	Current (mA)	Potential (V)	Time (h)	Electricity (F/mol)	Yield (%)
1	MDHA, 79.8/ 0.08	$LiClO_4$ 0.1 M	Acetone	10.0	-------	18.5	27.61	30
2	MAA, 158.6/ 0.08	LiClO4 0.1 M	Acetone	10.0	-------	17.88	13.35	n.d.
3	MAA, 79.8/ 0.08	LiClO4 0.1 M	Acetone	-------	2.0	24	2.92	n.d.
4	MAA, 79.9/ 0.08	LiClO4 0.15 M	Acetone	-------	1.0 – 1.6	8.48	0.61	n.d.
5	MAA, 79.9/ 0.08	LiClO4 0.1 M	Acetone	-------	1.0 – 1.4	4.33	1.6	n.d.

Comparing the data in **table 4 vs table 8**, some conclusions can arise about the oxidation of MDHA, as we are able to achieve the oxidation with the mediator, but

despite having a total conversion, it has a lower yield than direct electrolysis and takes longer to finish (**entry 1**).

Based on the data in **table 8**, a conclusion can be taken for the process of oxidation of MAA, which was that either using constant current as reported in the protocol (**entry 2**) (34), or using constant potential, with different potentials (**entries 3 to 5**), we were not able to achieve the oxidation of MAA and synthesize the product we were aiming for.

In **entries 2,3 and 5**, by NMR analysis, neither traces of the product nor of the starting material were observed. And in **entry 4**, by NMR analysis, traces of the starting material were observed.

3.6.2 Oxidation of Non-activated positions

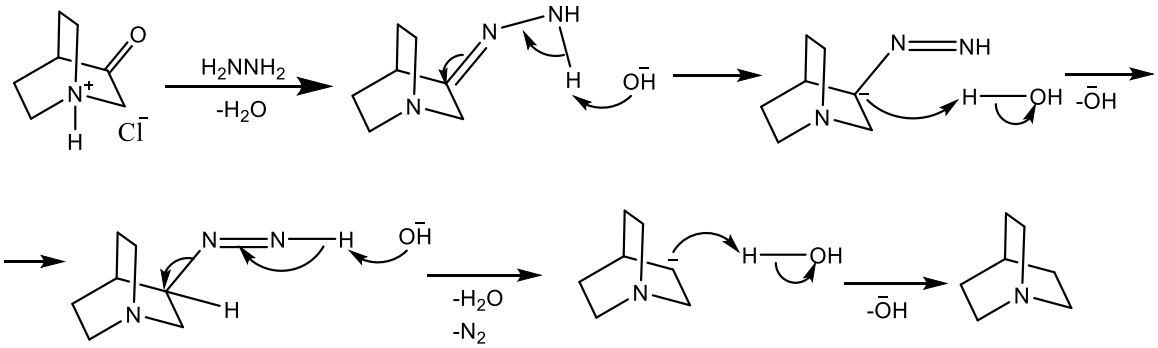
Non-activated positions are positions that have high redox potentials, so there are a couple of options, either we use high potentials to achieve their oxidation, and risk having the oxidative degradation of the solvent and other functionalities, or we can use strong oxidants, like methyl(trifluoromethyl)dioxirane (TFDO) and metal complexes, which have the problem of either have a complicated preparation or being expensive (60).

Baran's group studied the possibility of using mediators to obtain oxidation of non-activated positions, reaching the conclusion that with the use of quinuclidine as a mediator they were able to achieve the electrochemical oxidation at a relatively low potential (60).

We started by synthesizing quinuclidine from quinuclidone, based on protocols regarding that reaction (60,61). We followed a general procedure, although minimal changes were made in between the different attempts to synthesize the quinuclidine.

In regard to the different entries in **table 11**, we were always able to synthesize the quinuclidine, but we could not isolate it from the reaction medium, we tried to isolate the reaction product by distillation under low pressure, but the solvent from the reaction medium was always carried with the reaction product into the collection flask. Between the different entries we increased the reaction scale so that it would be easier to isolate the desired product, but always without success.

Regarding the mechanistic aspects of this reaction, a Wolff-Kishner reduction (**scheme 11**), the first step is the formation of the hydrazone, then deprotonation of the terminal nitrogen by the base with the formation of a hydrazone anion. The third step is the protonation of the carbon, and the fourth step is the deprotonation of nitrogen with the release of hydrogen, giving an alkyl anion, which undergoes protonation to give the alkane.



Scheme 11. Synthesis of quinuclidine.

3.7 Flow electrochemistry for oxidation of DHA

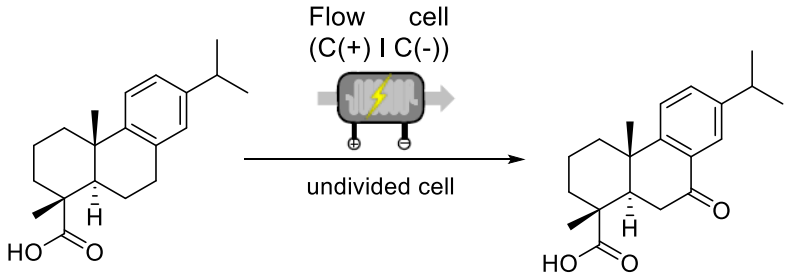
Since we were able to achieve and optimize the benzylic oxidation of DHA in batch, we started to work towards the scale up of this reaction, with the use of flow electrochemistry. As it can be seen in **table 9**, multiple experiments were performed in which the reaction conditions were changed to achieve oxidation of the starting material. All these reactions were done at room temperature without care to remove air or moisture. After adding the starting material, a white solid was observed that did not dissolve even after sonification. The reaction mechanism is the same as the one in **scheme 5**.

Based on the data in **table 9**, there are some conclusions to be taken regarding the oxidation of DHA, such as, the residence time, which is the amount of material in the flow cell divided by the flow rate, was the crucial factor for the oxidation to take place. When lower flow rates were used (**entries 4 and 5**), the residence time was higher, and we observed the conversion of the starting material in the product. While when we used higher flow rates (**entries 1 – 3**), this conversion was not observed, despite using optimal conditions for the benzylic oxidation to take place (use of constant current of 20 mA or constant potential of 2.4 V).

Nevertheless, this conversion in **entries 4 and 5** was not complete, there was still starting material present, and when compared between each other, we can observe that **entry 5** has a higher conversion than **entry 4**.

More experiments are needed to achieve the full conversion of the starting material in the product, to study if we can achieve this reaction without the presence of a supporting electrolyte, and to further optimize this reaction.

Table 9. Reaction conditions and results of the flow electrochemistry for oxidation reactions DHA.



Entry	DHA Quantity (mg)	Supporting electrolyte	Solvent	Current (mA)	Potential (V)	Flow rate (µL/min)	Residence time (min)	Final Result (starting material/desired product)
1	505.6	$LiClO_4$ 0.1 M	CH_3CN/H_2O 94:6	-------	2.4 V	500	2.4	No conversion observed
2				20	-------	500	2.4	No conversion observed
3				-------	2.4 V	100	12	No conversion observed
4	507.1	$LiClO_4$ 0.1 M	CH_3CN/H_2O 94:6	40	-------	50	24	Conversion observed (2:1)
5				40	-------	16.67	71.9	Conversion observed (1:3)

4 Experimental Design

4.1 Materials

All chemicals, and solvents for the synthesis of the compounds were either of analytical grade, purchased from commercial sources, namely Sigma-Aldrich®, Fluorochem, Merck, and Alfa Aesar, and these were used without further purification unless otherwise noted. The following list contains the material that was used for the basis of the development of the experimental design.

- Abietic Acid (AA)
- Dehydroabietic Acid (DHA)
- Quinuclidone
- Acetone
- Iodomethane
- Potassium carbonate
- Sodium borohydride
- Water
- Methyl *tert*-butyl ether (MTBE)
- Hexane
- Ethyl Acetate
- Formic Acid
- Dichloromethane (DCM)
- Acetonitrile
- Methanol
- Diethylene glycol
- HCl 1.0 M Solution
- Hydrazine
- Lithium perchlorate

- N-Hydroxytetrachlorophthalimide
- Pyridine
- 1,3,5-trimethylcyclohexane
- *Tert*-butyl hydroperoxide
- Anhydrous sodium sulfate
- Silica

4.2 Equipment

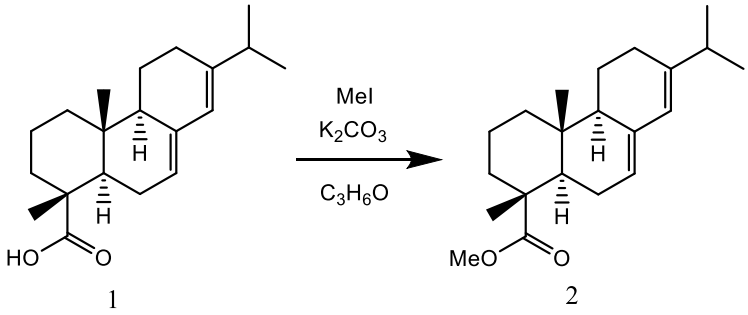
The following is the equipment that was the basis for the development of the experimental design.

- Stirring plate
- NMR (Bruker MX300 spectrometer)
- Rotary evaporator
- Vacuum pump
- IKA ElectraSyn 2.0
- Heating blanket
- Flow System consisting of two pumps, a mixing unit, IKA ElectraSyn Flow, and a power source.

5 Procedures

5.1 Esterification of AA

For the esterification of AA, we followed a reported protocol (41) which consisted in adding AA (6.03 g, 0.0199 mol), acetone (90.0 mL, 0.22 M), iodomethane (4.0 mL, 0.064 mol, 3.2 equiv.), and potassium carbonate (7.03 g, 0.0508 mol, 2.55 equiv.) to a 250 mL round-bottom flask equipped with a stir bar. It was left to stir for 48 hours at room temperature. The reaction was followed by TLCs (eluent – 9:1 or 8:2 Hexane/Ethyl Acetate).



1 2

Scheme 12. Esterification of the AA (1) with the formation of the MAA (2).

After, it was used a separatory funnel to separate the organic fraction from the aqueous fraction, starting by using 50 mL of water, then used MTBE, first 75 mL two times, and lastly 40 mL. We recovered the organic fraction to an Erlenmeyer flask, added anhydrous sodium sulfate, and filtered the organic fraction to a round-bottom flask. The organic fraction was dried in a rotary evaporator. A mixture of yellowish oil and brown oil was obtained which was washed with heated hexane (two times 6 mL and one time 10 mL), giving a yellow solution that was stored in the refrigerator for 10 hours.

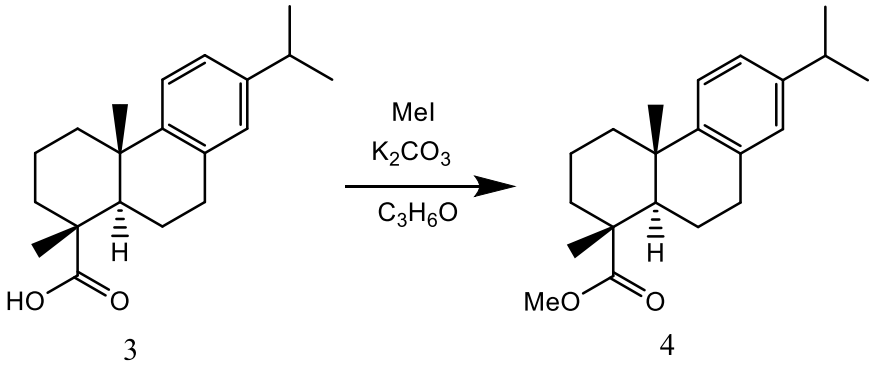
The solution was absorbed in silica and we did a column to isolate the products of the reaction. The fractions that had the products were collected to a round-bottom flask and then dried in a rotary evaporator. The eluent used was first 200 mL of Hexane/Ethyl Acetate (9.5:0.5) and then 600 mL of Hexane/Ethyl Acetate (9:1).

Two fractions were isolated, one first fraction that was a transparent oil (5.2962 g) and a second fraction that was a thick yellow oil (0.5012), both fractions had the methyl ester derivative, but the second fraction add less and was less pure, so the first fraction was the one used in the subsequent reactions. After being stored in the fridge both stayed as oil.

After isolating the methyl ester of abietic acid, it was isolated with 81.5 % yield as a transparent oil. It was characterized by ^{1}H-NMR and ^{13}C-NMR and were following the literature (37–39).

5.2 Esterification of DHA

For the esterification of DHA, we followed a reported protocol (41) which consisted in adding DHA (6.06 g, 0.0202 mol), acetone (90.0 mL, 0.22 M), iodomethane (4.0 mL, 0.064 mol, 3.2 equiv.), and potassium carbonate (7.07 g, 0.051 mol, 2.52 equiv.) to a 250 mL round-bottom flask equipped with a stir bar. It was left to stir for 48 hours at room temperature. The reaction was followed by TLCs (eluent – 9:1 or 8:2 Hexane/Ethyl Acetate).



3 4

Scheme 13. Esterification of the DHA (3) with the formation of the MDHA (4).

After, it was used a separatory funnel to separate the organic fraction from the aqueous fraction. We started by using 50 mL of water, then used MTBE, first 75 mL and second 50 mL. The organic fraction was recovered to an Erlenmeyer flask, added anhydrous sodium sulfate, and filtered the organic fraction to a round-bottom flask. The organic fraction was dried in a rotary evaporator. A transparent oil was obtained which was washed with heated hexane (two times 6 mL) and stored in the refrigerator for 10 hours.

The solution was absorbed in silica and we did a column to isolate the products of the reaction. The fractions that had the products were collected to a round-bottom flask and then dried in a rotary evaporator. The eluent used was first 100 mL of just Hexane, 300 mL of Hexane/Ethyl Acetate (9.5:0.5) and then 300 mL of Hexane/Ethyl Acetate (9:1).

Three fractions were isolated, one first fraction that was a transparent oil (0.0904 g), a second fraction that was also a transparent oil (3.1029 g), and a third fraction that was initially a transparent oil, but when stored in the fridge, it turned into a dense white oil,

close to a solid (4.0244 g). Only the second and third fractions did have the methyl ester derivative, but the second fraction add less and was less pure, so the third fraction was the one used in the subsequent reactions.

After isolating the methyl ester of dehydroabietic acid, it was isolated with 63.4 % yield as a transparent oil. It was characterized by ^{1}H-NMR and ^{13}C-NMR and were following the literature (37–41).

5.3 Cyclic Voltammetry studies of Abietanes AA, MAA, DHA and MDHA

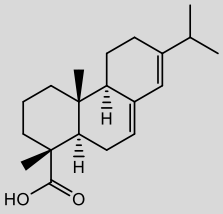
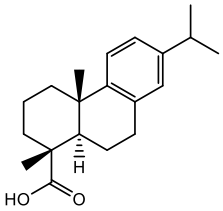
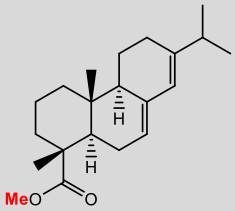
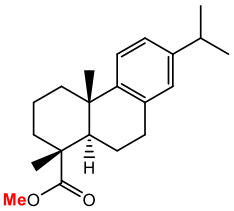
Cyclic voltammetry was performed using an IKA ElectraSyn 2.0.

The electrolyte used was 0.1 M $[Bu_4N][BF_4]$ and the solvent used was acetonitrile (3 mL).

The reference electrode was an AgCl/Ag electrode, the working electrode was made of glassy carbon, and the counter electrode of platinum.

The concentrations used in the experiments for each molecule are in **table 10**.

Table 10. Concentrations of Abietanes used in Cyclic voltammetry studies

Molecule				
Low concentration tested mmol/mL	2.6	3.9	5.3	5.6
High concentration tested mmol/mL	6.8	9.3	10.8	11.7

Regarding **figures 6 – 9**, each figure has the results obtained with cyclic voltammetry for each molecule, having an experiment with the background (no compound was added), and the multiple experiments performed where the concentration of the compound and the maximum potential of the experiment were varied.

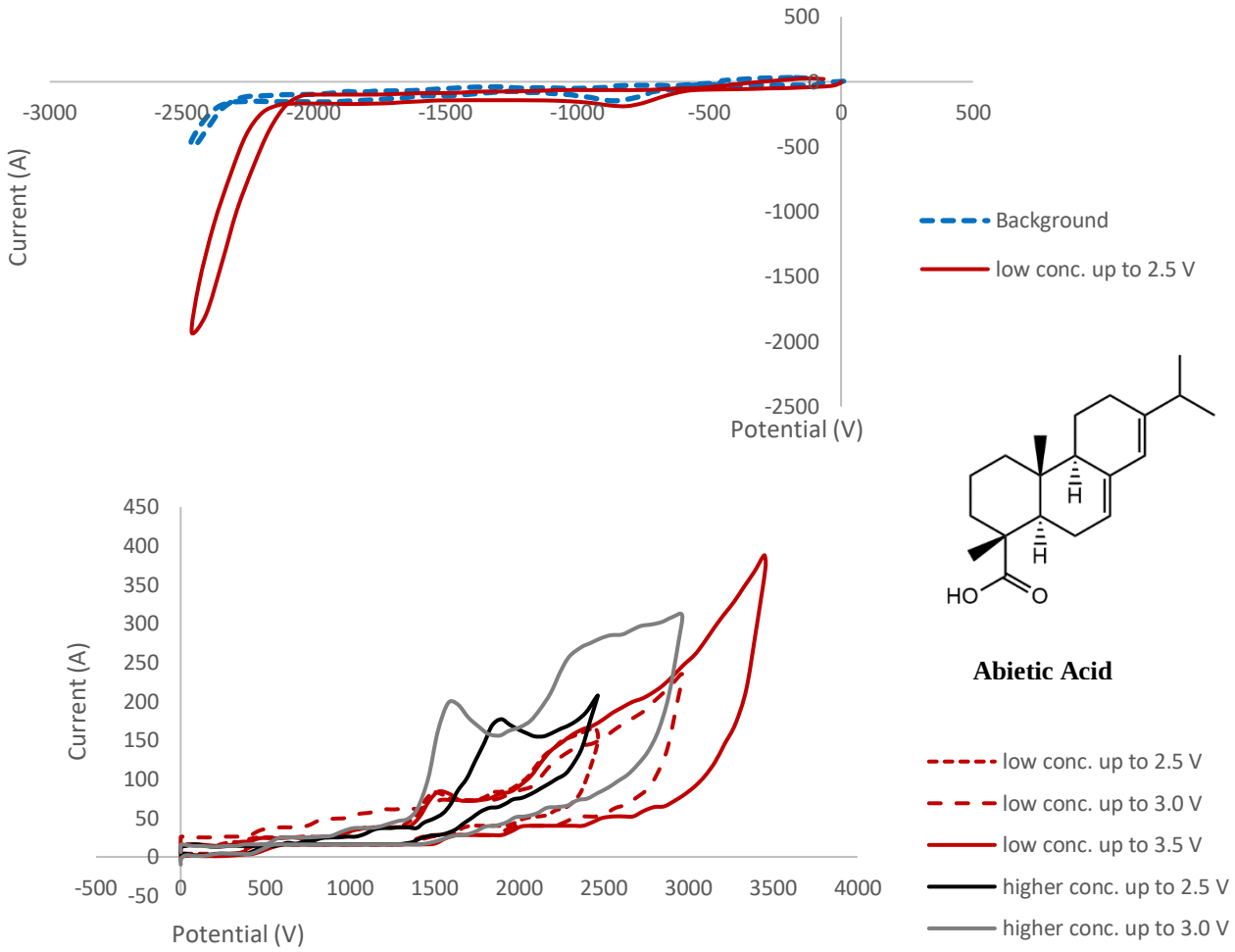


Figure 6. Cyclic voltammetry data of AA was obtained with a glassy carbon as working electrode and platinum as the counter electrode in acetonitrile containing $[Bu_4N][BF_4]$ as the electrolyte.

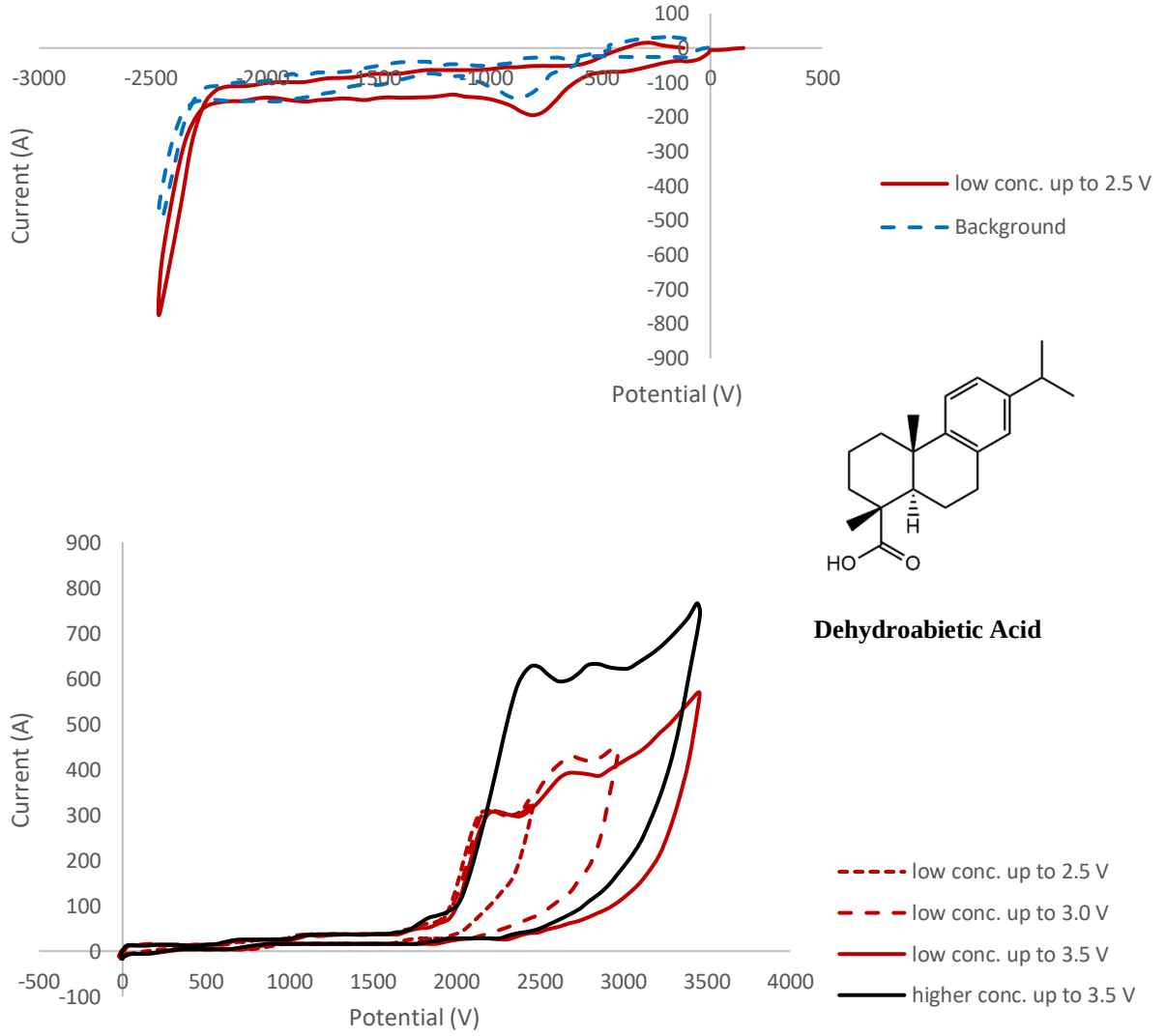


Figure 7. Cyclic voltammetry data of DHA was obtained with a glassy carbon as working electrode and platinum as the counter electrode in acetonitrile containing $[Bu_4N][BF_4]$ as the electrolyte.

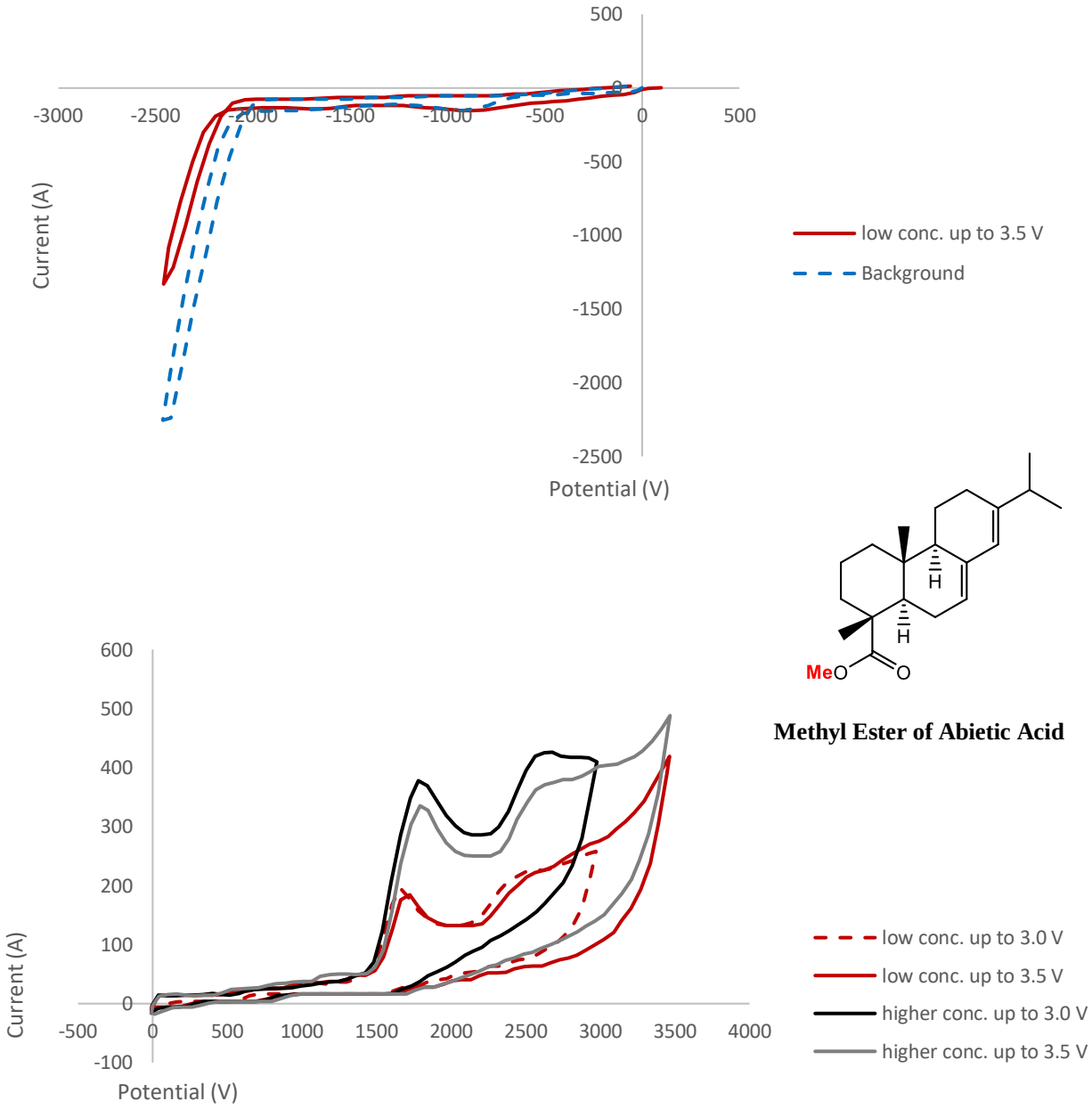


Figure 8. Cyclic voltammetry data of MAA was obtained with a glassy carbon as working electrode and platinum as the counter electrode in acetonitrile containing $[Bu_4N][BF_4]$ as the electrolyte.

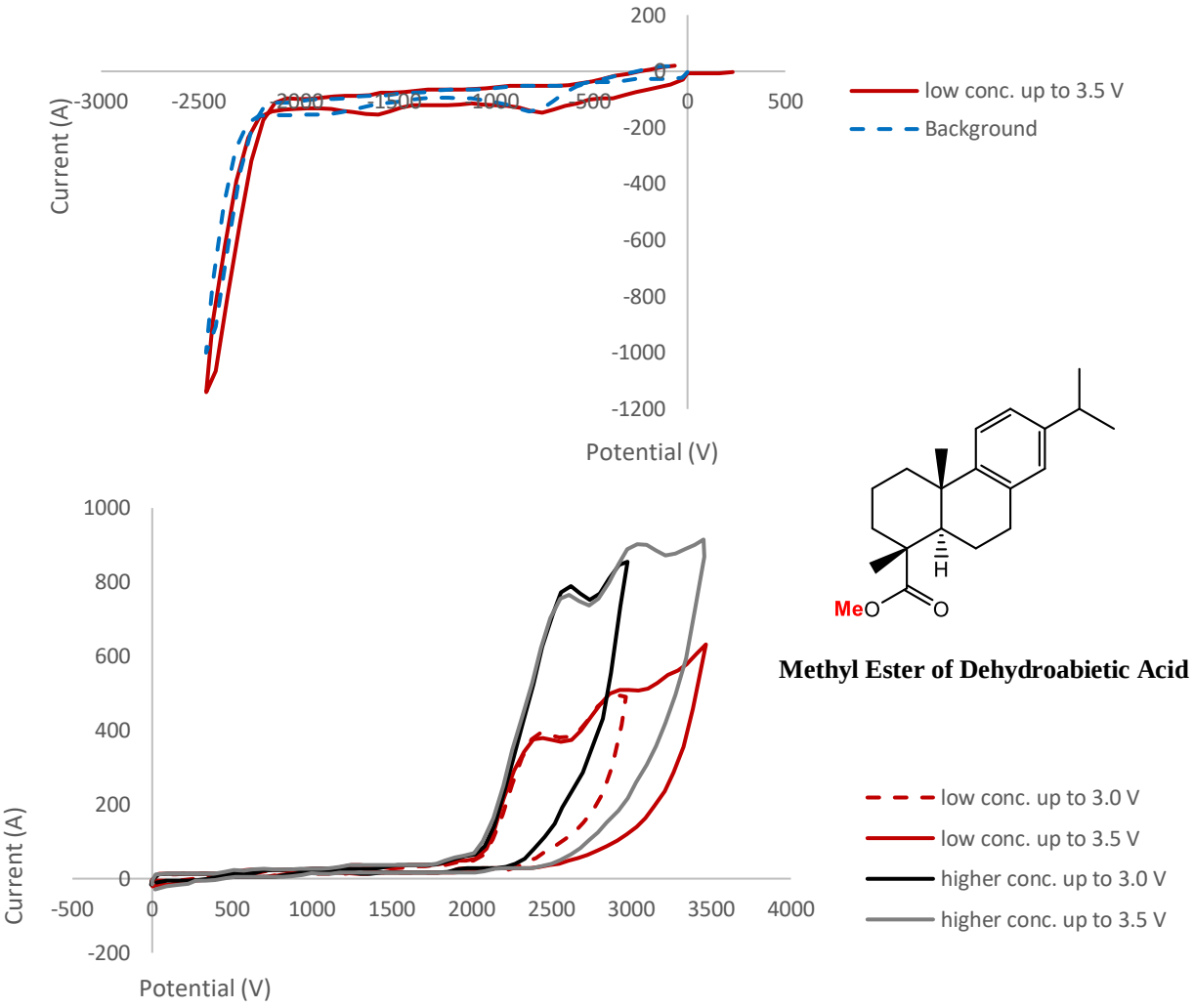
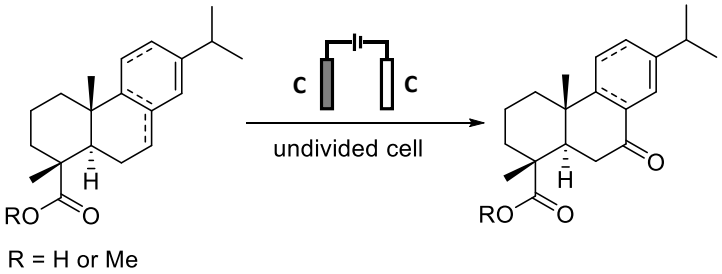


Figure 9. Cyclic voltammetry data of MDHA was obtained with a glassy carbon as working electrode and platinum as the counter electrode in acetonitrile containing [Bu_4N][BF_4] as the electrolyte.

5.4 Oxidation of Abietanes AA, MAA, DHA and MDHA

Regarding the oxidation of abietanes DHA and MDHA, the conditions are in **table 4**, and regarding the oxidation of abietanes AA and MAA, the conditions are in **table 6**.



Scheme 14. Oxidation of Abietanes DHA or AA (R = H) or MDHA or MAA (R = Me).

All these reactions were done at room temperature without care to remove air or moisture, unless stated otherwise. Also, in all these reactions the equipment used was an IKA ElectraSyn 2.0, graphite electrodes were used as both working and counter electrodes and let the reaction stir at 550 rpm during the whole experiment. All the reactions were followed by TLCs (eluent – 9:1 or 8:2 Hexane/Ethyl Acetate) and with an NMR of the reaction crude. The product isolated in all the experiments where conversion of the starting material to the product was observed was a yellowish oil. The products of the oxidation of DHA and MDHA was characterized by ^{1}H-NMR and ^{13}C-NMR and were following the literature (41). And the products of the oxidation of AA and MAA was characterized by ^{1}H-NMR and ^{13}C-NMR and were following the literature (51,52).

For oxidation of abietanes, uses as the foundation reported protocols (42–44), which consisted in adding the starting material, solvent, and supporting electrolyte in this order to a vial equipped with a stir bar. The reaction started as a transparent reaction medium with a white precipitate, and in the end, the reaction turned yellow. The reaction was followed through TLCs and then an NMR of the reaction crude.

Flash column chromatography was performed to isolate and purify the desired products of the reactions.

The eluent used for **entry 3** in **table 4**, was first 150 mL of just Hexane, 400 mL of Hexane/Ethyl Acetate (9.5:0.5), then 200 mL of Hexane/Ethyl Acetate (9:1) and at last 100 mL of Hexane/Ethyl Acetate (1:1). In the end, three fractions were collected and all of them had the product of the reaction, being that the second fraction was the purest one.

The eluent used for **entry 13** in **table 4**, was first 100 mL of just Hexane, then 300 mL of Hexane/Ethyl Acetate (9:1), and at last 200 mL of just Ethyl Acetate. In the end, five fractions were collected and all of them had the product of the reaction, being that the second fraction was the purest one.

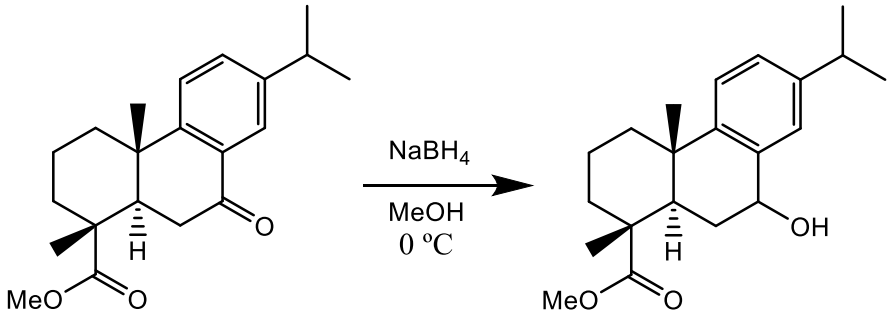
The eluent used for **entry 9** in **table 6**, was 600 mL of Hexane/Ethyl Acetate (9:1). In the end, three fractions were collected and the last two had the product of the reaction.

The eluent used for **entry 15** in **table 6**, was 400 mL of Hexane/Ethyl Acetate (9:1) plus 10 mL of formic acid. In the end, four fractions were collected and the two in the middle had the product of the reaction.

For the selective oxidation of MAA in a mixture of MAA and MDHA, we started by adding MDHA (100.2 mg, mol), MAA (100.7 mg, mol), acetonitrile (9.5 mL), water (0.6 mL), and lithium perchlorate (0.15 M) to a vail equipped with a stir bar. It was left to stir for 2.55 hours with a constant potential of 2.0 V at room temperature. The electricity was 2.0 F/mol.

5.5 Reduction of the ketone to the secondary alcohol

For the reduction of the ketone to the secondary alcohol, we followed a reported protocol (55), which consisted in adding the product of the oxidation of MDHA (60.5 mg, 0.18 mmol), methanol (2 mL, 0.09 M), and sodium borohydride (7.07 g, 0.051 mol, 2.52 equiv.) to a small round-bottom flask equipped with a stir bar. It was left to stir for 1 hour in a cold bath. At the end of the reaction, quench with saturated NH_4Cl (1 mL).



Scheme 15. Reduction of the ketone to the secondary alcohol.

After, a separatory funnel was used to separate the organic fraction from the aqueous fraction. Started by adding 5 mL of water, then extracted with DCM three times (5 mL). The organic fraction was recovered to an Erlenmeyer flask, added anhydrous sodium sulfate, and filtered the organic fraction to a round-bottom flask. The organic fraction was evaporated in a rotary evaporator.

At the end of the reaction, we observed the total conversion to the secondary alcohol with a 92% yield. The product isolated was a whiteish oil. It was characterized by ^{1}H-NMR and ^{13}C-NMR and were following the literature (55).

5.6 Cyclic Voltammetry studies of the secondary alcohol derivative

Cyclic voltammetry was performed using an IKA ElectraSyn 2.0.

The electrolyte used was 0.1 M $[Bu_4N][BF_4]$ and the solvent used was acetonitrile (3 mL).

The reference electrode was an AgCl/Ag electrode, the working electrode was made of glassy carbon, and the counter electrode of platinum.

Regarding **figure 10**, each figure has the results obtained with cyclic voltammetry for each molecule, having an experiment with the background (no compound was added), and the multiple experiments performed where the concentration of the compound and the maximum potential of the experiment were varied.

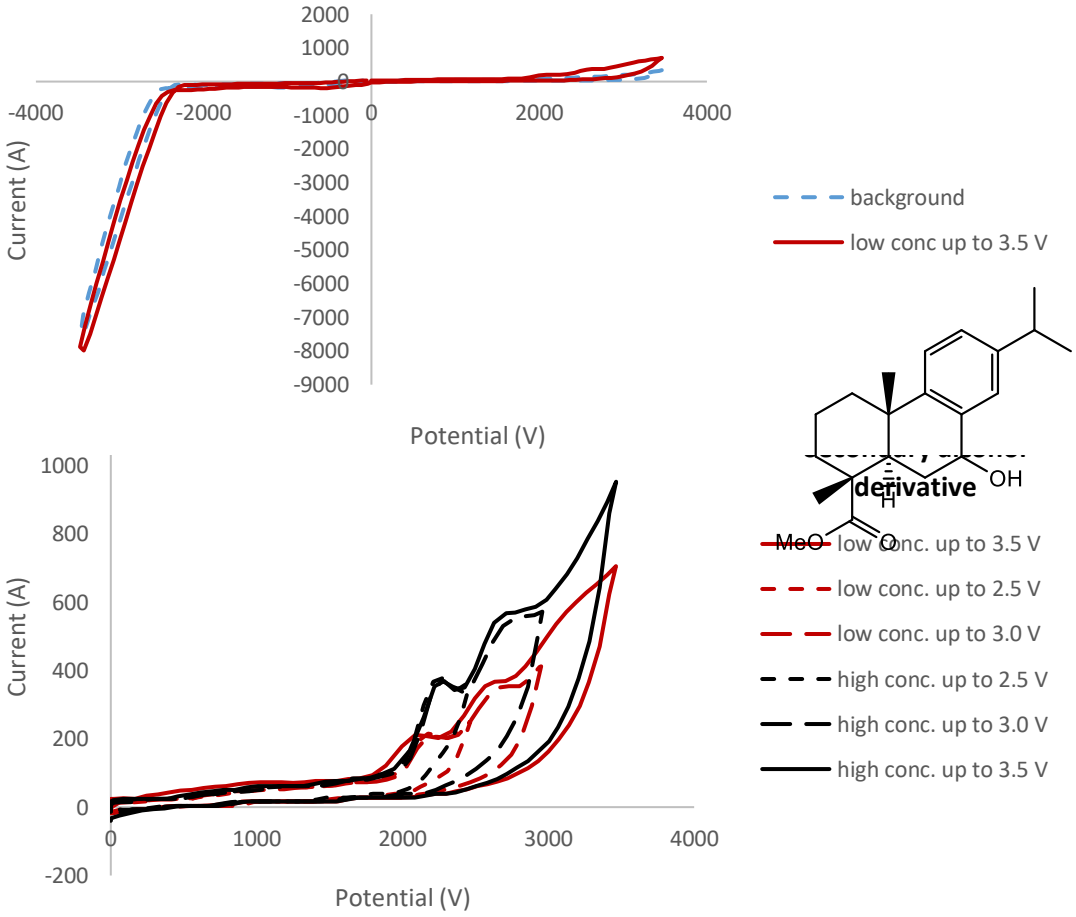
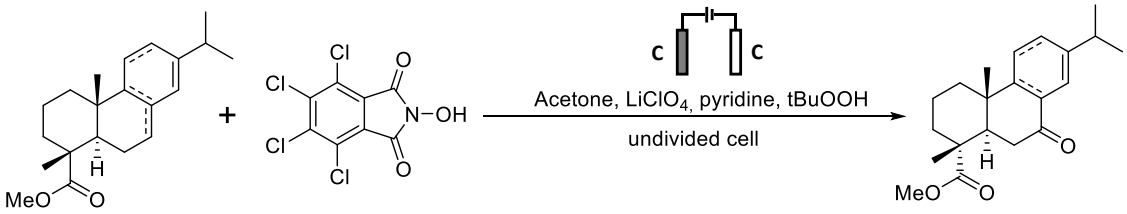


Figure 10. Cyclic voltammetry data of Secondary alcohol derivative was obtained with a glassy carbon as working electrode and platinum as the counter electrode in acetonitrile containing $[Bu_4N][BF_4]$ as the electrolyte.

5.7 Electrochemical Oxidation with Mediators

5.7.1 Allylic and Benzylic Oxidation

Regarding the oxidation with mediators of abietanes MDHA and MAA, the conditions are in **table 8**.



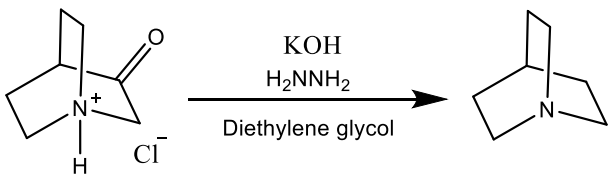
Scheme 16. Oxidation of Abietanes MDHA or MAA.

All these reactions were done at room temperature without care to remove air or moisture, unless stated otherwise. Also, in all these reactions the equipment used was an IKA ElectraSyn 2.0, graphite electrodes were used as both working and counter electrodes and let the reaction stir at 550 rpm during the whole experiment. All the reactions were followed by TLCs (eluent – 9:1 or 8:2 Hexane/Ethyl Acetate) and with an NMR of the reaction crude.

For oxidation of abietanes, we followed the protocols reported by Baran's group (34), which consisted in adding the starting material, acetone, lithium perchlorate, and Cl_4NHPI in this order to a vial equipped with a stir bar. Then we added pyridine, where it is observed a change of the solution from pale yellow to red, which resulted from the formation of a suspension of the same color. The reaction was followed through TLCs and then an NMR of the reaction crude.

5.7.2 Synthesis of quinuclidine

For the synthesis of quinuclidine we followed a general protocol (60,61), which consisted in adding to a round-bottom flask quinuclidone, then diethylene glycol, potassium hydroxide, and at last hydrazine. All the reactions were conducted under an inert atmosphere (N_2) and heated to reflux. The conditions of each experiment are in **table 11**.



Scheme 17. Synthesis of quinuclidine.

Regarding **entries 1** and **2**, we used the same conditions as the ones described by Baran's group (60), except for the use of Dean-Stark apparatus. We started by heating the reaction mixture to reflux (135ºC) for 2 hours, then increased the temperature (165ºC) for another 4 hours.

Regarding **entries 3** to **5**, we started by heating the mixture to reflux for 2 hours (165ºC).

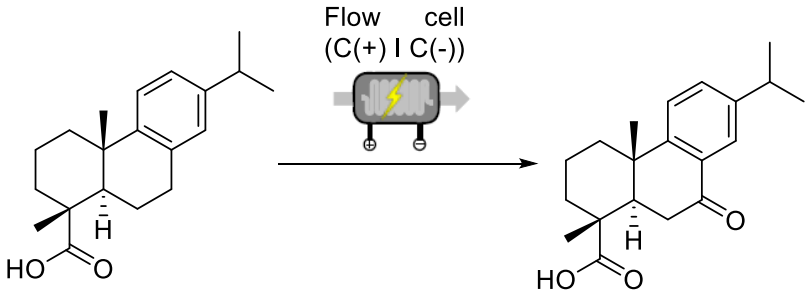
Concerning the isolation and purification of quinuclidine, three different paths were followed, first was combining **entries 1** to **3** and did a distillation under vacuum at low pressure and high temperature, second try, **entry 4**, did a distillation with a more powerful vacuum pump, or third try, **entry 5**, did a distillation with a more powerful vacuum pump, with cold water bath passing through the condenser, and afterwards, the reaction crude was transferred to a separating funnel to which different organic solvents (four times MTBE 10 mL, one time Hexane 10 mL, one time Ethyl Acetate 10 mL) or an acid solution (10 mL of HCl) were added.

Table 11. Reaction conditions synthesis of quinuclidine.

Entry	Subtract (mg)	Diethylene Glycol (mL)	Potassium hydroxide (mg)	Hydrazine (μL)
1	103.9	0.7	195	90
2	102.1	0.7	207.2	90
3	100.5	0.7	133.1	90
4	507.3	3.5	700.2	450
5	2002.0	14	2805.8	1800

5.8 Flow electrochemistry for oxidation of DHA

Regarding the oxidation of DHA with flow electrochemistry, all these reactions were done at room temperature without care to remove air or moisture. Also, in all these reactions the equipment used was an IKA ElectraSyn flow, graphite electrodes as both working and counter electrode, undivided, with an interelectrode gap which volume was 1.2 mL. All the reactions were followed by TLCs (eluent – 9:1 Hexane/Ethyl Acetate) and with an ^{1}H NMR of the reaction crude.

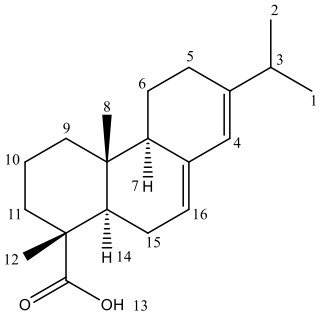


Scheme 18. Oxidation of Abietanes DHA.

The reaction conditions for the oxidation of DHA are described in **table 9** and the general procedure consisted in adding the starting material, solvent, and supporting electrolyte in this order to an Erlenmeyer flask equipped with a stir bar, resulting in a white suspension. Then 10 mL of the reaction mixture were taken to a disposable syringe and were pumped throughout the electrochemical setup with a fixed flow rate. In the beginning and in between experiments under different reaction conditions we had discarded 2 mL of the reaction mixture.

6 Product Characterization

Abietic Acid (AA). **^{1}H NMR (300 MHz, $CDCl_3$) δ** 5.77 (s, 1H), 5.38, 5.38 (d, 1H, *J* = 3.8 Hz), 2.24-2.20 (m, 1H), 2.09 – 2.07 (m, 4H), 1.92 – 1.90 (m, 2H), 1.86 (s, 1H), 1.83 – 1.76 (m, 4H), 1.70 – 1.69 (s, 1H), 1.61 – 1.58 (m, 2H), 1.26 (s, 3H), 1.02, 1.01 (d, 3H, *J* = 2.71Hz), 1.00 – 0.99 (d, 3H, *J* = 2.76 Hz), 0.83 (s, 3H).



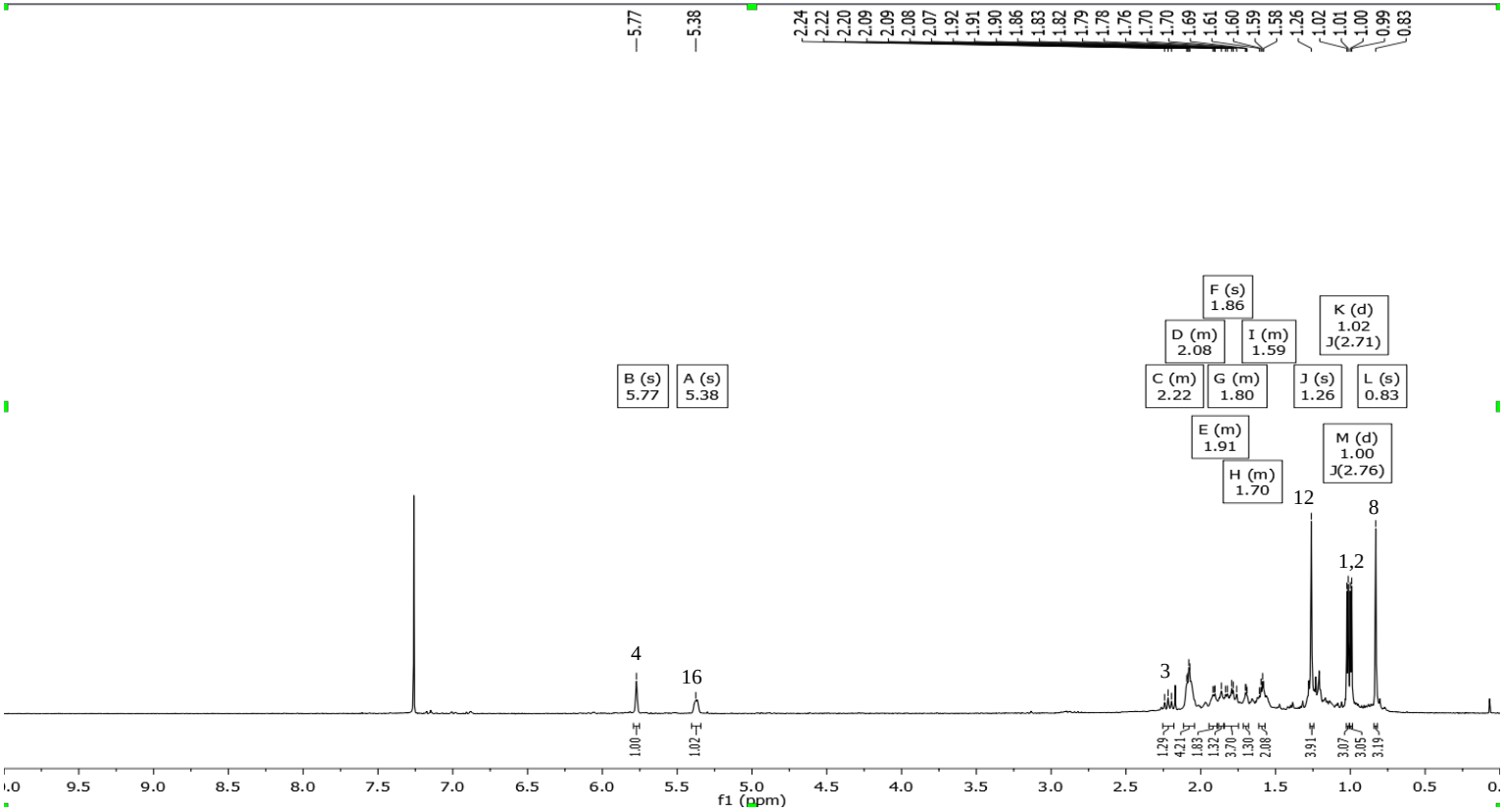


Figure 11. ^{1}H NMR spectrum of AA.

Dehydroabietic Acid (DHA). **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.19, 7.16 (d, 1H, *J* = 8.18 Hz), 7.02 – 6.99 (dd, 1H, *J* = 8.14, 2.04), 6.89 (d, 1H, *J* = 2.04 Hz), 2.95 – 2.88 (m, 2H), 2.85 – 2.76 (sept, 1H), 2.28 – 2.23 (dd, 1H, *J* = 12.45, 2.20 Hz), 1.83 (m, 2H), 1.80 – 1.74 (m, 3H), 1.71 - 166 (m, 2H), 1.61 – 1.59 (m, 1H), 1.57 – 1.50 (m, 2H), 1.29 (s, 3H), 1.24 (s, 3H), 1.23 (s, 3H).

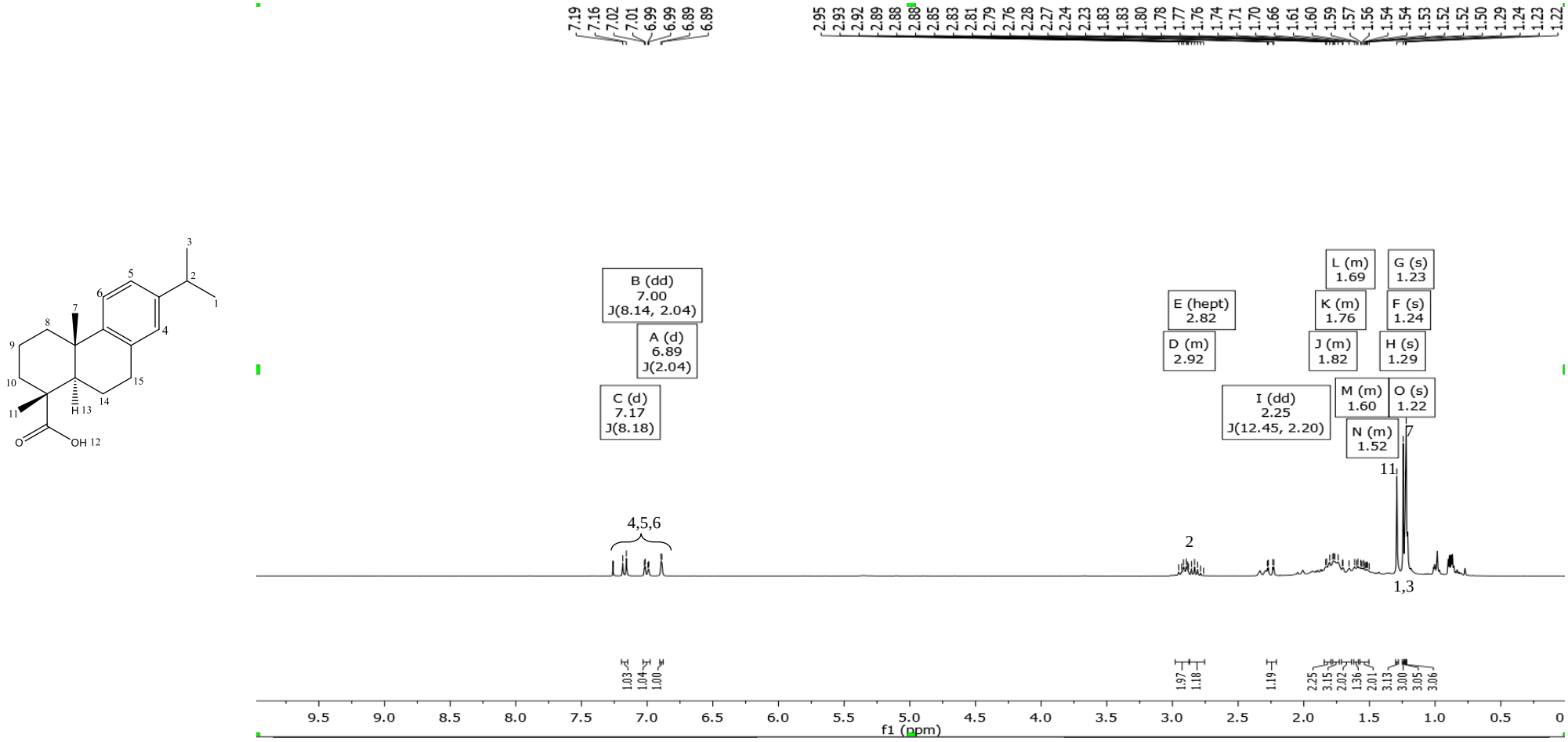


Figure 12. ^{1}H NMR spectrum of DHA.

Methyl Ester of Abietic Acid (MAA). **^{1}H NMR (300 MHz, $CDCl_3$) δ** 5.77 (s, 1H), 5.36, 5.34 (d, 1H, *J* = 5.4 Hz), 3.61 (s, 3H), 2.27 – 2.14 (sept, 1H, *J* = 6.9, 6.4 Hz), 2.07 – 2.03 (m, 4H), 1.90 – 1.69 (m, 5H), 1.61 – 1.52 (m, 3H), 1.24 (s, 3H), 1.01 (d, 3H, *J* = 2.6 Hz), 0.98 (d, 3H, *J* = 2.7 Hz), 0.81 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 179.1, 145.5, 135.7, 122.5, 121.4, 52.9, 51.7, 51.0, 50.5, 46.7, 35.1, 34.7, 27.6, 22.4, 21.9, 20.7, 19.6, 18.3, 17.9, 16.3, 14.9.

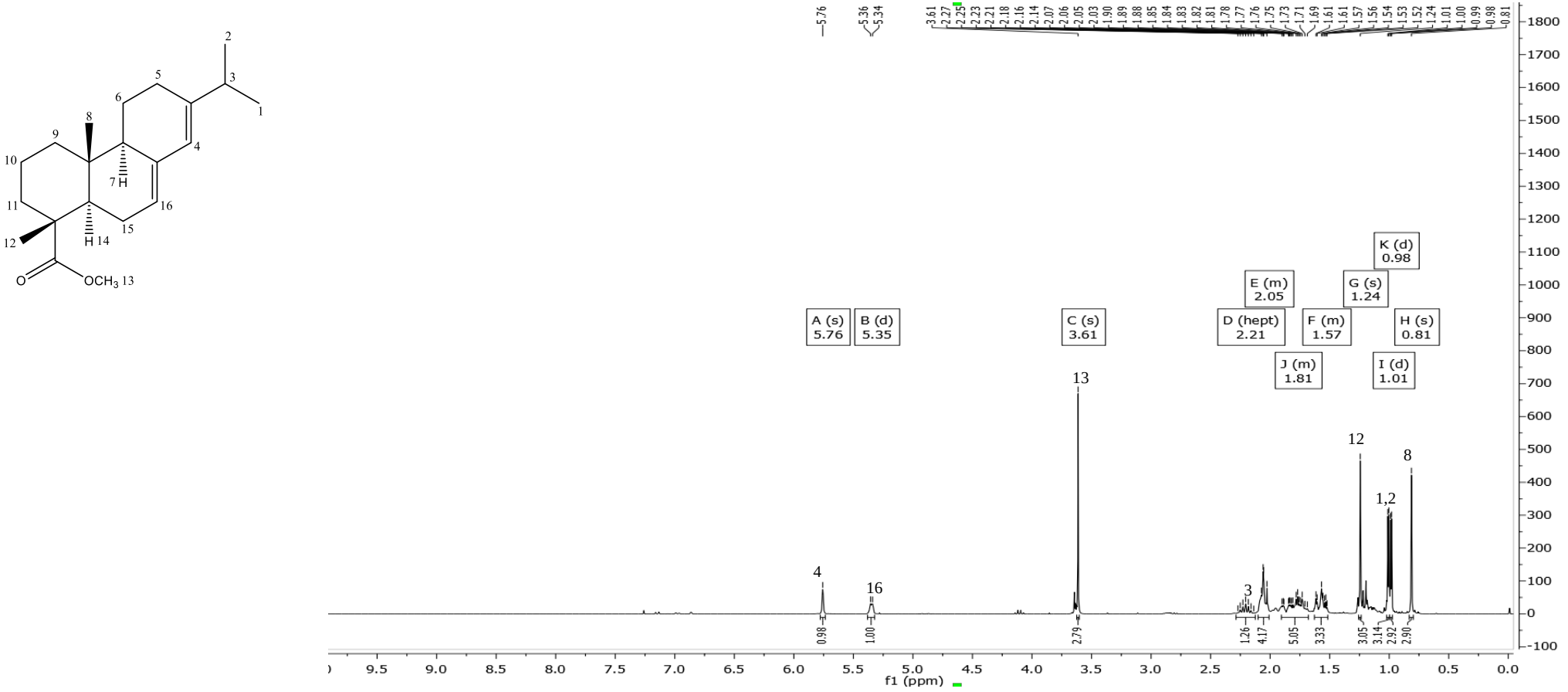


Figure 13. ^{1}H NMR spectrum of MAA.

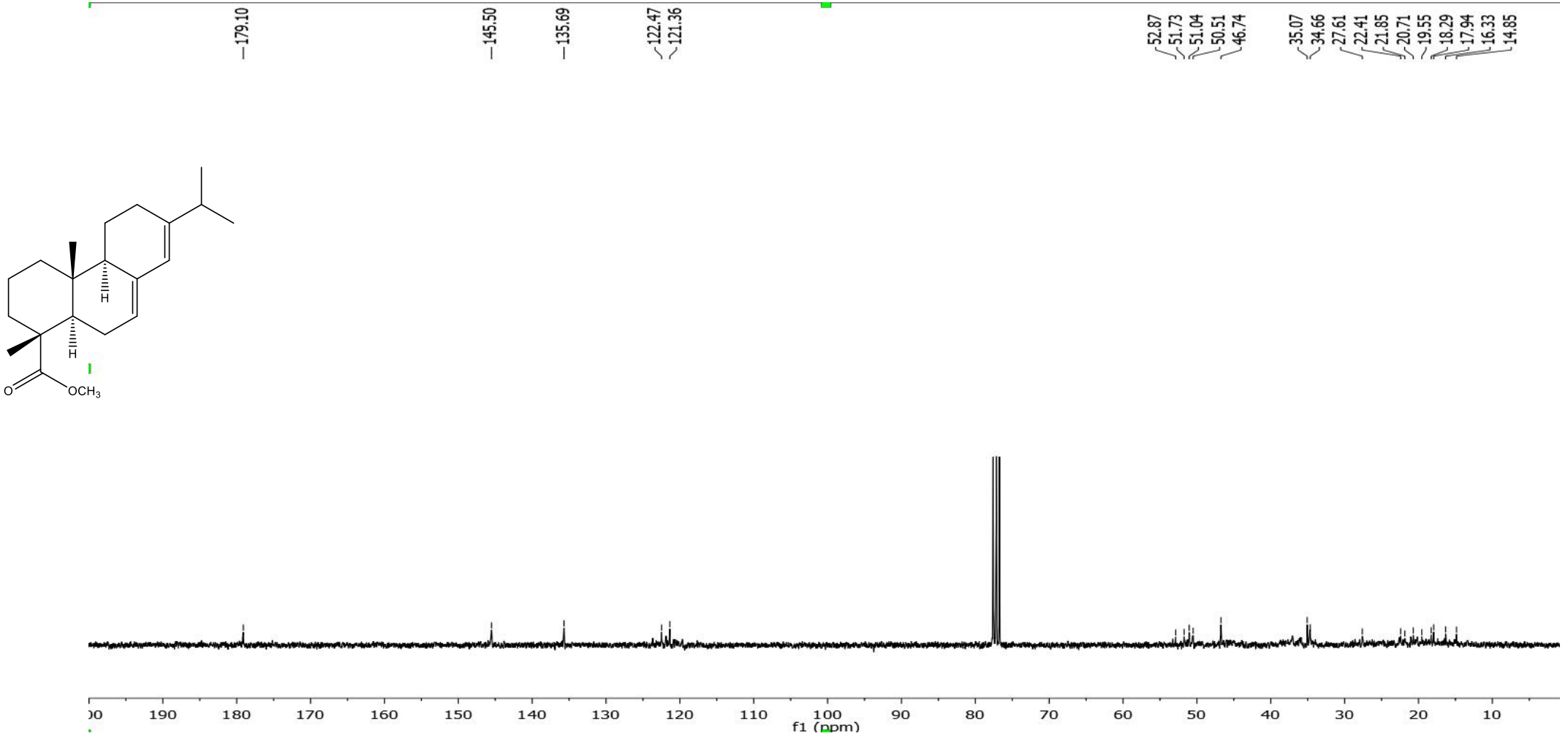
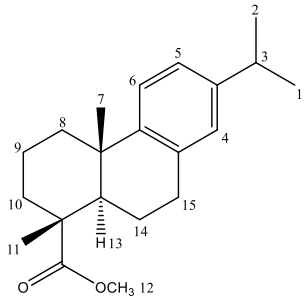


Figure 14. ^{13}C NMR spectrum of MAA.

Methyl Ester of Dehydroabietic Acid (MDHA). **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.18, 7.15 (d, 1H, *J* = 8.2 Hz), 7.01 – 6.88 (d, 1H, *J* = 8.2, 1.6 Hz), 6.88 (s, 1H), 3.66 (s, 3H), 2.91 – 2.76 (m, 3H), 2.33 – 2.28 (m, 1 H), 2.27 – 2.22 (m, 1H), 1.75 – 1.58 (m, 7H), 1.28 (s, 3H), 1.24 (s, 3H), 1.21 (s, 6H). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 179.3, 147.0, 145.8, 134.8, 128.0, 125.0, 123.3, 52.9, 51.0, 47.8, 45.9, 45.1, 37.1, 33.9, 33.8, 30.1, 24.9, 24.5, 23.3, 21.7, 18.72.



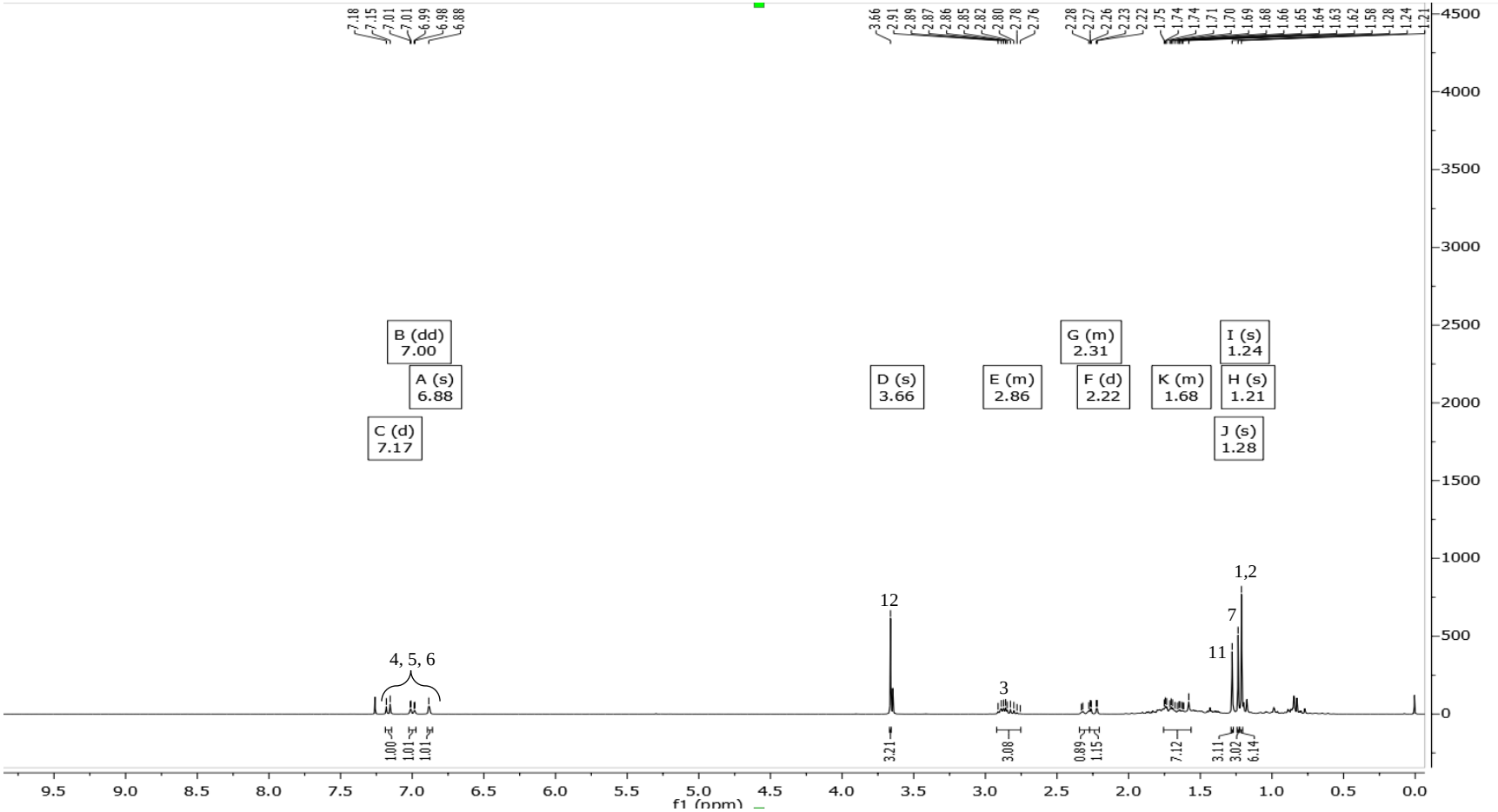


Figure 15. ^{1}H NMR spectrum of MDHA.

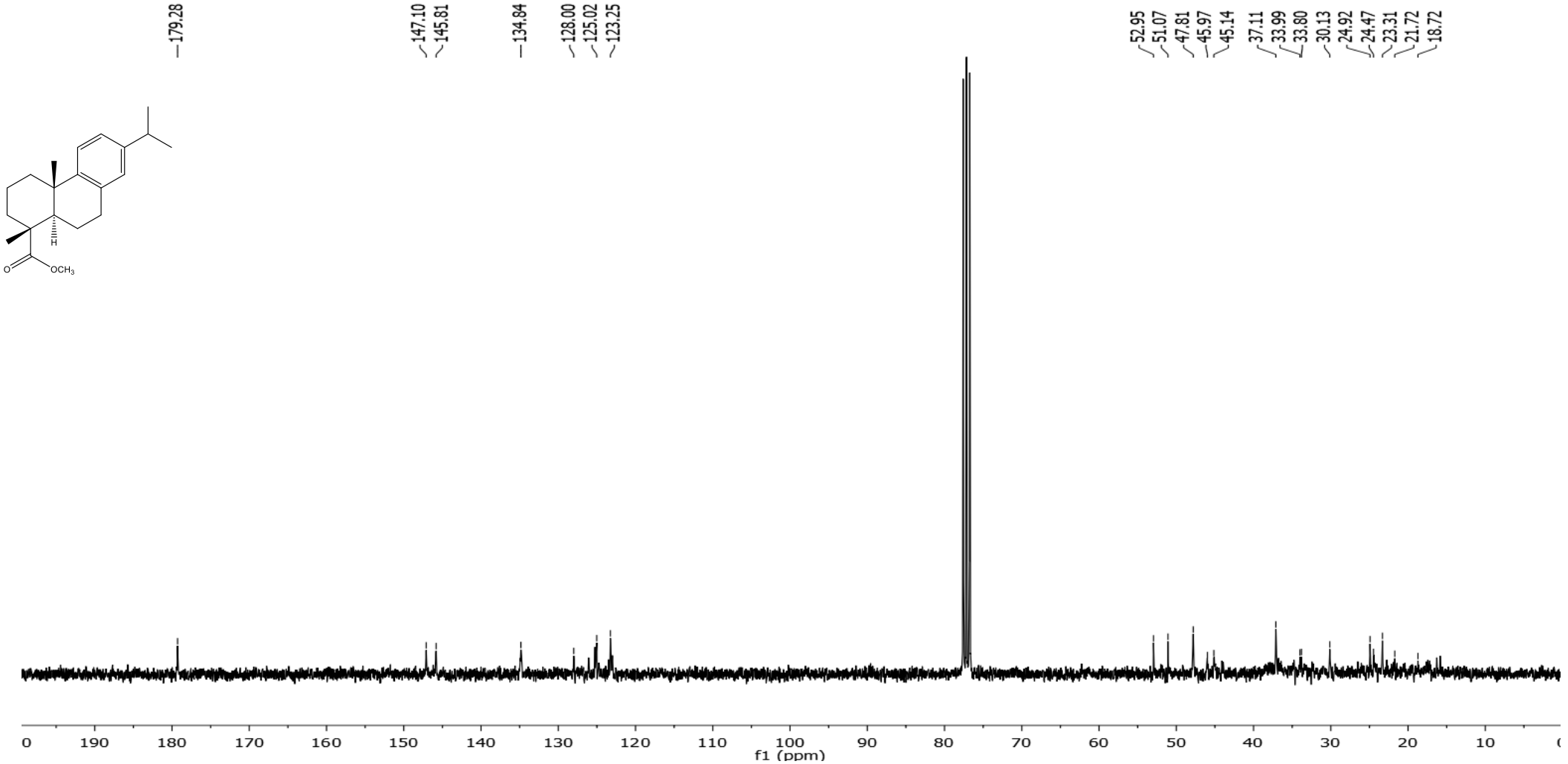


Figure 16. ^{13}C NMR spectrum of MDHA.

Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.79 (d, 1H, *J* = 2.2Hz), 7.32 (dd, 1H, *J* = 8.1, 2.1), 7.21 (d, 1H, *J* = 8.2), 3.56 (s, 3H), 2.84 (sept, 1H, *J* = 6.9 Hz), 2.71 – 2.56 (m, 2H), 2.35 – 2.17 (m, 2H), 1.82 – 1.44 (m, 5H), 1.26 (s, 3H), 1.17 (s, 6H), 1.15 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 198.5, 177.8, 152.9, 146.9, 132.6, 130.7, 125.0, 123.5, 52.2, 46.7, 43.8, 37.8, 37.3, 37.1, 36.5, 33.6, 23.8-23.7, 18.2, 16.4.

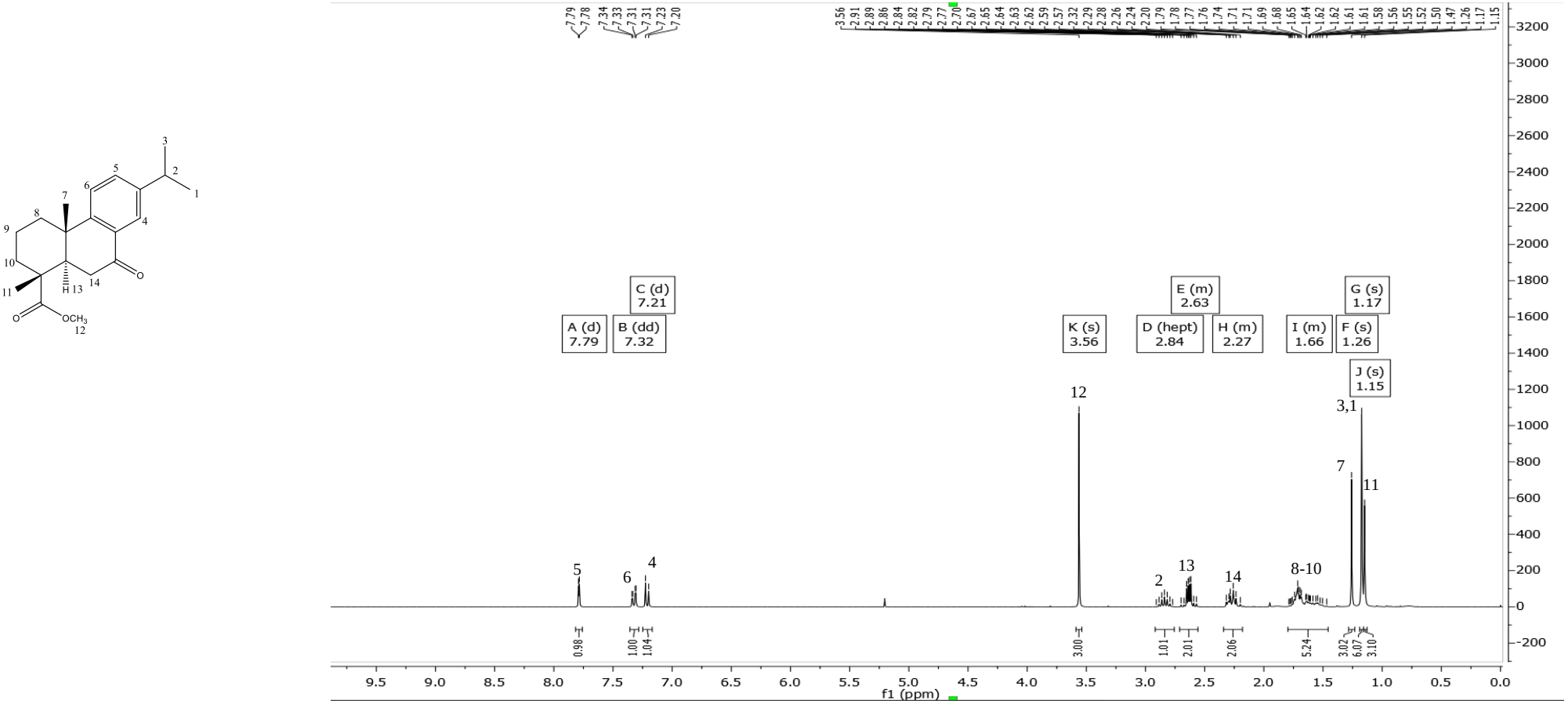


Figure 17. ^{1}H NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

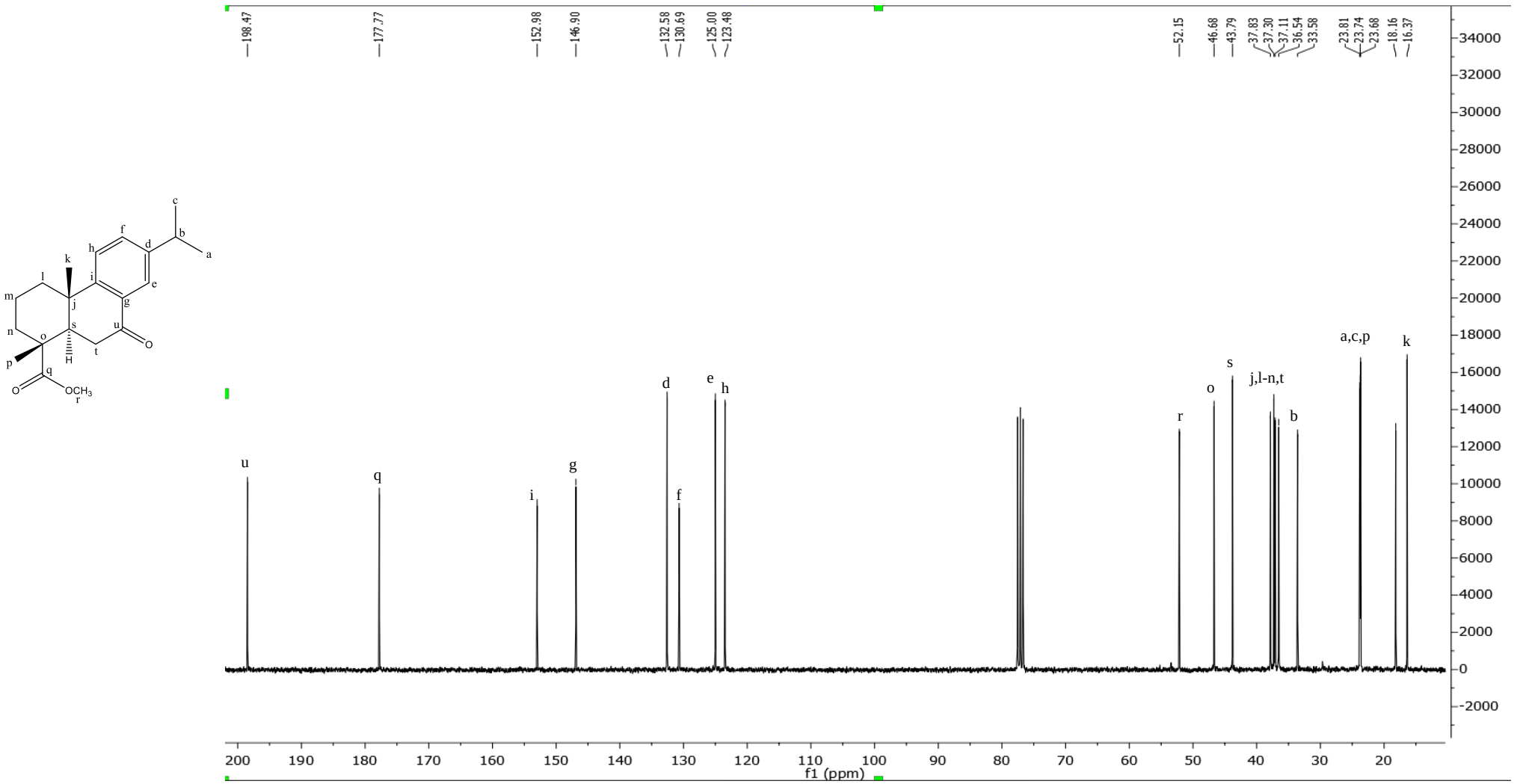


Figure 18. ^{13}C NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

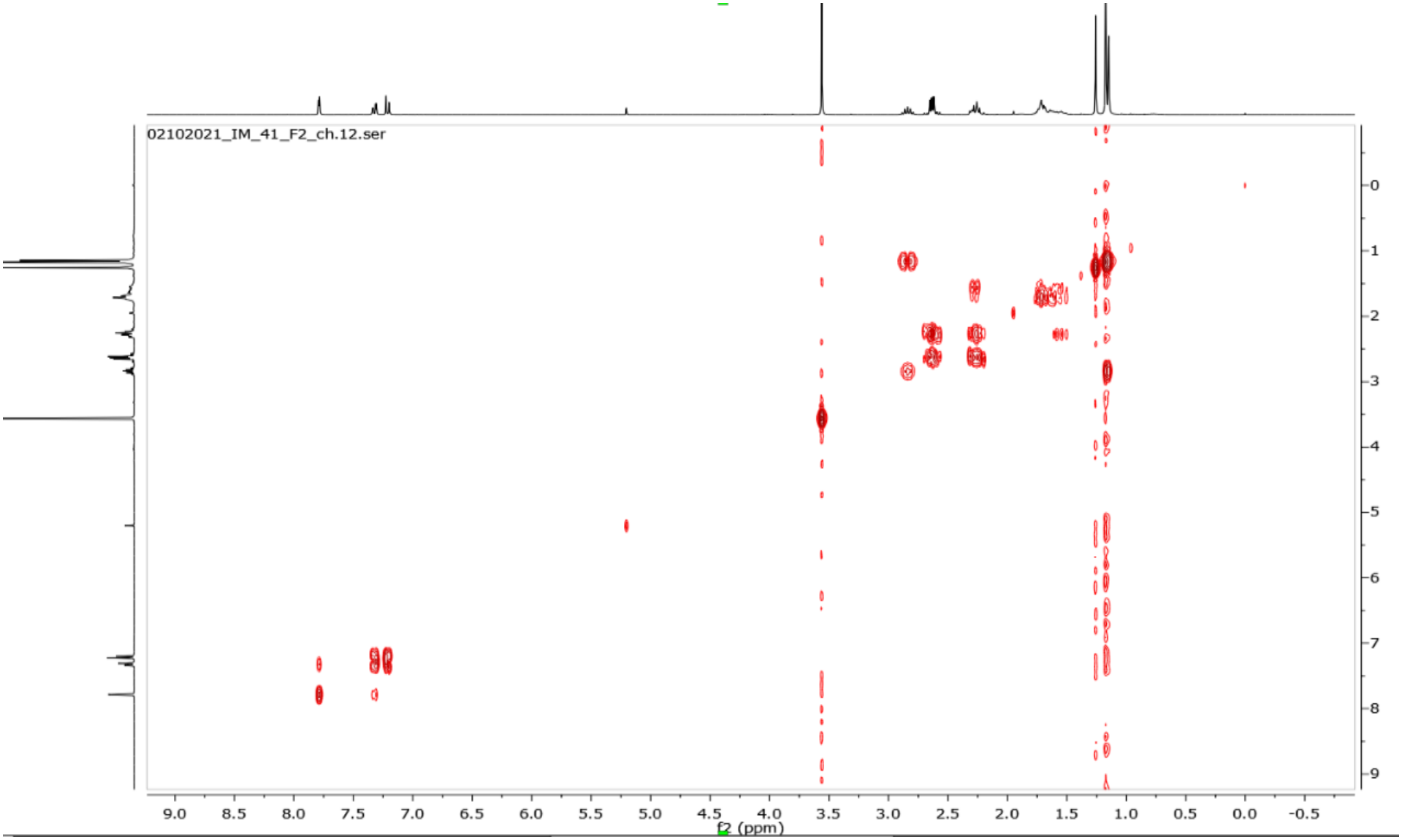


Figure 19. COSY NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

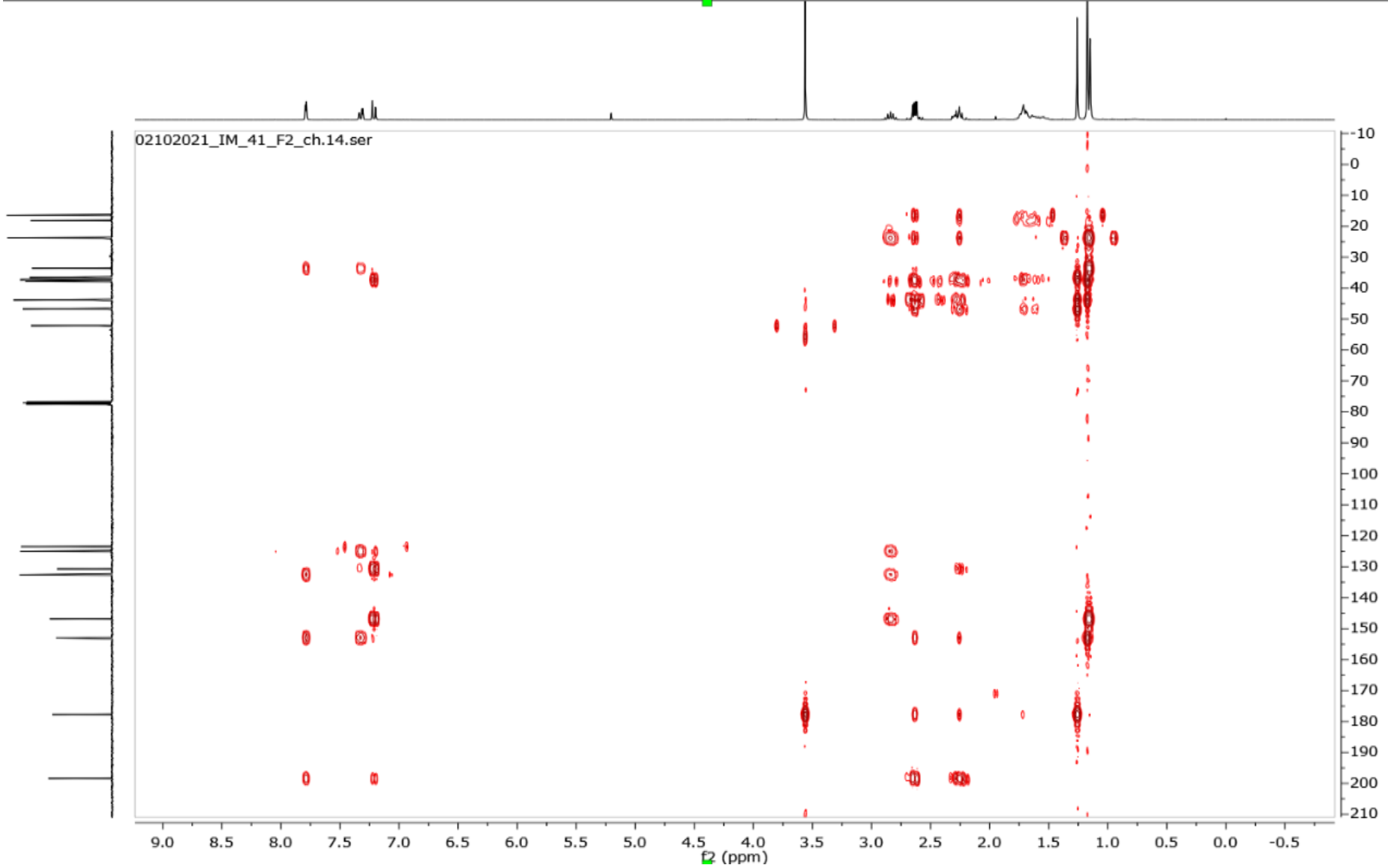


Figure 20. HMQC NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

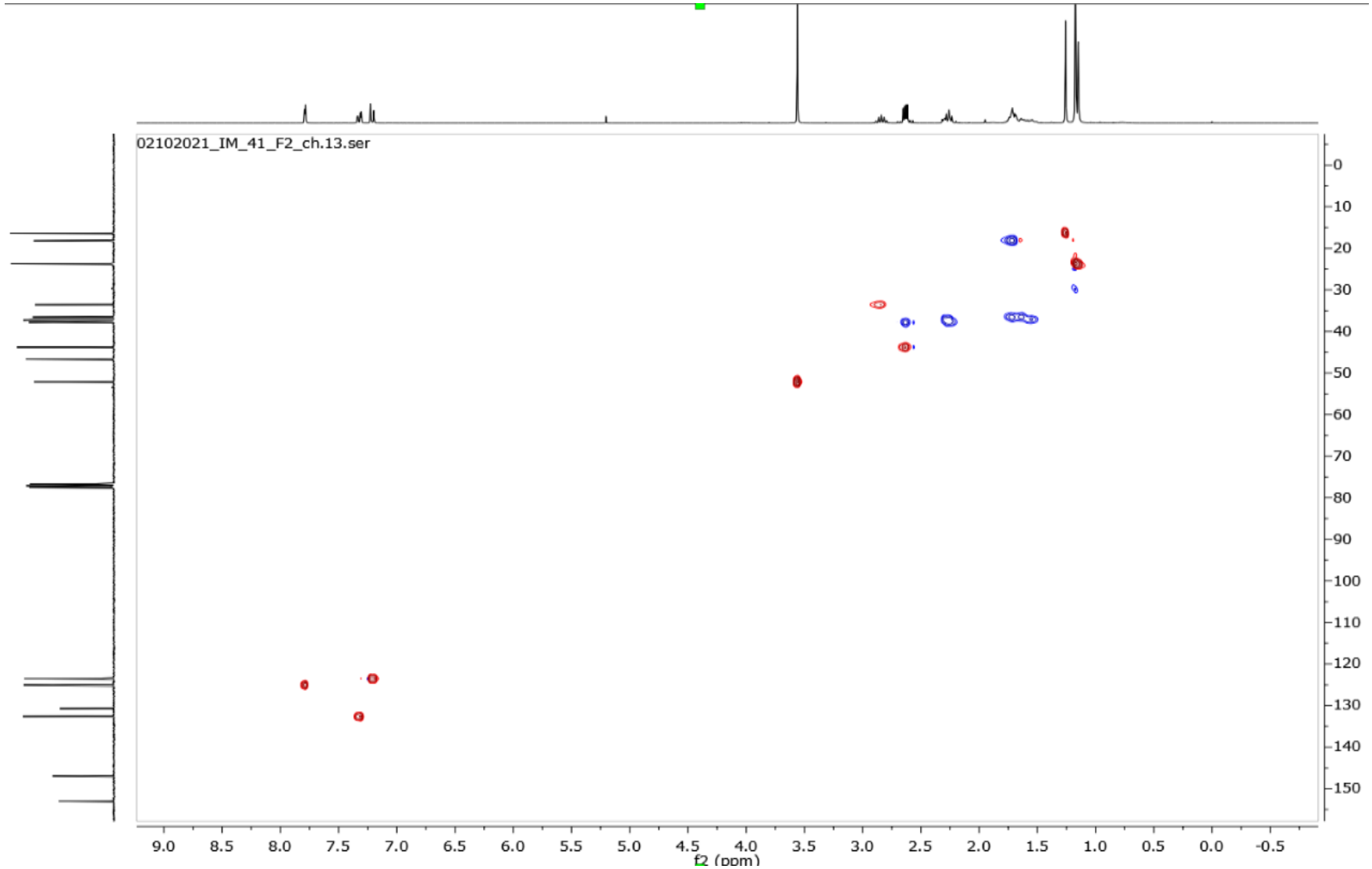


Figure 21. HSQC NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.86 (d, 1H, *J* = 2.1 Hz), 7.41 – 7.37 (dd, 1H, *J* = 8.2, 2.1 Hz), 7.28 (d, 1H, *J* = 8.26 Hz), 2.97 – 2.83 (sept, 1H, *J* = 6.90), 2.75 – 2.66 (m, 2H), 2.50 – 2.46 (m, 1H), 2.37 – 2.24 (m, 2H), 1.86 – 1.76 (m, 5H), 1.33 (s, 3H), 1.25 (s, 3H), 1.23 (s, 3H), 1.21 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 199.1, 183.1, 153.1, 147.0, 132.8, 130.7, 125.2, 123.6, 46.5, 43.7, 37.8, 37.3, 37.2, 36.6, 33.7, 23.9, 23.8, 23.7, 18.2, 16.2.

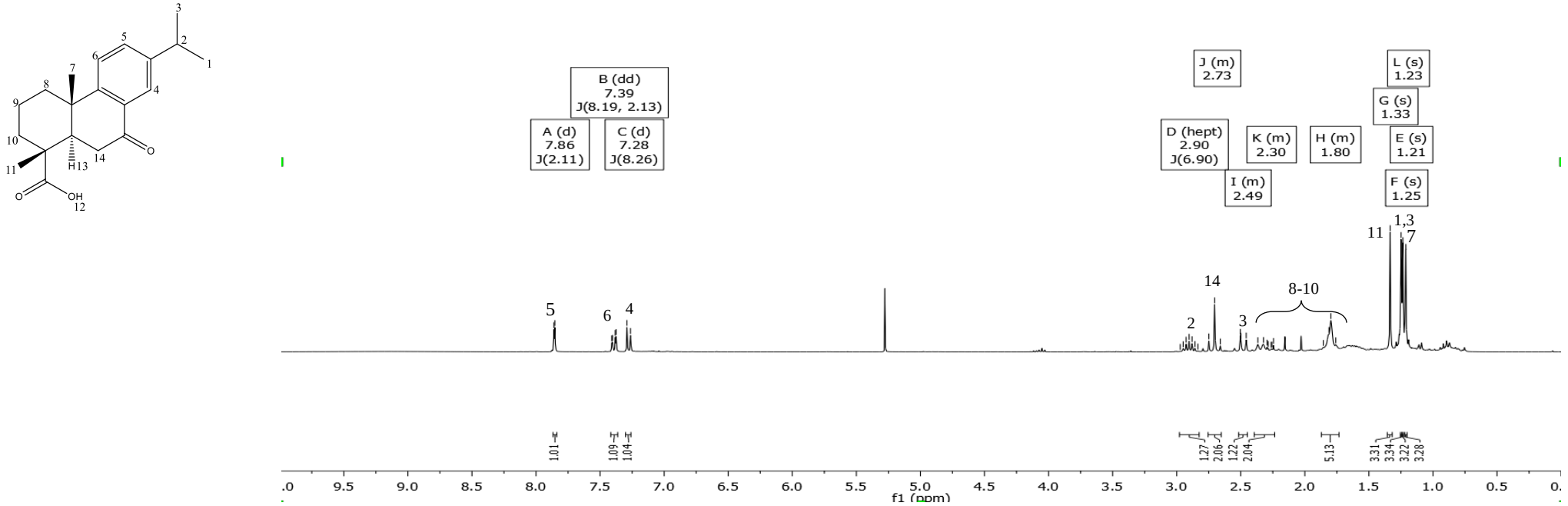


Figure 22. ^{1}H NMR spectra of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid.

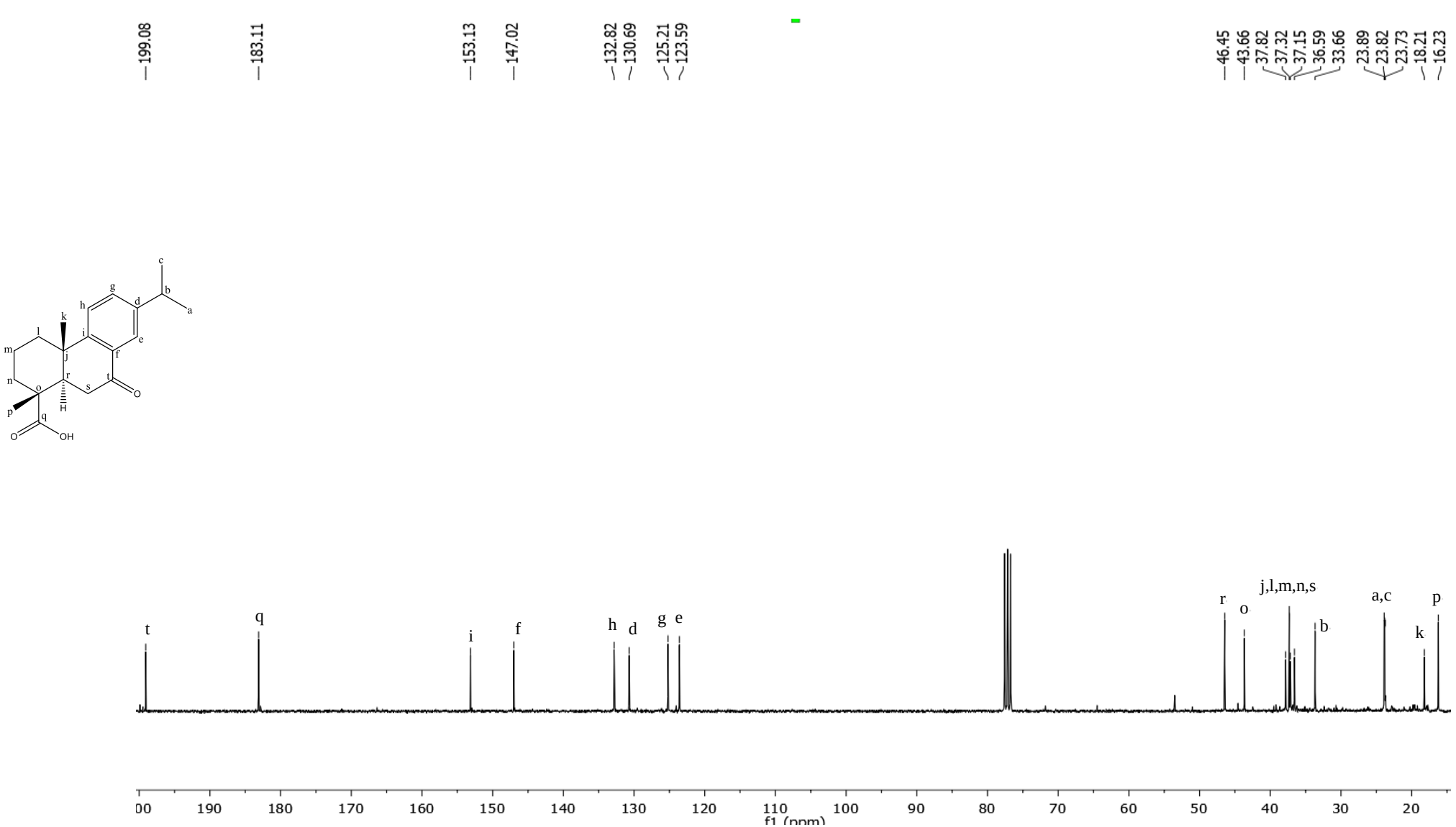


Figure 23. ^{13}C NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid.

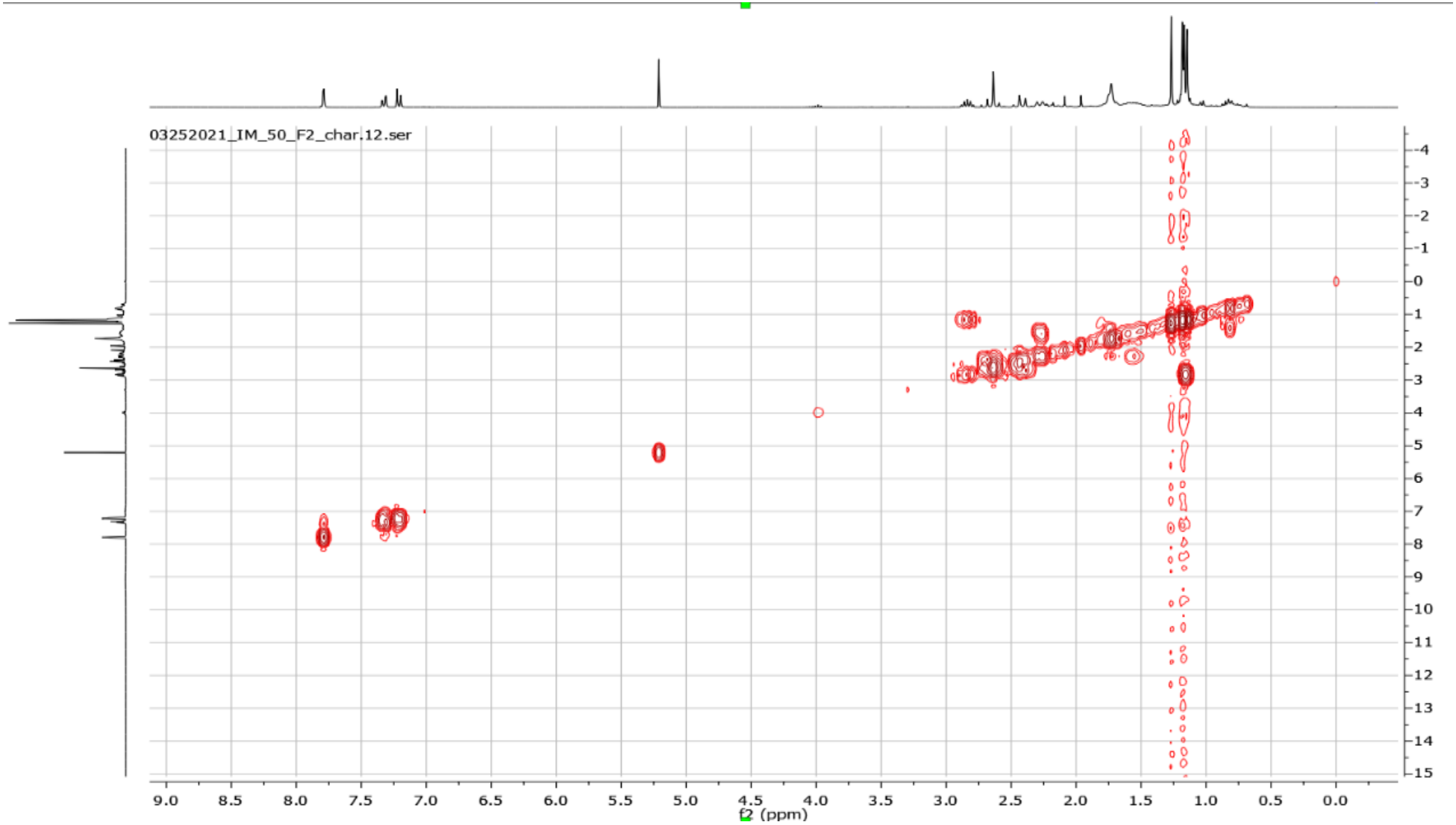


Figure 24. COSY NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid.

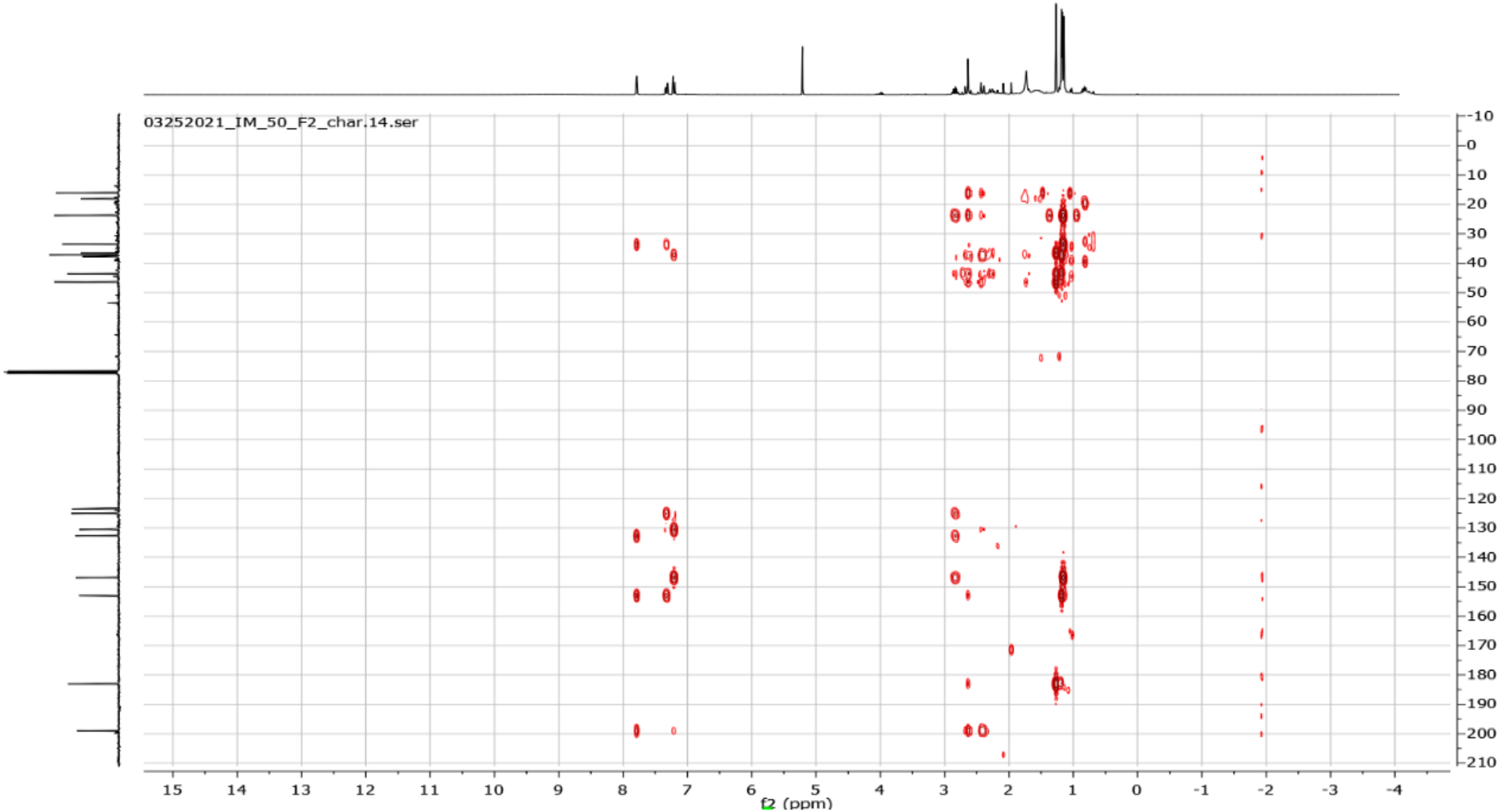


Figure 25. HMQC NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid.

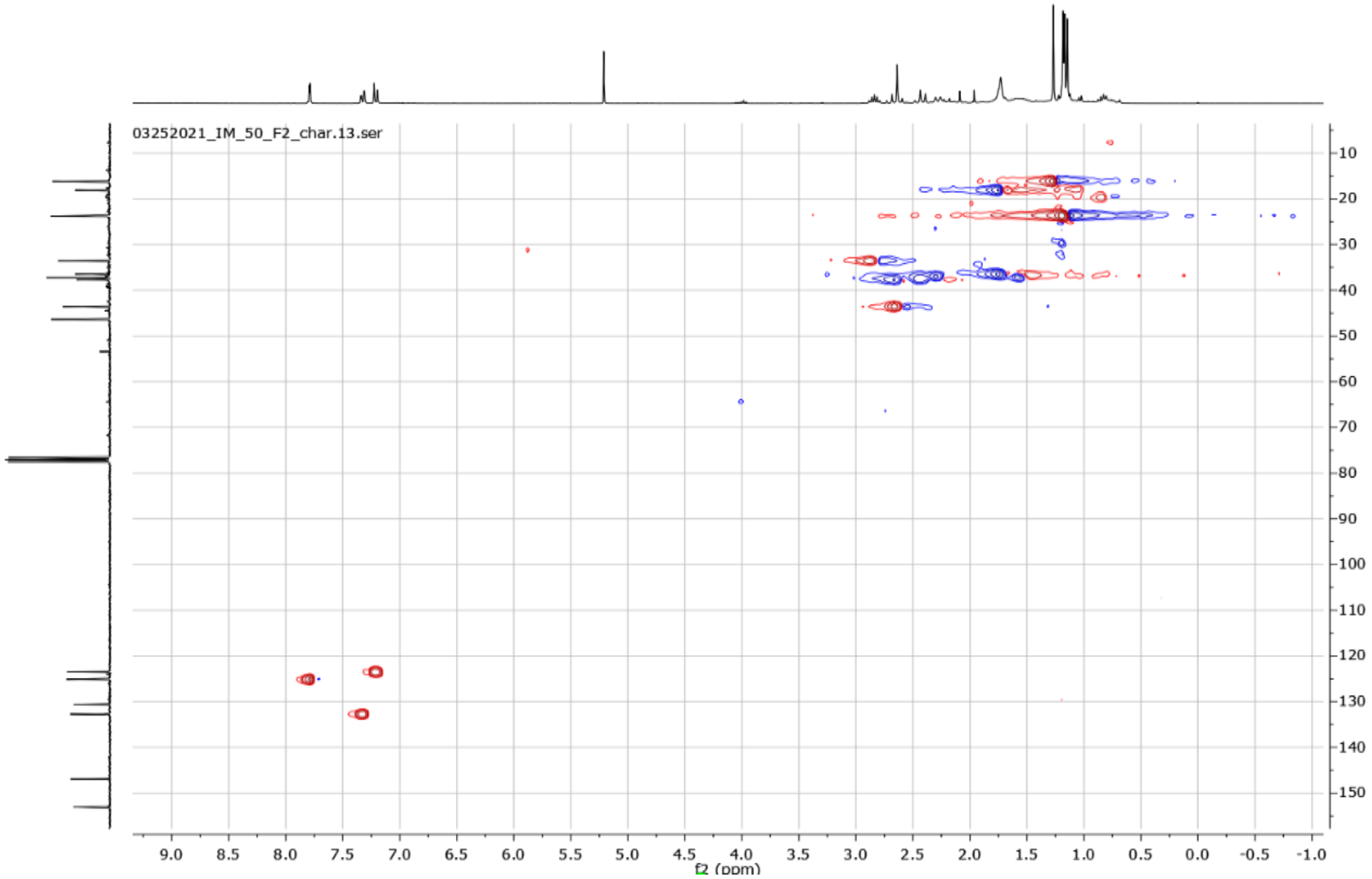


Figure 26. HSQC NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylic acid.

Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylate.**^{1}H NMR (300 MHz, $CDCl_3$) δ** 5.80 (s, 1H), 3.63 (s, 3H), 2.89, 2.86 (d, 1H, *J* = 9.9 Hz), 2.45 – 2.36 (td, 1H, *J* = 14.1, 1.2 Hz), 2.27 – 2.19 (m, 1H), 2.17 – 2.12 (dd, 1H, *J* = 14.16, 2.98 Hz), 1.97 – 1.91 (dd, 2H, *J* = 14.1, 2.7 Hz), 1.89 – 1.82 (m, 2H), 1.79 – 1.69 (m, 2H), 1.67 – 1.60 (m, 3H), 1.35 – 1.30 (m, 2H), 1.26 – 1.23 (m, 1H), 1.21 (s, 3H), 1.06 (s, 3H), 1.00 (s, 3H), 0.98 (s, 3H). **^{13}C NMR (100 MHz, $CDCl_3$) δ** 208.8, 177.9, 144.7, 115.3, 53.1, 52.1, 50.2, 50.0, 49.3, 49.0, 47.3, 36.9, 36.0, 34.9, 26.5, 24.8, 23.3, 17.9, 16.8, 16.0, 13.7.

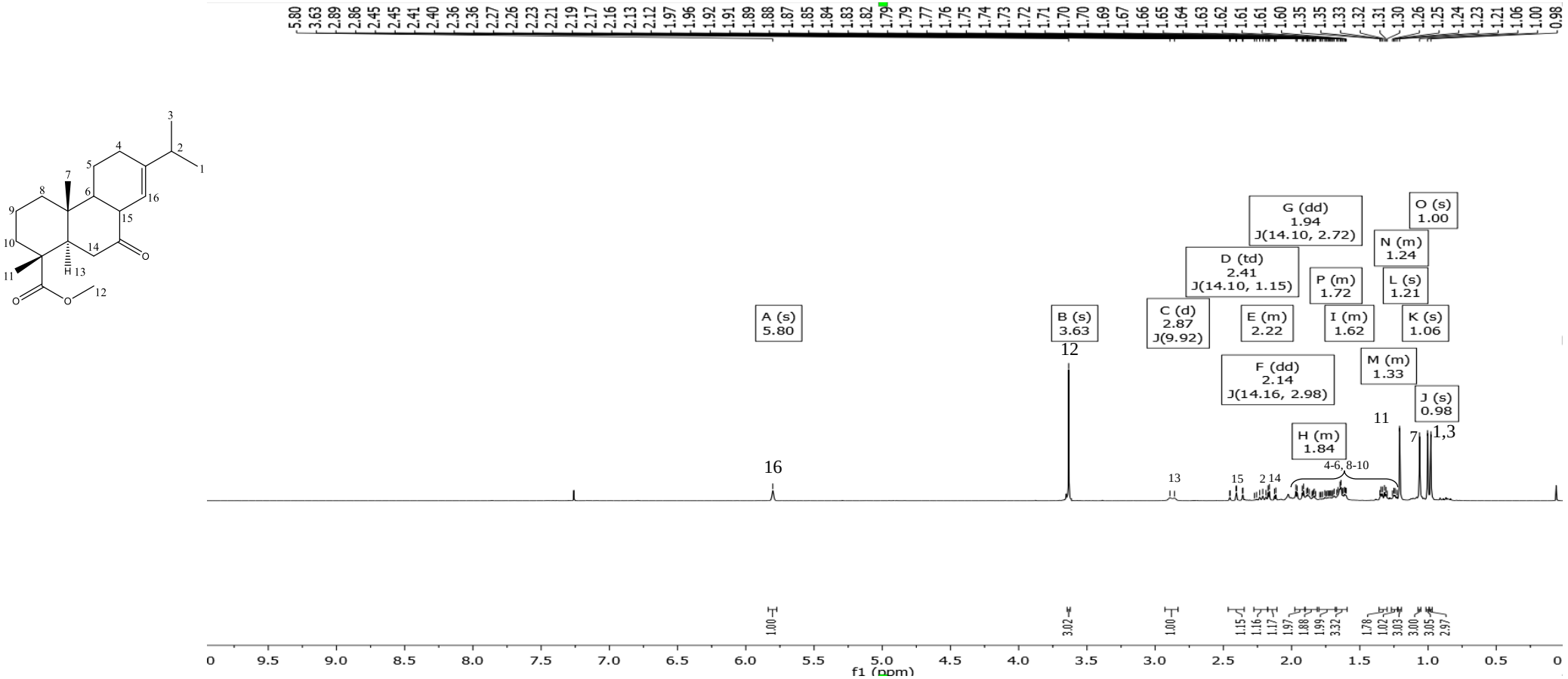


Figure 27. ^{1}H NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylate.

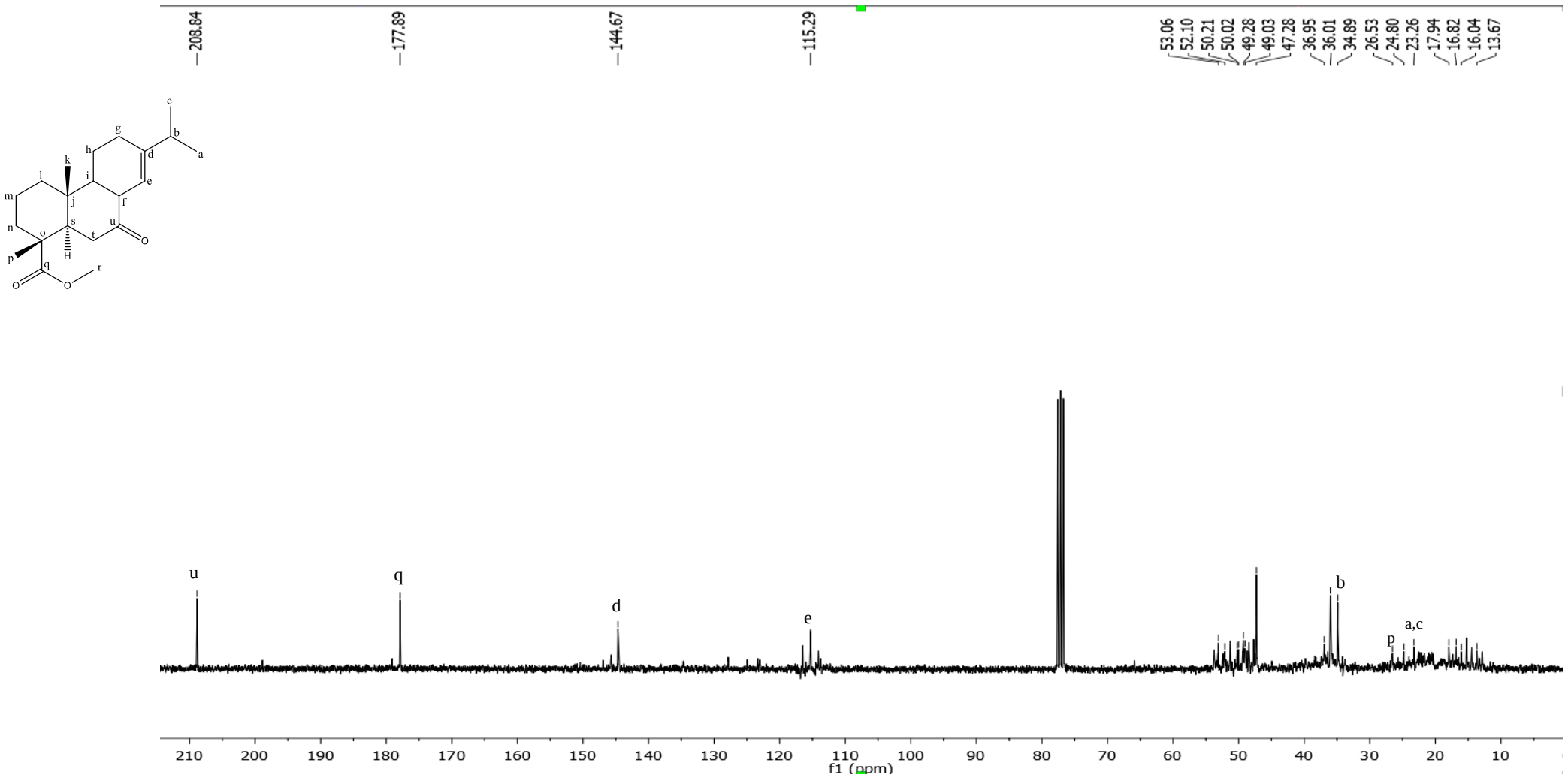


Figure 28. ^{13}C NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylate.

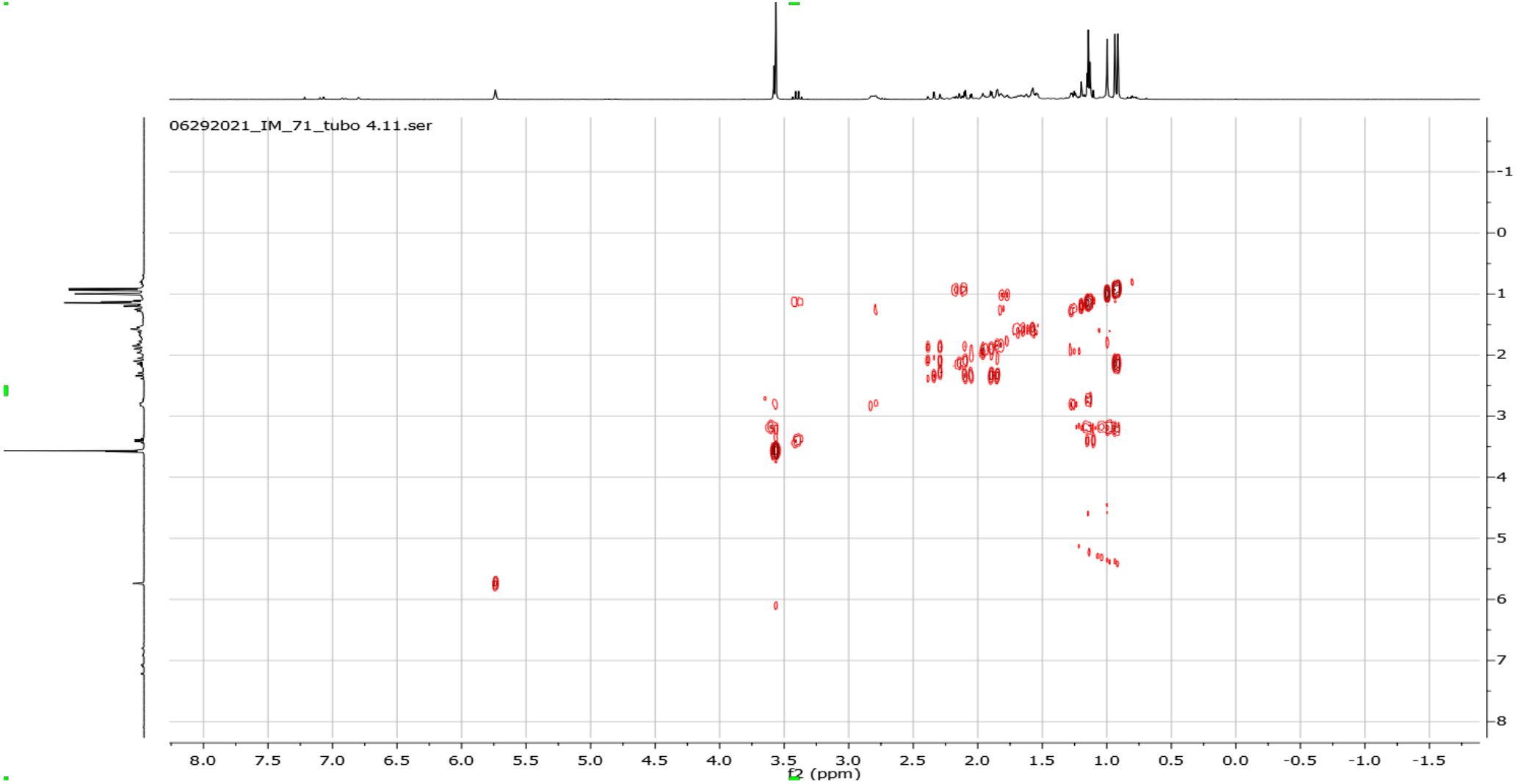


Figure 29. COSY NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylate.

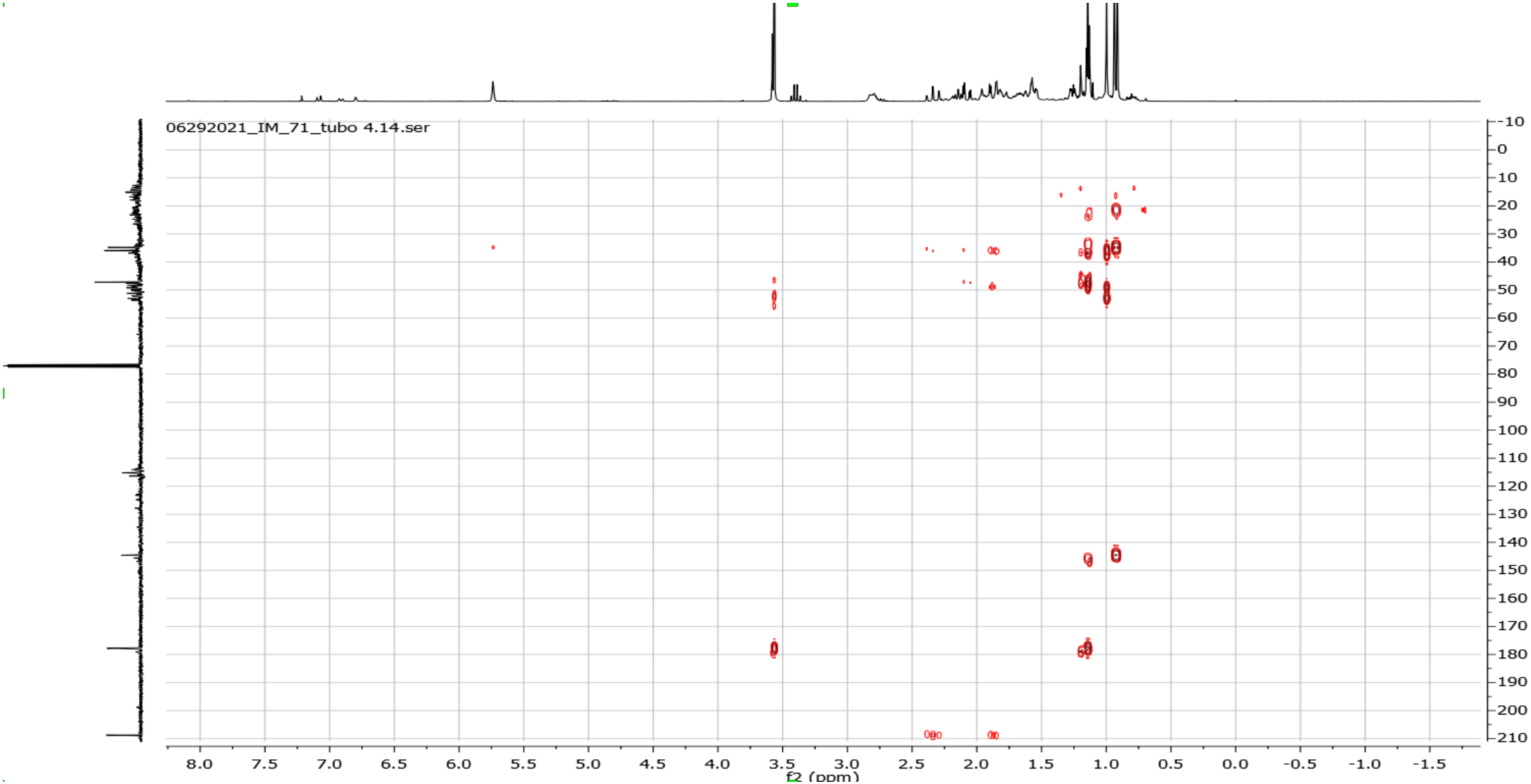


Figure 30. HMQC NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylate.

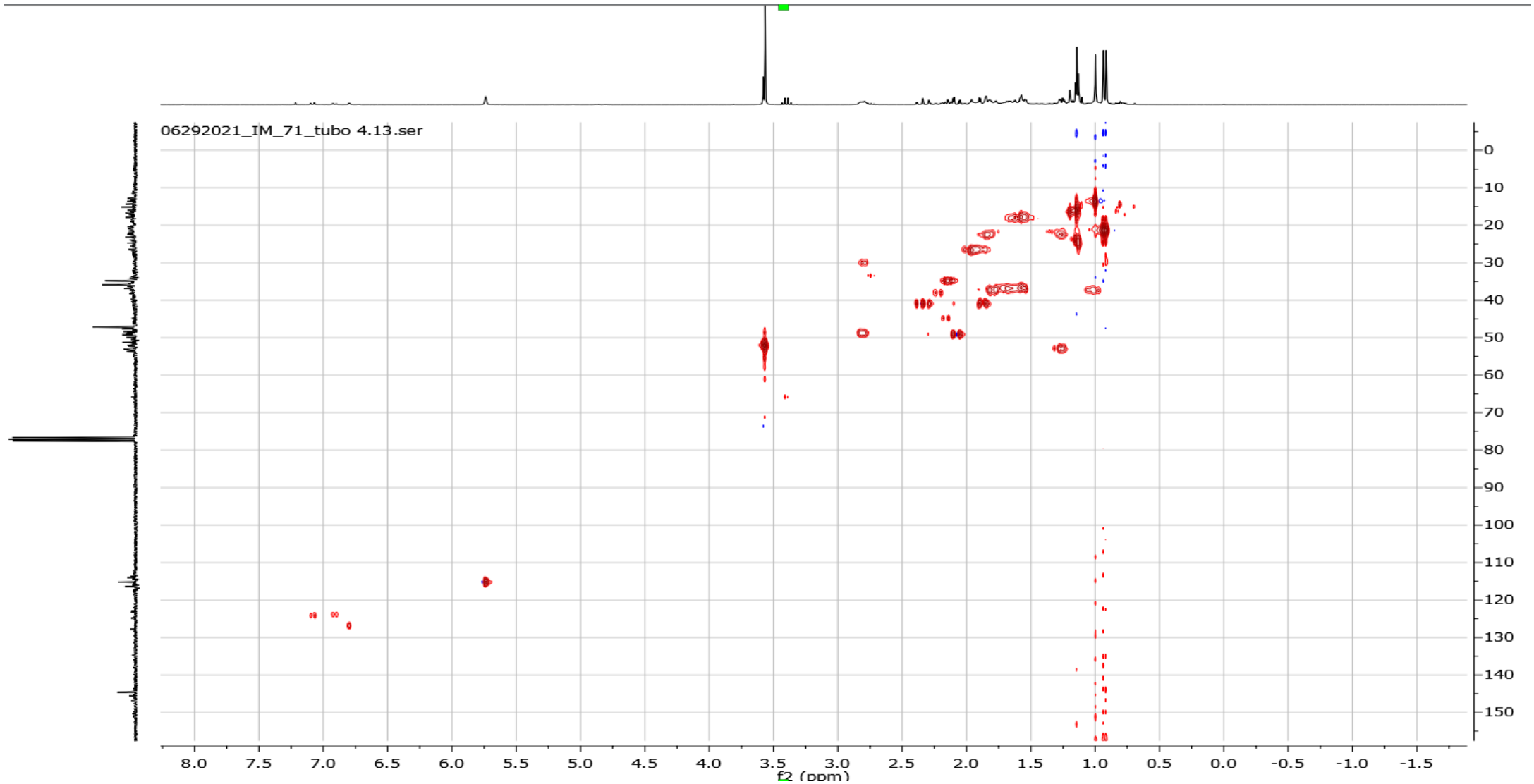


Figure 31. HSQC NMR spectrum of Methyl-7-isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylate.

7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylic acid. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 5.29 (s, 1H), 2.92 – 2.88 (m, 2H), 2.45 – 2-40 (dd, 2H, *J* = 14.80, 1.15 Hz), 2.24 – 2.19 (m, 2H), 2.15 – 2.10 (dd, 2H, *J* = 10.88, 3.92 Hz), 1.73 – 1.66 (dt, 6H, *J* = 15.02, 2.98 Hz), 1.36 – 1.32 (m, 3H), 1.21 (s, 3H), 1.07 (s, 3H), 1.01 (s, 3H), 0.98 (s, 3H). **^{13}C NMR (300 MHz, $CDCl_3$) δ** 209.8, 183.2, 144.8, 127.0, 115.2, 49.1, 48.9, 47.1, 44.7, 40.9, 36.0, 34.9, 33.6, 26.7, 25.3, 22.7, 21.8, 21.3, 17.9, 13.8.

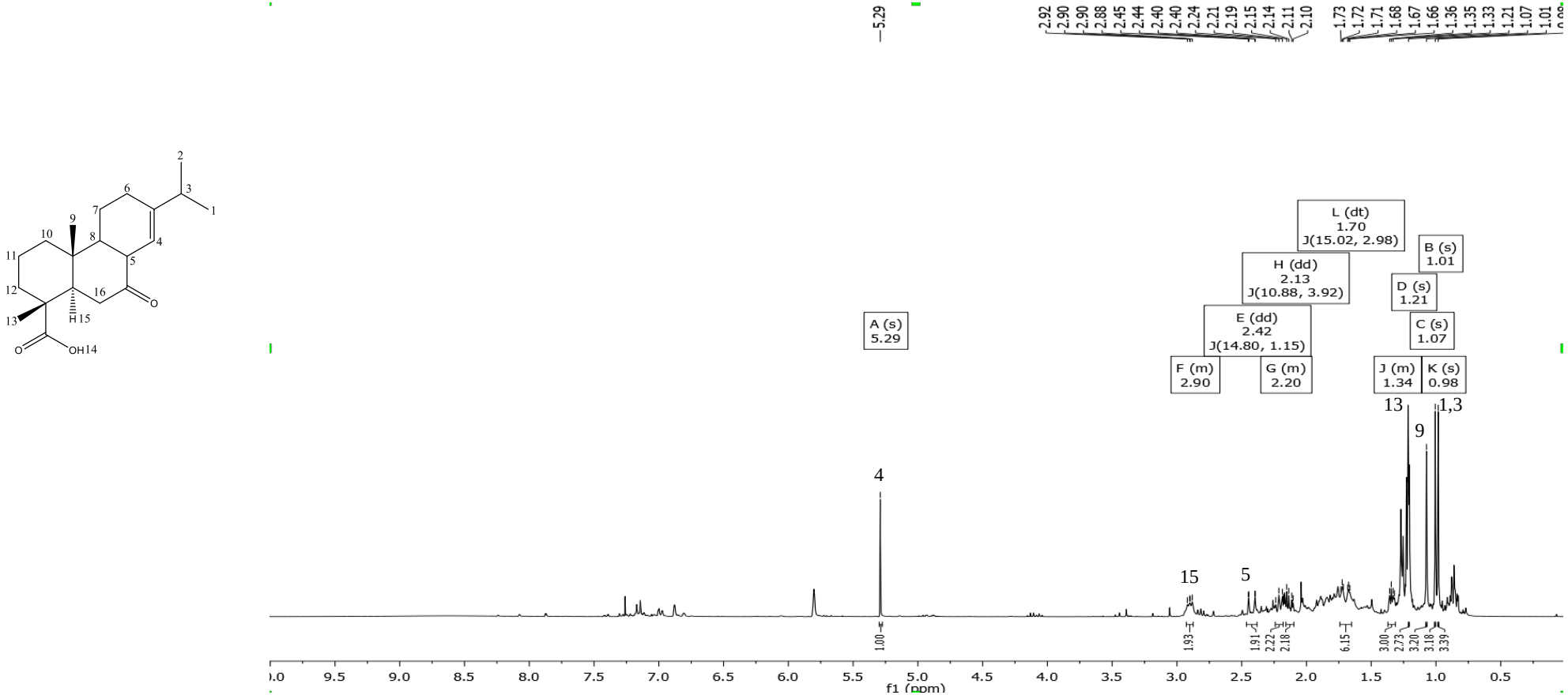


Figure 32. ^{1}H NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylic acid.

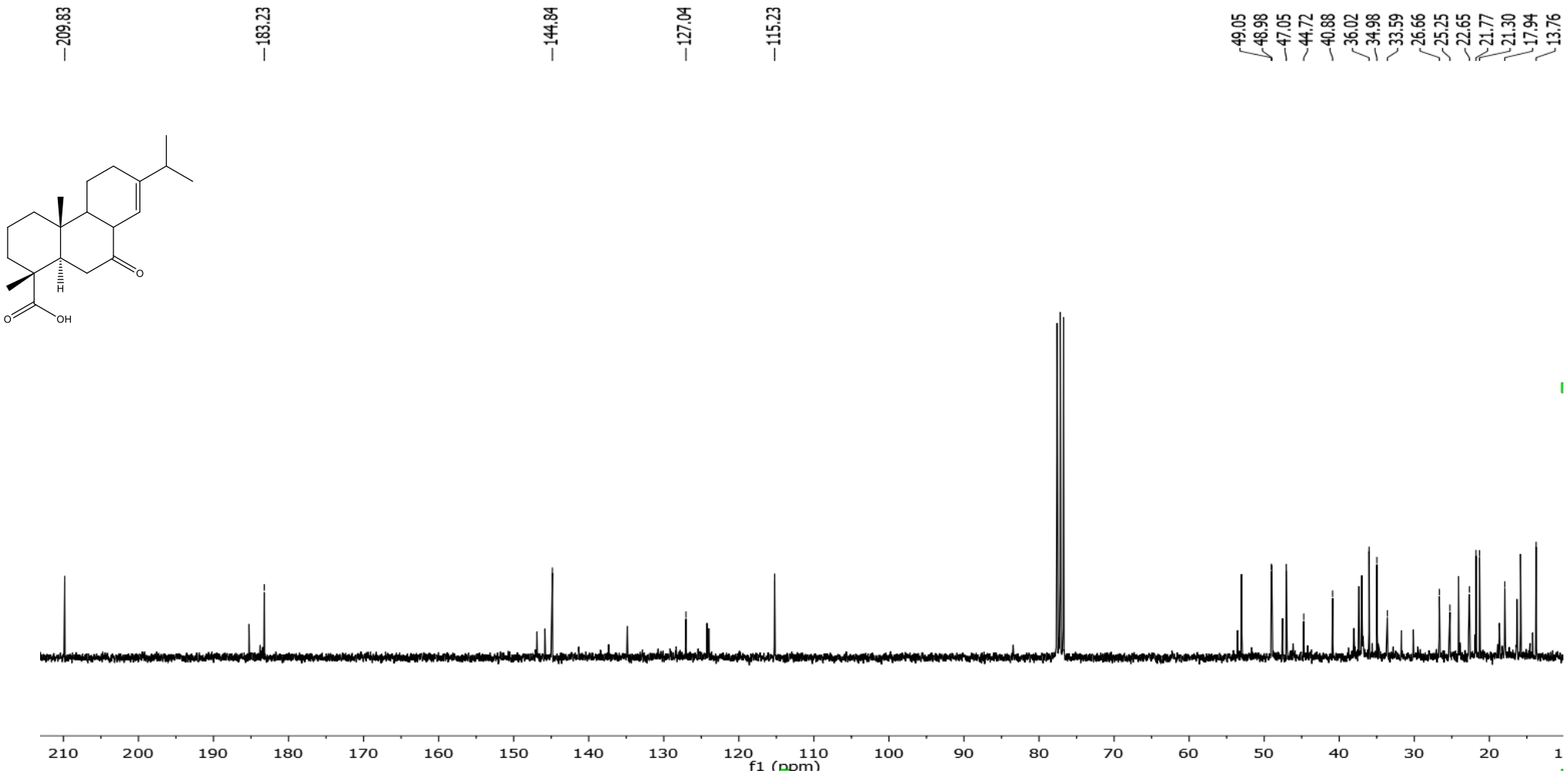


Figure 33. ^{13}C NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylic acid.

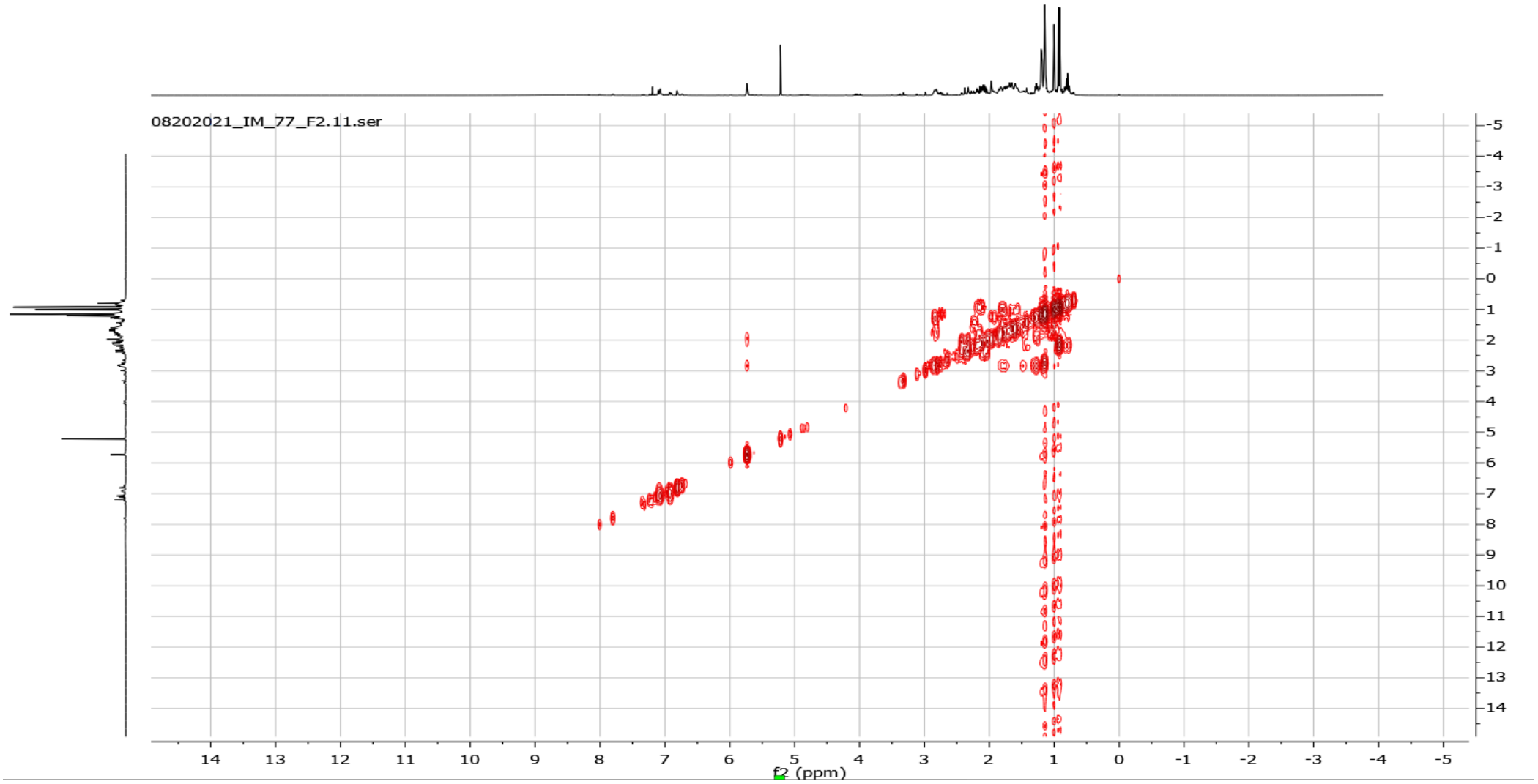


Figure 34. COSY NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylic acid.

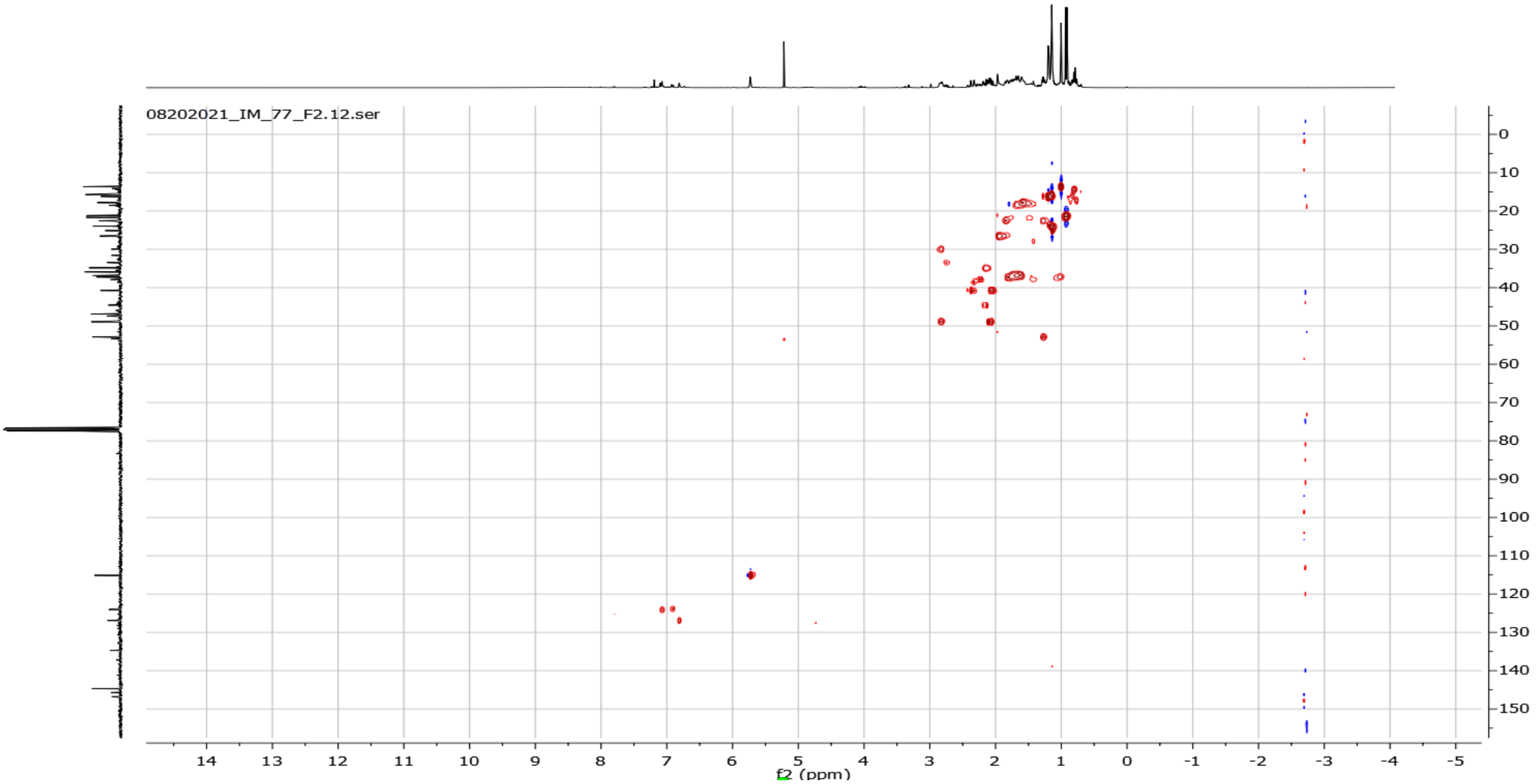


Figure 35. HSQC NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylic acid.

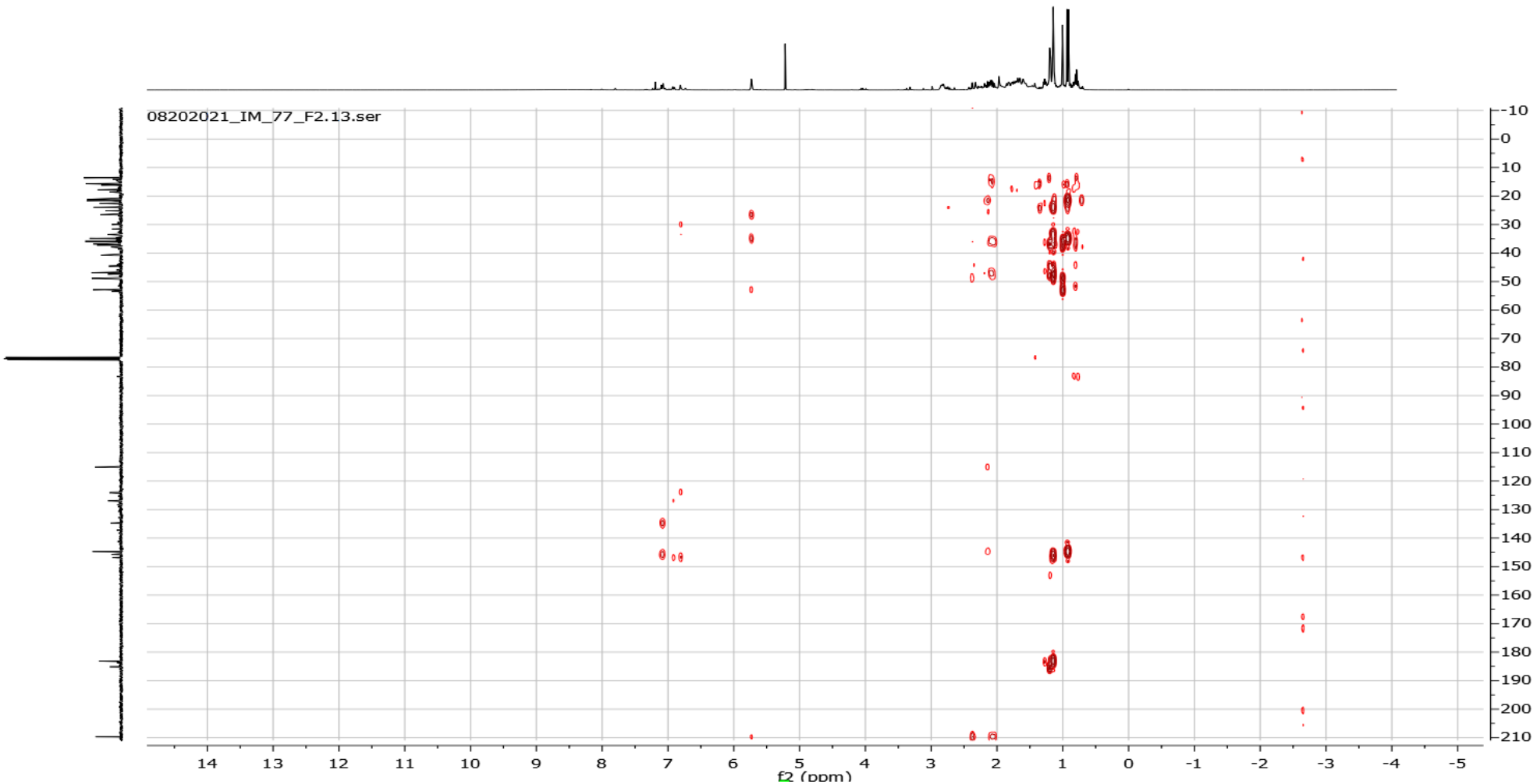


Figure 36. HMQC NMR spectrum of 7-Isopropyl-1,4a-dimethyl-9-oxo-1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydrophenanthrene-1-carboxylic acid.

Methyl-9-hydroxy-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate. **^{1}H NMR (300 MHz, $CDCl_3$) δ** 7.38, 7.37 (d, 1H, *J* = 1.96 Hz), 7.17, 7.14 (d, 1H, *J* = 8.28 Hz), 7.11 – 7.08 (dd, 1H, *J* = 8.23, 2.01 Hz), 4.89 – 4.83 (t, 1H, *J* = 8.68 Hz), 3.67 (s, 3H), 2.96 – 2.81 (sept, 1H, *J* = 6.94 Hz), 2.30, 2.29 (d, 1H, *J* = 4.24), 2.26, 2.25 (d, 1H, *J* = 3.57), 1.84 – 1.79 (dd, 2H, *J* = 8.26, 4.41 Hz), 1.77, 1.73 (d, 2H, *J* = 11.98 Hz), 1.68 – 1.64 (m, 2H), 1.48, 1.44 (d, 1H, *J* = 13.03 Hz), 1.29 (s, 3H), 1.28 (s, 3H), 1.25 (s, 3H), 1.23 (s, 3H). **^{13}C NMR (300 MHz, $CDCl_3$) δ** 178.8, 146.7, 146.5, 137.6, 125.2, 124.9, 123.3, 71.5, 69.9, 52.9, 51.1, 47.3, 37.6, 26.8, 26.31, 26.2, 24.6, 23.0, 21.7, 16.1, 15.7.

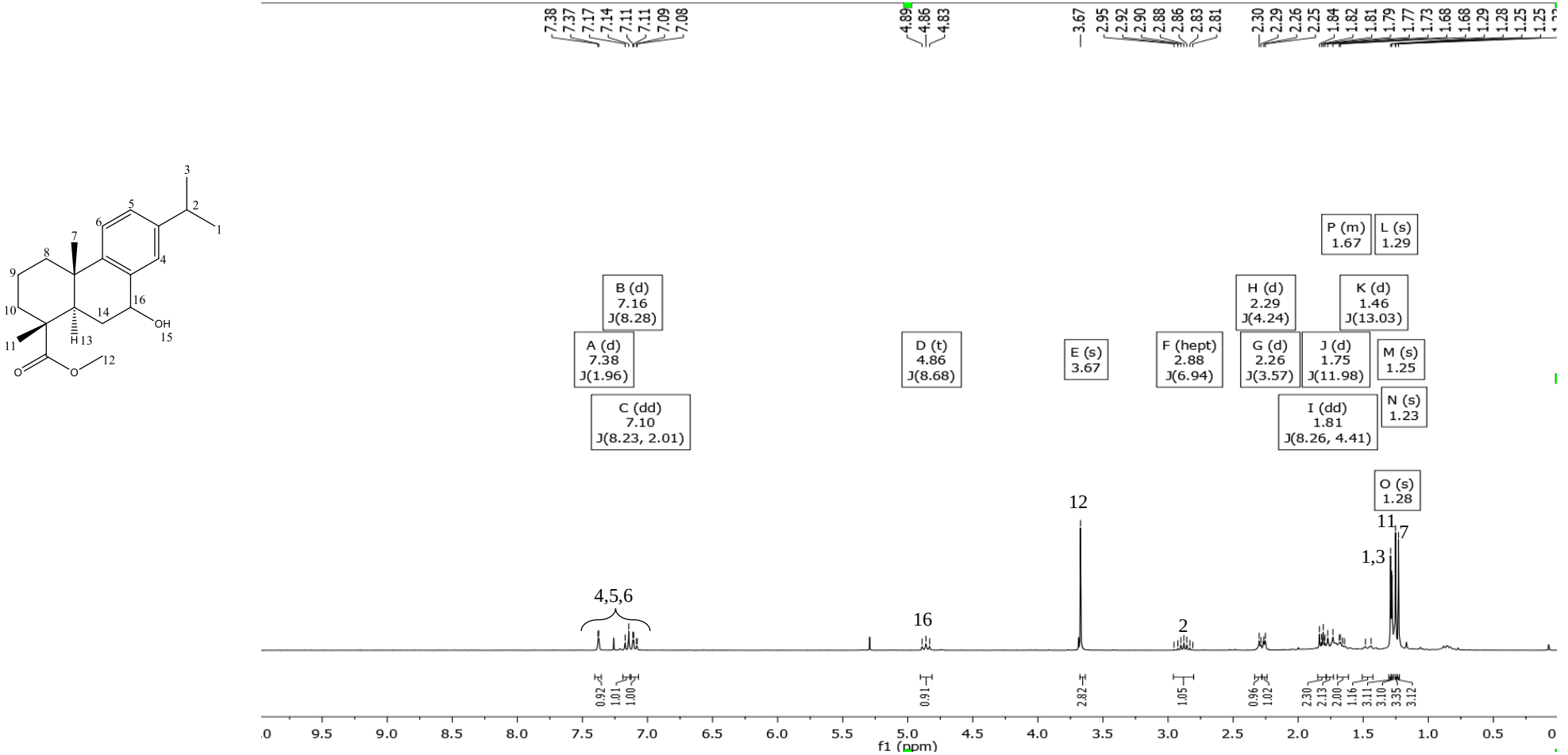


Figure 37. ^{1}H NMR spectrum of Methyl-9-hydroxy-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

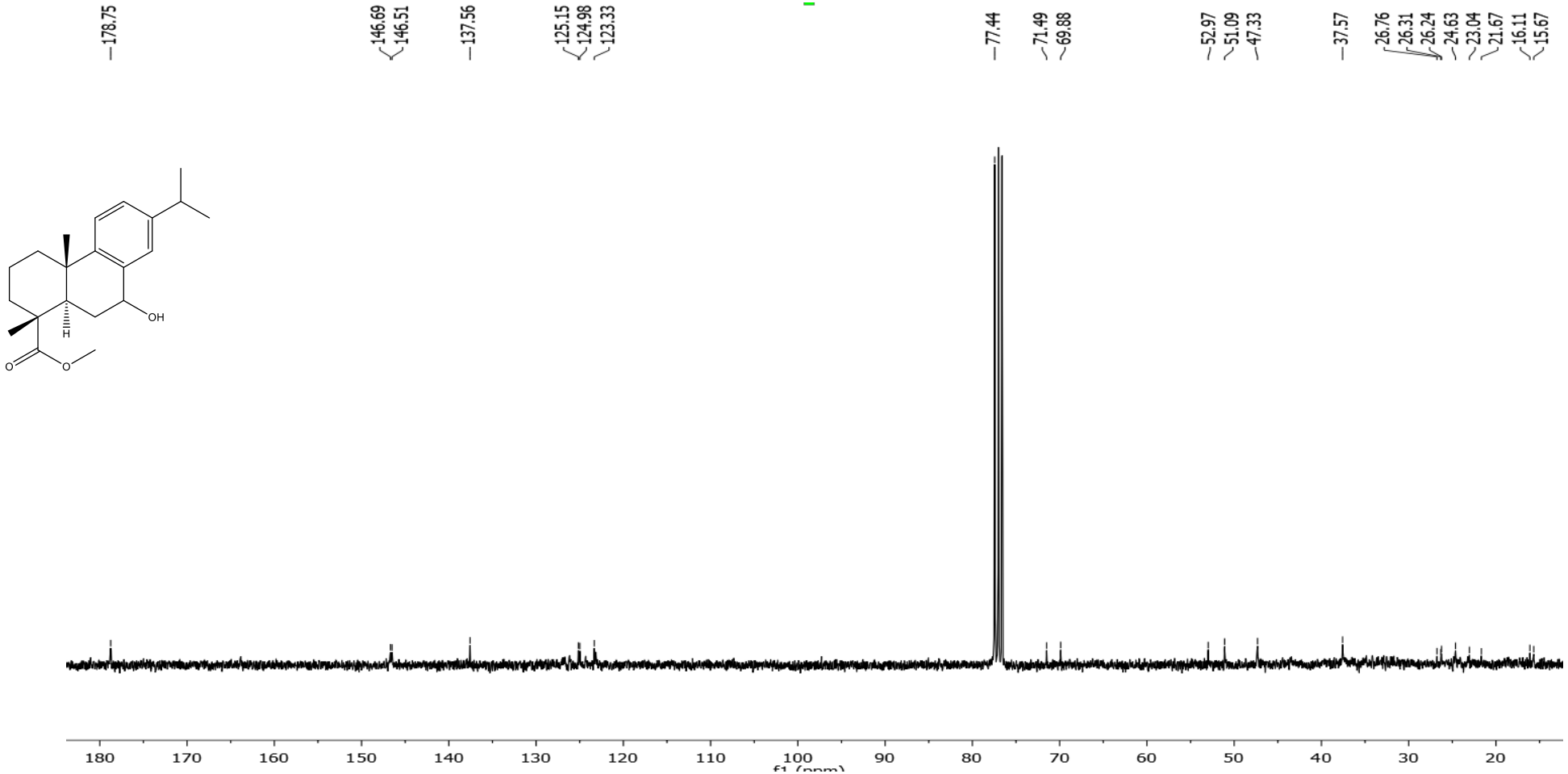


Figure 38. ^{13}C NMR spectrum of Methyl-9-hydroxy-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-1-carboxylate.

7 Conclusions

The main objective of this work was to add biosynthetic value to colophony and its abietanes (mainly AA and DHA) by developing protocols for electrochemical oxidations.

We started the experimental work by preparing the corresponding methyl esters of AA and DHA, as they were potentially more stable and easier to handle starting materials than the carboxylic acids. Next, we performed cyclic voltammetry studies on these compounds, which showed two irreversible oxidation potentials for each molecule. Furthermore, comparing AA or DHA with their derivatives (MAA and MDHA, respectively), both show similar oxidation potentials, and that comparing AA/MAA with DHA/MDHA, the latter has higher oxidation potentials. Then we used electrochemistry to evaluate if AA could be converted into DHA, which we concluded it was not feasible.

Finally, we developed protocols for the electrochemical benzylic oxidation of DHA and MDHA, and the oxidation of AA and MAA. Regarding these oxidations, we were able to achieve the direct benzylic oxidation of both DHA and MDHA with good yields, the indirect oxidation of MDHA (using Cl_4NHPI as a mediator) and were also able to achieve the oxidation of AA and MAA with moderated yields, performed the structure elucidation of the reaction products.

Regarding these reactions, when the reaction conditions were changed, it affected the outcome of the reactions, such as the concentration and type of supporting electrolyte affected the yield of the reaction, being that with higher concentrations and lithium perchlorate as supporting electrolyte we observed higher. The use of constant current or constant potential to control the reaction affected the yield and the specificity of the reaction, for instance, when we used constant currents to oxidize MDHA, the reaction was selective and had good yields. However, when constant current was used for the oxidation of DHA, the reaction was not selective, while with constant potentials it was. Also, by controlling the potential of the reaction, with a mixture of MAA and MDHA, we were able to achieve selective oxidation of only MAA.

We proposed that for the oxidation to take place, water is an important source of oxygen since when we did the reaction without adding water, the oxidation of the starting material was not observed.

Another goal of this project was to perform electrochemical oxidations of non-activated C-H bonds of the abietanes by using redox mediators. Towards this end, we synthesized the mediator quinuclidine by Wolff-Kishner reduction of 3-quinuclidone, but we were not able to isolate it from the reaction medium during the project period, thusly, these reactions were not carried out.

To elucidate more about the mechanism behind the oxidation of abietanes, the reduction of the product of the oxidation of MDHA, we were able to reduce its ketone to the secondary alcohol, remaining to be tested if we can oxidize the alcohol back to the ketone under the same electrochemical conditions.

To start studying the scale up of these oxidations, we started to develop protocols for the flow electrochemical oxidation, in specific the benzylic oxidation of DHA. Regarding these reactions, we could conclude that the residence time was the crucial factor for the oxidation to take place, since with lower flow rates we observed the conversion of the starting material in the product, while the same was not observed with higher flow rates.

For follow-up work to continue to add biosynthetic value to colophony and its abietanes, as future research, we will continue to study the use of electrochemistry in different reactions to synthesize more derivatives of abietanes such as to carry out the etherification of allylic and benzylic positions, carry out the esterification of carboxylic acid group, carry out the oxidation of non-activated positions, either by using different mediators or by using quinuclidine itself. We also aim to use colophony itself in the electrochemical reactions. Will also study the ability to achieve these oxidations with other supporting electrolytes. And regarding flow electrochemistry, more experiments are needed to achieve the full conversion and to study if we can achieve this reaction without the presence of a supporting electrolyte.

8 References

1. Lopes CMO. Caracterização de resinas naturais e seus derivados por análise multivariada. 2008.
2. Neto Í. Abietane Cationic Amphiphiles (Aca)-Loaded Polymeric Beads To Tackle Resistant Bacteria. 2017.
3. Karlberg A -T, Boman A, Hacksell U, Jacobsson S, Nilsson JLG. Contact allergy to dehydroabietic acid derivatives isolated from Portuguese colophony. Vol. 19, Contact Dermatitis. 1988. p. 166–74.
4. Downs AMR, Sansom JE. Colophony allergy: A review. Contact Dermatitis. 1999;41(6):305–10.
5. González MA, Correa-Royero J, Agudelo L, Mesa A, Betancur-Galvis L. Synthesis and biological evaluation of abietic acid derivatives. Eur J Med Chem. 2009;44(6):2468–72.
6. Castells AA. The role of terpenes in the defensive responses of conifers against herbivores and pathogens. 2015.
7. McKeon L. Characterisation and determination of rosin compositions using analytical approaches. Doctor Thesis, Dublin City University. 2014.
8. Farm G. Contact Allergy To Colophony - Clinical and Experimental Studies with Emphasis on Clinical Relevance. 1997.
9. Karlberg A -T, Bergstedt E, Boman A, Bohlinder K, Lidén C, Lars J, et al. Is abietic acid the allergenic component of colophony? Vol. 13, Contact Dermatitis. 1985. p. 209–15.
10. Keeling CI, Bohlmann J. Diterpene resin acids in conifers. Phytochemistry. 2006;67(22):2415–23.
11. Salts R, Esters R, Products R. REACH registrations of Rosin , Rosin Salts and Rosin Esters H4R Position Statement on One Substance Registration. 2019;(February).
12. Fieser LF, Campbell WP. Substitution Reactions of Dehydroabietic Acid. J Am Chem Soc. 1938;60(11):2631–6.
13. Haslinger E, Hofner D. Synthetic Transformation of Abietic Acid. Monatshefte für Chemie / Chem Mon. 1998;129(3):297–308.
14. Prinz S, Müllner U, Heilmann J, Winkelmann K, Sticher O, Haslinger E, et al. Oxidation products of abietic acid and its methyl ester. J Nat Prod. 2002;65(11):1530–4.

15. Presser A, Haslinger E, Weis R, Hüfner A. Synthetic transformations of abietic acid IV [1]. B- and C-ring oxidation. Monatshefte fur Chemie. 1998;129(8–9):921–30.

16. Haslinger E, Hüfner A. New chiral synthons from abietic acid: Oxidation of the C-ring and degradation of the carbon skeleton. Monatshefte für Chemie Chem Mon. 1995;126(10):1109–23.

17. Eksi G, Kurbanoglu S, Erdem SA. Analysis of diterpenes and diterpenoids. Recent Advances in Natural Products Analysis. Elsevier Inc.; 2020. 313–345 p.

18. Gigante B, Silva AM, Marcelo-Curto MJ, Savluschinske Feio S, Roseiro J, Reis L V. Structural effects on the bioactivity of dehydroabietic acid derivatives. Planta Med. 2002;68(8):680–4.

19. Ioannidis K. ME& MP. Resonance-Based Screening for the Identification and Quantification of Heartwood Diterpenic Acids in Four. Molecules. 2019;3603(24):1–14.

20. Pujiwidodo D. Synthesis of Abietic Acid and Dehydroabietic Acid Derivatives to Target Bacterial Biofilms. Vol. III. 2016.

21. Perveen S. Introductory Chapter: Terpenes and Terpenoids. Terpenes and Terpenoids. 2018. 1–12 p.

22. Feliu DA De. Análise de terpenóides de espécies de Croton sect. Lamprocrotin (Mull. Arg.) Pax (Euphorbiaceae). 2011.

23. Fonseca T, Gigante B, Marques MM, Gilchrist TL, De Clercq E. Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietic acid. Bioorganic Med Chem. 2004;12(1):103–12.

24. San Feliciano A, Gordaliza M, Salinero MA, Miguel Del Corral JM. Abietane acids: Sources, biological activities, and therapeutic uses. Planta Med. 1993;59(6):485–90.

25. Krogerus S, Maria S, Krogerus L. Benzylic C-H oxidations of dehydroabietic acid derivatives at positions 7 and 15. 2016.

26. Park JY, Lee YK, Lee DS, Yoo JE, Shin MS, Yamabe N, et al. Abietic acid isolated from pine resin (Resina Pini) enhances angiogenesis in HUVECs and accelerates cutaneous wound healing in mice [Internet]. Vol. 203, Journal of Ethnopharmacology. Elsevier Ireland Ltd; 2017. 279–287 p. Available from: http://dx.doi.org/10.1016/j.jep.2017.03.055

27. Schäfer HJ. Contributions of organic electrosynthesis to green chemistry. Comptes Rendus Chim. 2011;14(7–8):745–65.

28. Tanbouza N, Ollevier T, Lam K. Bridging Lab and Industry with Flow Electrochemistry.

iScience [Internet]. 2020;23(11):101720. Available from: https://doi.org/10.1016/j.isci.2020.101720

29. Kingston C, Palkowitz MD, Takahira Y, Vantourout JC, Peters BK, Kawamata Y, et al. A Survival Guide for the “electro-curious.” Acc Chem Res. 2020;53(1):72–83.

30. Moeller KD. Synthetic applications of anodic electrochemistry. Tetrahedron. 2000;56(49):9527–54.

31. Folgueiras-Amador AA, Wirth T. Perspectives in Flow Electrochemistry. J Flow Chem. 2017;7(3–4):94–5.

32. Wills AG, Charvet S, Battilocchio C, Scarborough CC, Wheelhouse KMP, Poole DL, et al. High-Throughput Electrochemistry: State of the Art, Challenges, and Perspective. Org Process Res Dev. 2021;

33. Maljuric S, Jud W, Kappe CO, Cantillo D. Translating batch electrochemistry to single-pass continuous flow conditions: an organic chemist’s guide. J Flow Chem. 2020;10(1):181–90.

34. Horn EJ, Rosen BR, Chen Y, Tang J, Chen K, Eastgate MD, et al. Scalable and sustainable electrochemical allylic C-H oxidation. Nature. 2016;533(7601):77–81.

35. Wang F, Stahl SS. Electrochemical Oxidation of Organic Molecules at Lower Overpotential: Accessing Broader Functional Group Compatibility with Electron-Proton Transfer Mediators. Acc Chem Res. 2020;53(3):561–74.

36. Noël T, Cao Y, Laudadio G. The Fundamentals behind the Use of Flow Reactors in Electrochemistry. Acc Chem Res. 2019;52(10):2858–69.

37. Krohn K, Budianto E, Flörke U, Hausen BM. Untersuchung der allergenen Prinzipien aus Kolophonium: Autoxidation, Synthese und Sensibilisierung. Liebigs Ann der Chemie. 1992;1992(9):911–9.

38. Alvarez-Manzaneda E, Chahboun R, Bentaleb F, Alvarez E, Escobar MA, Sad-Diki S, et al. Regioselective routes towards 14-hydroxyabietane diterpenes. A formal synthesis of immunosuppressant (-)-triptolide from (+)-abietic acid. Tetrahedron. 2007;63(45):11204–12.

39. Cambie RC, Mitchell LH, Rutledge PS. of Chemistry of Benzannulated Lactones. Csiro Publ. 1998;51.

40. Alvarez-Manzaneda E, Chahboun R, Alvarez E, Alvarez-Manzaneda R, Muñoz PE, Jimenez F, et al. Lead(IV) acetate mediated cleavage of β-hydroxy ethers: Enantioselective synthesis of α-

acetoxy carbonyl compounds. Tetrahedron. 2011;67(46):8910–7.

41. Rafferty RJ, Hicklin RW, Maloof KA, Hergenrother PJ. Synthesis of complex and diverse compounds through ring distortion of abietic acid. Angew Chemie - Int Ed. 2014;53(1):220–4.

42. Meng L, Su J, Zha Z, Zhang L, Zhang Z, Wang Z. Direct electrosynthesis of ketones from benzylic methylenes by electrooxidative C-H activation. Chem - A Eur J. 2013;19(18):5542–5.

43. Wang H, Liang K, Xiong W, Samanta S, Li W, Lei A. Electrochemical oxidation-induced etherification via C(sp3)-H/O-H cross-coupling. Sci Adv. 2020;6(20):1–7.

44. Marko JA, Durgham A, Bretz SL, Liu W. Electrochemical benzylic oxidation of C-H bonds. Chem Commun [Internet]. 2019;55(7):937–40. Available from: http://dx.doi.org/10.1039/C8CC08768G

45. Monteiro SMCS, Silvestre AJD, Silva AMS, Cavaleiro JAS, Félix V, Drew MGB. Synthesis and structural characterisation of ring B oxidised derivatives of dehydroabietic acid. New J Chem. 2001;25(8):1091–7.

46. Zhou Z, Wang X, Zhou T. Synthesis and Antibacterial Activity of Benzenesulfonylhydrazone Derivatives of Methyl Dehydroabietate. Russ J Gen Chem. 2019;89(4):819–23.

47. Korinek K, McKillop TFW. Electro-organic chemistry. Annu Reports Prog Chem - Sect B. 1973;70:285–309.

48. Masnyk M, Butkiewicz A, Górecki M, Luboradzki R, Paluch P, Potrzebowski MJ, et al. In depth analysis of chiroptical properties of enones derived from abietic acid. J Org Chem. 2018;83(7):3547–61.

49. Masnyk M, Kuśmirek D, Trzybiński D, Frelek J. Research into the oxidation of abietic acid–derived enone with atmospheric oxygen. Chirality. 2020;32(4):437–45.

50. Amato ME, Ballistreri FP, Pappalardo A, Tomaselli GA, Toscano RM, Sfrazzetto GT. Selective oxidation reactions of natural compounds with hydrogen peroxide mediated by methyltrioxorhenium. Molecules. 2013;18(11):13754–63.

51. Zhang J, Wang Z, Wang Y, Wan C, Zheng X, Wang Z. A metal-free catalytic system for the oxidation of benzylic methylenes and primary amines under solvent-free conditions. Green Chem. 2009;11(12):1973–8.

52. Jin K, Maalouf JH, Lazouski N, Corbin N, Yang D, Manthiram K. Epoxidation of Cyclooctene Using Water as the Oxygen Atom Source at Manganese Oxide Electrocatalysts. J Am Chem Soc.

2019;141(15):6413–8.

53. Buglioni L, Beslać M, Noël T. Dehydrogenative Azolation of Arenes in a Microflow Electrochemical Reactor. J Org Chem. 2021;

54. Jud W, Kappe CO, Cantillo D. One-pot multistep electrochemical strategy for the modular synthesis of epoxides, glycols, and aldehydes from alkenes. Electrochem Sci Adv. 2021;1(3):1–9.

55. Zhang SX, Shen XL, Li ZQ, Zou LW, Wang FQ, Zhang H Bin, et al. Enantioselective total synthesis of (-)-limaspermidine and formal synthesis of (-)-1-acetylaspidoalbidine. J Org Chem. 2013;78(22):11444–9.

56. Kayser MM, Eliev S, Eisenstein O. Reduction of ketones by sodium borohydride in the absence of protic solvents. Inter versus intramolecular mechanism. Tetrahedron Lett. 1983;24(10):1015–8.

57. Ichikawa HCB and K. The Effect of Ring Size on the Rate of Reaction of the Cyclanones with Sodium Borohydride. Tetrahedron. 1957;1(3):221–30.

58. Kärkäs MD. Electrochemical strategies for C-H functionalization and C-N bond formation. Chem Soc Rev. 2018;47(15):5786–865.

59. Steven M. Singer, Marc Y. Fink VVA. Synthetic Organic Electrochemical Methods Since 2000: On the Verge of a Renaissance. Vol. 176, Physiology & behavior. 2019. 139–148 p.

60. Kawamata Y, Yan M, Liu Z, Bao DH, Chen J, Starr JT, et al. Scalable, Electrochemical Oxidation of Unactivated C-H Bonds. J Am Chem Soc. 2017;139(22):7448–51.

61. Forsyth DA, Prapansiri V. Conformational Analysis via NMR Isotope Shifts. Side-Chain Equilibria in N-Alkylpiperidines. J Am Chem Soc. 1989;111(13):4548–52.