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Labdane Aldehyde Diterpenoids from *Curcuma mangga* Rhizome

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ABSTRACT

Curcuma mangga val. (Zingiberaceae) is one of the plants that used as traditional medicine by Indonesian. Several studies have been reported on the content of compounds of *C. mangga*, but it is not yet known which compounds have medicinal properties. In this study, two labdane diterpenes were isolated from the extract of rhizome of *C. mangga*. Determination of the structure conducted by NMR (^{1}H, ^{13}C, HSQC and HMBC) that obtained two compounds, calcaratarin A (1) and labda-8(17),12-diene-15,16-dial (2). Both compounds have an aldehyde functional group. However, both compounds did not show antibacterial activity on *Escherichia coli*.

Keywords: *Curcuma mangga*; labdane, diterpene, antibacterial, Zingiberaceae

INTRODUCTION

Escherichia coli are bacteria of gram-negative type. In general, these bacteria can be found in the large intestine of humans. These microbes are one of the most common causes of several bacterial infections in humans and animals [1]. These bacteria are the main cause of enteritis, urinary tract infections, septicemia or sepsis and other clinical infections, such as neonatal meningitis [2]. Most strains of *E. coli* live harmlessly in the gut and rarely cause disease in otherwise healthy individuals. However, number of pathogenic strains can cause diarrhea or extraintestinal disease in both healthy and immune compromised individuals [3]. Diarrhea is a public health problem and a major cause of morbidity and mortality in infants and young children, especially in developing countries. Antibiotic drugs have been developed, but the treatment of *E. coli* infection is threatened by the emergence of antimicrobial resistance. The prevalence of multidrug-resistant *E. coli* strains is increasing worldwide mainly due to the spread of mobile genetic elements, such as plasmids and the overuse and lack of targeted antibiotics [2]. The high cases of infection and increasing cases of resistance indicate the need developing new antibiotics, especially from natural products.

Indonesia is a country that rich in diversity of flora and fauna, due to its strategic geographical location and consists of various islands and rich tropical forests. The diversity of flora in Indonesia can be utilized, one of which is Zingiberaceae whose potential needs to be developed for the source of natural medicine. Zingiberaceae consists of many species, including 50 genera and 1600 species which are widely distributed in tropical and subtropical areas [4]. One of the genera of Zingiberaceae, *Curcuma* species consists of 70 species that spread across tropical Asia and Africa [5]. One of these plants is *C. mangga* or '*temu mangga*'.

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The leaves of *C. mangga* have an appearance like *C. longa*, which is a light green colour, there is no brownish strip to the midrib of the leaves like in *C. xanthorrhiza* and *C. heyneana*. On rhizome, the differences are very clear, *C. mangga* is pale yellow, whereas *C. xanthorrhiza* and *C. longa* are yellow orange. The rizhome of *C. mangga* has a distinctive aroma like mango or *Mangifera odorata* and the taste is not bitter [6].

Searching on the antimicrobial activity in *C. mangga* has been carried out on both the rhizome extract [7, 8] and the essential oil [9, 10]. The compound that has the potential as an antibacterial in the *C. mangga* is not yet known. In some reports, rhizome of *C. mangga* is rich of terpenoids, phenyl propanoids and curcuminoids. Some compounds have been reported such as labda-8,12-diene-15,16-dial, calcaratarin A, zerumin B, demethoxycurcumin, bisdemethoxycurcumin, 1,7-cis-(4- hydroxyphenyl)-1,4,6-heptatriene-3-one, curcumin, and p-hydroxycinnamic acid [11]. *C. mangga* extract from Yogyakarta was reported composed of seven compounds, namely (E)-labda-8(17),12-dien-15,16-dial, (E)-15,16-bisnor-labda-8(17),11-dien-13-on, zerumin A, β-sitosterol, curcumin, demethoxycurcumin and bis-demethoxycurcumin [12]. From Thailand was obtained as 4-[(1*R*,4a*R*,8a*R*)-decahydro-5,5,8a-trimethyl-2-methylene-1-naphthalenyl]-(3*E*)-rel-3-buten-2-one, demethoxycurcumin, and bisdemethoxycurcumin [13]. In this paper, is disclosed our recent isolation of two labdane type of diterpenoide contains the aldehyde functionality. The structure characterization is based on their nuclear magnetic resonance spectra. In addition, a low antibacterial activity is indicated to inhibit the growth of *E. coli*.

EXPERIMENT

Chemicals and instrumentation

Rhizome of *C. mangga* was collected from Surakarta. Specimen was identified by staff Department of Biology FMIPA UNS. Solvents used for maceration and chromatography were *n*-hexane, EtOAc and MeOH, $CHCl_3$ and Acetone. Vacuum liquid chromatography (KCV) using silica gel Merck Si-gel 60 G, flash chromatography used silica gel Merck Si-gel 60 (0.04-0.063 mm). Thin layer chromatography (TLC) analysis on silica-coated aluminum plate (Merck Kieselgel 60 F_{254} 0.25 mm). A solution of 1.5% $Ce(SO_4)_2$ in 2N H_2SO_4 was used as TLC visualization reagent.

The molecular structure was determined by nuclear magnetic resonance spectroscopy method (^{1}H NMR, ^{13}C NMR, HSQC and HMBC) recorded by Agilent VNMR DDR 400 MHz spectrophotometer using TMS as an internal standard. Deuterated chloroform was applied as solvent.

Procedure for isolation

Rizhomes of *C. mangga*, a total of 5 kg was sliced thinly, then dried and made into powder until 1.5 kg of dry powder. This powder is then extracted using a soxhlet extractor with acetone as a solvent. The extracted solution was then evaporated to obtain a dark brown acetone extract (78.03 g). 20 g of acetone extract then fractionated using vacuum liquid chromatography (VLC), using *n*-hexane:ethyl acetate as eluent by gradual composition. After combining the VLC fractions, 8 fractions were obtained (A-H). Fraction C (1.3 g) was purified using flash chromatography and 46 fractions were obtained. The results of the analysis by thin layer chromatography (TLC) obtained two single spots on fraction $C_{19\text{-}20}$ (35 mg) as isolate-**1** and Fraction $C_{25\text{-}26}$ (73 mg) as isolate-**2**. After being tested for purity, the isolated compound was analysis by NMR spectroscopy including ^{1}H NMR, ^{13}C NMR, HSQC and HMBC.

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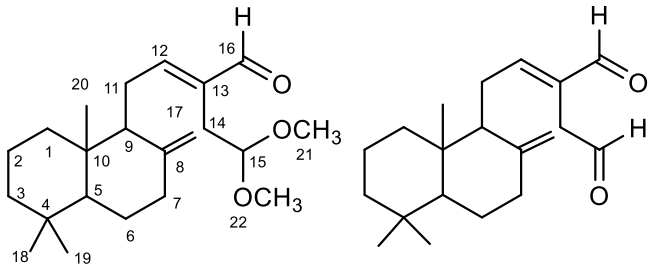
Antibacterial evaluation

The method used in the antibacterial test of pure compounds isolated from *C. manga* is a diffusion agar method with a well technique, whereas the bacteria used is gram-negative bacteria *E. coli*. The incubation was undergone for overnight. This antibacterial test aims to determine the ability of isolated compounds to inhibit bacterial growth and to determine the Minimum Inhibitory concentration (MIC) of these compounds. Antibacterial potency testing was carried out at concentrations (μg/ml) of 1000, 800, 600, 500, 400, 200, and 100.

RESULT AND DISCUSSION

Isolate 1 is yellowish gum. The ^{13}C NMR spectrum of isolate 1 (Table 1) gives the presence of 22 carbons. The carbonyl of conjugated aldehyde group was shown at δ_C 194.9. This aldehyde signal is supported by the ^{1}H NMR peak at δ_H 9.32 ppm. Four peak of alkene carbons at δ_C 159.9, 147.2, 134.1 and 107.8, where δ_C 147.2 and 107.8 indicated terminal alkenes. This is supported by ^{1}H NMR at δ_H 4.41 (*d*, 1.2), 4.82 (*d*, 1.2) and 6.54 (t, 6.3). One alkene at δ_C 159.9 (δ_H 6.54) and 134.1 indicated an alkene that conjugated to an aldehyde. Peak at δ_C 103.9 is not an alkene but an acetal carbon. ^{1}H NMR spectra also showed the presence of three singlet peak of methyl at δ_H 0.87, 0.80, 0.73 and two peaks of methoxy at δ_H 3.34 and 3.35. Based on the HSQC spectrum, there are seven methylene, three methynes and two quaternary carbons.

Isolate 1 have total 22 carbons, while two are methoxy, indicated a diterpenoid derivative. From the HSQC data, based on the types of carbon atoms in each chemical shift of the compound structure, isolate 1 has the molecular formula $C_{22}H_{36}O_3$. The results of the calculation show that isolate 1 has double bond equivalent (DBE) of five, of which, there are four double bonds (1 carbonyl and 2 alkenes) and the other are two rings. Based on the references, compounds have been isolated from the rhizome of *C. mangga* that has two rings are labdane diterpenoids. The presence of three singlet peaks indicated that from six methyls of the labdane skeleton, three methyl carbons were unchanged, one changed to an aldehyde, one to a terminal alkene and one to acetal carbon.



1 2

Figures 1. Structures of calcaratarin A (**1**) and labda-8(17),12-diene-15,16-dial (**2**)

HMBC spectrum (Figure 2) showed a correlation between proton aldehyde at δ_H 9.32 with δ_C 159.9 and 134.1 indicating conjugated aldehyde. The correlation of methylene protons at δ_H 2.56 with δ_C 194.9 and 107.8 indicates that aldehyde and two methoxy groups are located close together so that the terminal alkene is believed to be located on the labdane ring. Compound 1 which has two alkenes, one aldehyde and two methoxy is suggested to be calcaratarin A, a compound were first isolated from the rhizome of *Alpinia calcarata* [14].

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Tabel 1. ^{1}H and ^{13}C NMR of compound **1** and **2.** Data are summarized from the spectra in **Figure 1a-d** (supporting information)

No. C	δ_H (m, *j* Hz) **(CDCl$_3$, 400MHz)**		δ_C **(ppm)**	
	1	**2**	**1**	**2**
1	1.07 (*m*), 1.74 (*m*)	1.04 (*m*); 1.68 (*m*)	39.2	39.2
2	1.50 (*m*); 1.54 (*m*)	1.54 (*m*); 1.57 (*m*)	19.3	19.3
3	1.17 (*m*); 1.38 (*m*)	1.18 (*m*); 1.35 (*m*)	42.1	41.9
4	-	-	33.6	33.5
5	1.13 (*dd*, 12.7, 2.5)	1.13 (*m*)	55.4	55.4
6	1.32 (*m*); 1.74 (*m*)	1.32 (*m*); 1.74 (*m*)	24.1	24.1
7	2.01 (*dt*, 11.9, 3.9)	2.02 (*td*,12.8, 4.8)	37.9	37.8
	2.38 (*m*)	2.40 (ddd, 12.8; 3.9; 3.5)		
8	-	-	147.2	148.0
9	1.9 (*d*, 10.1)	1.89 (*d*, 11.0)	56.6	56.4
10	-	-	39.6	39.6
11	2.44 (*m*, 17.7; 11.3; 6.7)	2.30 (*ddd*, 17.9; 11.0; 6.8)	24.5	24.7
	2.64 (*ddd*, 17.3; 5.9; 3.1)	2.47 (*ddd*, 16.8, 6.3, 3.0)		
12	6.54 (*t*, 6.3)	6.75 (*t*, 6.5)	159.9	159.9
13	-	-	134.1	134.8
14	2.56 (*m*)	3.40 (*m*)	29.1	39.3
15	4.42 (*t*, 5.6)	9.61 (*s*)	103.9	197.3
16	9.32 (*s*)	9.40 (*brs*)	194.9	193.5
17	4.41 (*d*, 1.2)	4.35 (*s*)	107.8	107.8
	4.82 (*d*, 1.2)	4.85 (*s*)		
18	0.87 (*s*)	0.87 (*s*)	33.6	33.5
19	0.80 (*s*)	0.80 (*s*)	21.7	21.7
20	0.73 (*s*)	0.71 (*s*)	14.4	14.4
21	3.34 (*s*)	-	54.3	-
22	3.35 (*s*)	-	54.3	-

The ^{13}C NMR spectrum of isolate-2 showed twenty peaks indicating the presence of 20 carbons (Table 1). Two carbonyl aldehydes indicated by peak at δ_C 197.3 and 193.5 ppm. The presence of the two aldehydes was also supported by the proton signal at δ_H 9.61 and 9.40, which was seen in the correlation with the HSQC spectrum. Four carbon alkenes appear at δ_C 159.9, 148.0 134.8 and 107.8 ppm, of which one is a terminal alkene. Terminal alkenes are supported by peak at δ_H 4.35 and 4.85. Three methyls were indicated by singlet signals at δ_H 0.87, 0.80 and 0.71 ppm and δ_C 33.5, 21.7 and 14.4 implying the same skeleton as compound 1. HSQC spectrum indicated there were two methine of peak δ_C 55.4 and 56.4 ppm correlated with protons δ_H 1.13 and 1.89 ppm. There are seven methylene carbons at the δ_C 41.9, 39.3, 39.2, 37.8, 24.7, 24.1 and 19.3. Two quaternary carbon peaks presence at δ_C 39.6 and 29.7 ppm. The presence of 20 carbons indicates a diterpenoid compound for isolate-2. The number of H atoms read in the ^{1}H-NMR spectra is 30 and O atoms are 2, thus, the molecular formula of

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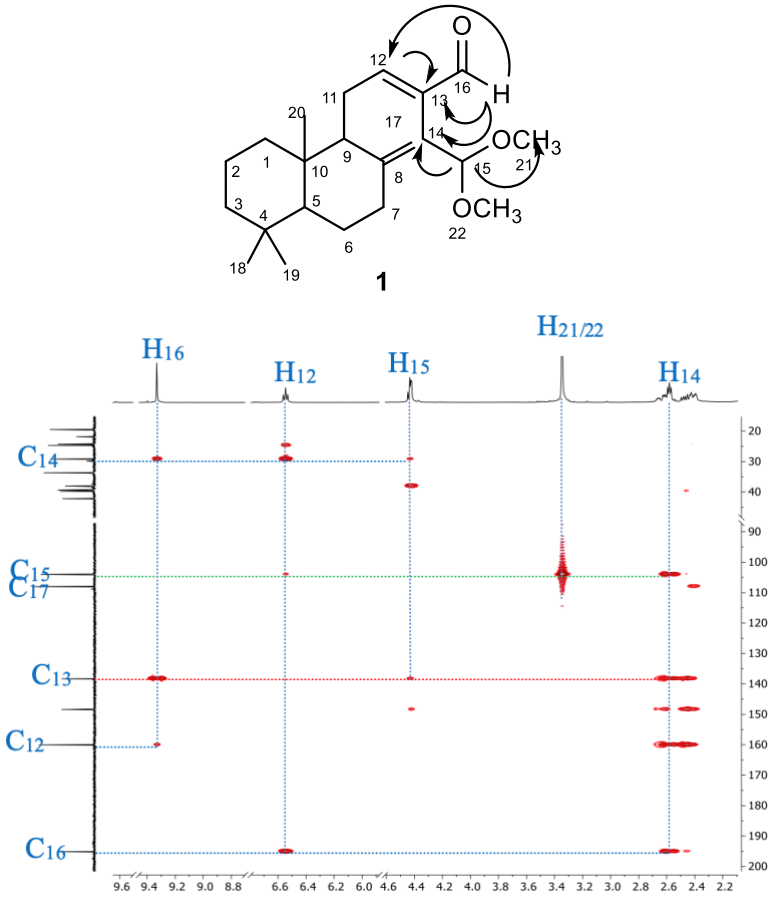
isolate-2 is $C_{20}H_{30}O_2$. Compound-2 has two aldehyde groups, three methyl, eight methylene, three methine and four quaternary carbons. Isolate-2 can be suggested as a compound labda-8(17),12-diene-15,16-dial. Based on data analysis of ^{1}H-NMR, ^{13}C-NMR, and HSQC, as well as comparison reference [15]. This compound was first isolated from the chloroform extract of *Curcuma amada* rhizome originating from India [15].



Figures 2. Selected HMBC correlation for compound **1**

The two isolated compounds, namely calcaratarin A (isolate-1) and labda-8(17),12-diene-15,16-dial (isolate-2) were tested for antibacterial using the agar diffusion method with a well technique where the bacteria used were gram-negative bacteria *E. coli*. Antibacterial testing was carried out at concentrations of 1000, 800, 600, 500, 400, 200, and 100 (µg/ml). The diffusion method was chosen because in this method the presence or absence of bacterial growth could be clearly observed. The diameter of the inhibition of bacterial growth was indicated by the presence of a clear zone around the well, while the cloudy color of the media indicated the growth of bacteria. The criteria for the strength of antibacterial power are as follows: an inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone of 5-10 mm is categorized as moderate, an inhibition zone of 10-20 mm is categorized as strong and an inhibition zone of 20 mm or more is categorized as very strong [16].

The results of the inhibition zone test on compounds 1 and 2 showed that there was no diameter of the inhibition zone in all the concentration variations that had been made (Table 2). The two compounds did not have potential as antibacterial when compared to the antibiotic

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chloramphenicol which could inhibit at a concentration of 30 g/ml. The positive control of chloramphenicol showed that the inhibition zone on compound 1 disc was 12 mm while that on disc 2 was 10 mm.

Table 2. Inhibition zone diameter of antibacterial test results

Inhibition zone	**Concentration (µg/ml)**							
(mm)	1000	800	600	500	400	200	100	30
1	0	0	0	0	0	0	0	-
2	0	0	0	0	0	0	0	-
chloramphenicol	-	-	-	-	-	-	-	10/12

CONCLUSION

Compounds from the rizhome of *C. mangga* has been isolated two labdane diterpenoids, i.e. calcaratarin A (1) and labda-8(17),12-diene-15,16-dial (2). The compounds have aldehyde groups at the structures. Antibacterial testing using *E. coli* to the compounds conclude that two compounds did not have potent to antibacterial activity.

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Supporting information

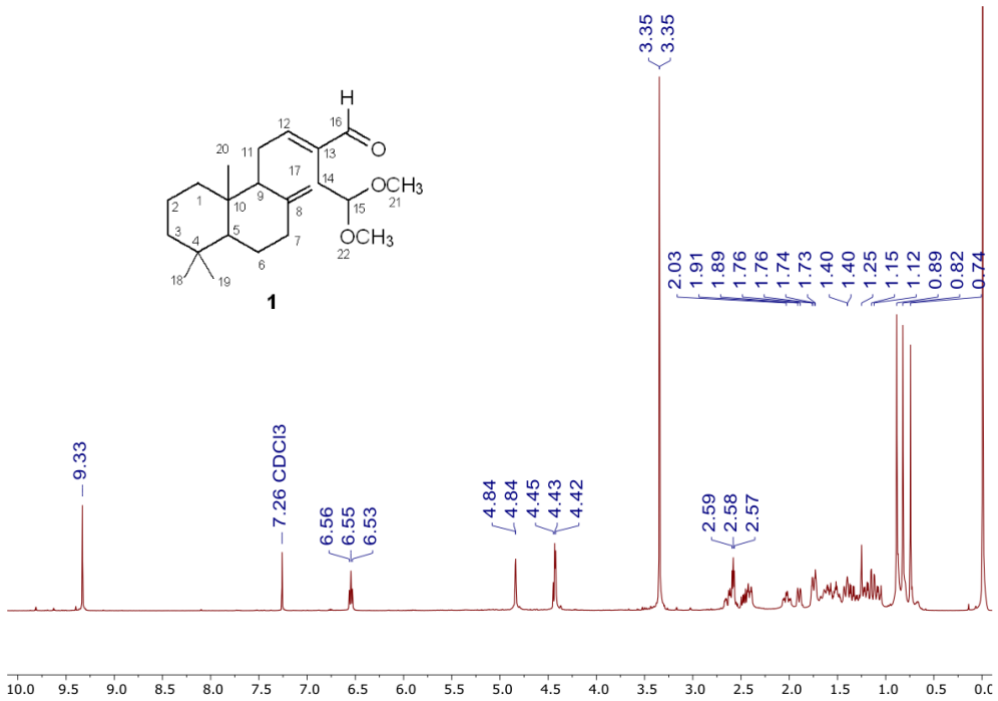

Figure 1a. Spectra ^{1}H-NMR compound-1 (400 MHz, $CDCl_3$)

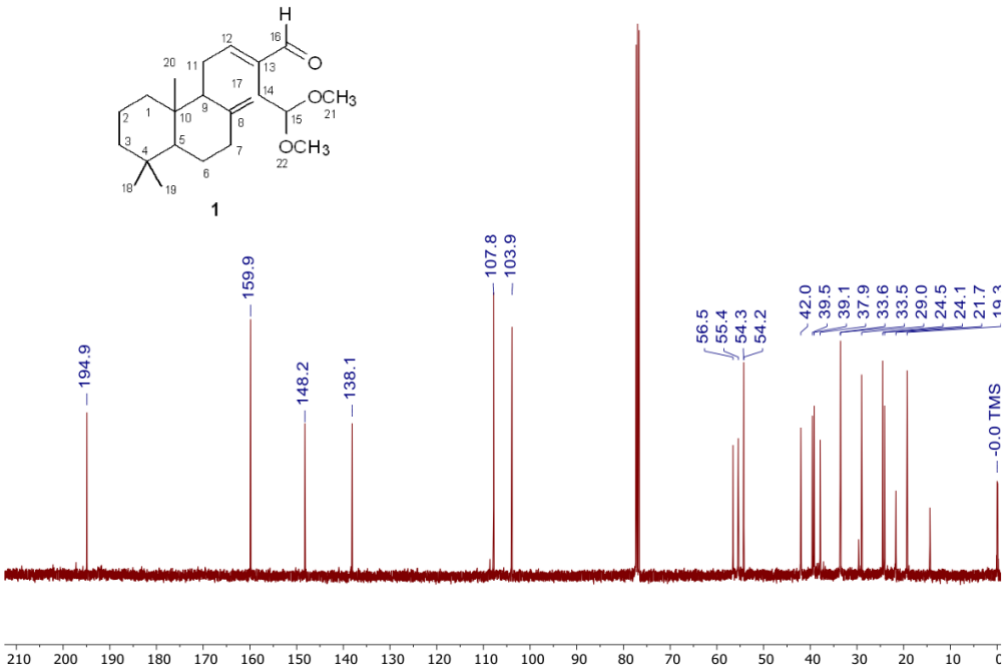

Figure 1b. Spectra ^{13}C-NMR compound-1 (125 MHz, $CDCl_3$)

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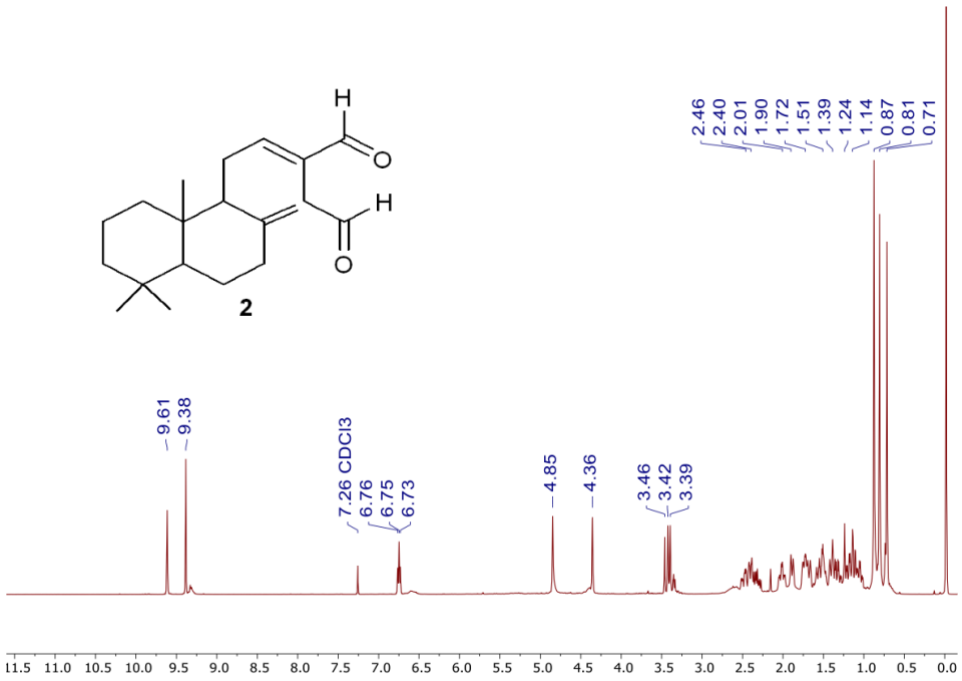

Figure 1c. Spectra ^{1}H-NMR compound-2 (400 MHz, $CDCl_3$)

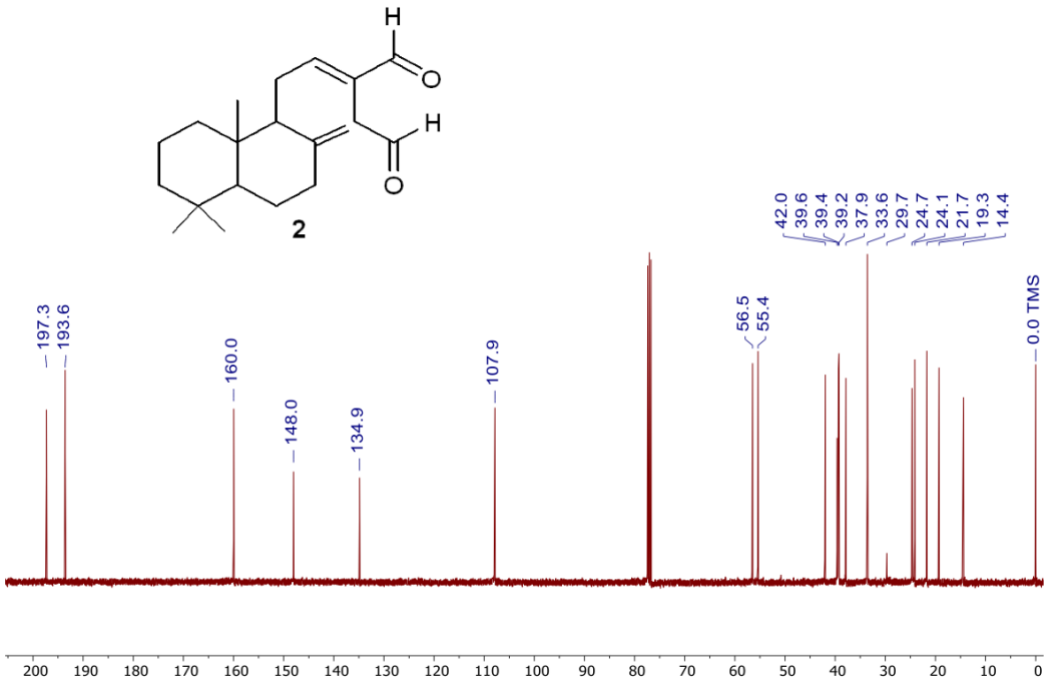

Figure 1d. Spectra ^{13}C-NMR compound-2 (125 MHz, $CDCl_3$)

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