Study on the antihyperglycemic activity of triterpenes in banana peel

Doctoral Dissertation

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Abstract

Banana including dessert banana and cooking banana, is the second largest produced fruit after citrus. Banana peel as by-product, which is about 35% of the total fresh weight, usually used as organic fertilizer or simply discarded as waste. Many reports have shown that banana peel is an important resource which contains many kinds of nutrients and active components. These active components have been reported to exert multiple bioactivities, such as antihyperglycemic activity. Diabetes mellitus which is mainly divided into type 1 and type 2, has been becoming an important global health problem. Under most circumstances, the current antidiabetic medicines are often responsible for various side effects which means that we need to develop less toxicity and side effects natural compounds. Triterpenes are good candidates that have shown the antihyperglycemic activity with several mechanisms. Banana peels are rich in triterpenes with cycloeucalenone and 31-norcyclolaudenone as the major components. The purpose of this research is to develop potential antidiabetic natural compounds in banana peel and take full advantage of banana peel resource by investigating the antihyperglycemic activity of its main triterpenes.

In chapter 2, four compounds were purified from banana peel ethyl acetate extract by HPLC based on the proton NMR. Compound **1** (Fr. 2-2-1) maybe 31-norcyclolaudenone and its recovery rate is 0.17%. Compound **2** (Fr. 2-2-2) maybe cycloeucalenone and its recovery rate is 0.52%. Compound **3** (Fr. 3-3-3-2-1) maybe the isomer of cycloeucalenol and its recovery rate is 0.005%. Compound **4** (Fr. 3-3-3-2-2) maybe cycloeucalenol and its recovery rate is 0.014%.

In chapter 3, the structures of four purified compounds were analyzed and confirmed by 1D and 2D NMR. Based on the ^{1}H and ^{13}C along with COSY, HMQC, HMBC, DEPT 90, DEPT 135 and comparison with literature data, compound **1** is 31-norcyclolaudenone (isoCE-one), compound **2** is cycloeucalenone (CE-one), compound **3** is isomer of cycloeucalenol (isoCE-ol), compound **4** is cycloeucalenol (CE-ol). The structural differences of these four triterpenes are the carbonyl group or hydroxy group at C-3 and the position of double bond (Δ25(27) or Δ24(28)) in the side chain.

In chapter 4, the α-glucosidase and α-amylase inhibitory activities of four triterpenes were measured and compared *in vitro*. And their structure-activity relationships were analyzed. In addition, the kinetic analysis of CE-one in α-glucosidase inhibitory assay was also elucidated. CE-one and isoCE-one exhibited both α-glucosidase and α-amylase inhibitory activities. And CE-one showed higher α-glucosidase and α-amylase inhibitory activities than that of isoCE-one. In contrast, CE-ol and isoCE-ol had insufficient inhibitory activity against either enzyme. The IC_{50} values of CE-one and isoCE-one against α-glucosidase were 31.83 ± 2.46 μM and 38.85 ± 1.54 μM, respectively, and their IC_{50} values against α-amylase were 20.33 ± 0.59 μM and 27.63 ± 0.83 μM, respectively. The main active sites of CE-one and isoCE-one are the carbonyl group at C-3 and double bond in the side chain. And CE-one induced a parabolic mixed-type inhibition with the K_i value of 73.86 μM in α-glucosidase inhibitory assay.

In chapter 5, the carbohydrate-loading tests of CE-one in normal mice and the antihyperglycemic activity of CE-one in STZ-induced diabetic mice were investigated. The 80 mg/kg dose of CE-one showed 26.7% decrease of blood glucose compared with control group at 30 min which is similar with positive control acarbose (23.3% decrease) in oral maltose tolerance test. The 80 mg/kg dose of CE-one showed 39.2% decrease of blood glucose compared with control group at 30 min which is similar with positive control acarbose (40.4% decrease) in oral starch tolerance test. These results indicated that CE-one can suppress the blood glucose level through inhibition of α-glucosidase and α-amylase activity in small intestine of normal mice. And 80 mg/kg dose of CE-one can also suppress the blood glucose level in oral glucose tolerance test which indicated that it maybe could inhibit the absorption of glucose. The results of CE-one in STZ-induced diabetic mice indicated that CE-one can reduce the blood glucose level, food intake, water consumption and AST level, improve the liver index, and increase the content of hepatic glycogen. CE-one cannot affect the body weight, kidney index, ALT level, and insulin level of diabetic mice. The underlying mechanisms maybe because of CE-one can improve the condition of liver injury and the content of liver glycogen. In addition, CE-one can also decrease total cholesterol level in plasma while has no effect on triglyceride level.

In chapter 6, the stability of CE-one was investigated *in vitro* and *in vivo*. The stability of CE-one is high below 60˚C and low beyond 100˚C, especially 200˚C. These indicated that

banana peel can be dried by oven at 50-60 ˚C for CE-one preparation process. CE-one can be used as ingredient for functional food. But it cannot be used for baked food. Furthermore, CE-one is relatively stable in acidic and neutral conditions. There are still around 75% of CE-one remain after kept in SGF and SIF conditions for 4 hours. Moreover, the stability of CE-one is also relatively high *in vivo*. After oral administration of CE-one for 2 h in normal mice, there are still around 77% of relative amount remain in the content of stomach and small intestine. These results provide some evidence of CE-one for food processing and effectivity in intestine as health promoting ingredient.

The present study first time showed the antihyperglycemic activity and mechanisms and stability of CE-one *in vitro* and *in vivo*. It not only provides the possibility to use CE-one as food ingredient or potential antidiabetic agent, but also provides an alternative way to recycle banana peel resource. Nevertheless, further studies about the absorption and metabolism of CE-one *in vivo* still need to be investigated in the future.

Abbreviation

DM: diabetes mellitus.

EtOAc: ethyl acetate.

Hex: hexane.

MeOH: methanol.

$CDCl_3$: chloroform-*d*.

NMR: nuclear magnetic resonance.

HPLC: high-performance liquid chromatography.

CE-one: cycloeucalenone.

isoCE-one: 31-norcyclolaudenone.

CE-ol: cycloeucalenol.

isoCE-ol: isomer of cycloeucalenol.

DMSO: dimethyl sulfoxide.

SGF: simulated gastric fluid.

SIF: simulated intestinal fluid.

CMC: carboxymethyl cellulose sodium salt.

OMTT: oral maltose tolerance test.

OSTT: oral starch tolerance test.

OGTT: oral glucose tolerance test.

STZ: streptozotocin.

FBG: fasting blood glucose.

FIN: fasting plasma insulin.

ALT: alanine transaminase.

AST: aspartate transaminase.

TC: total cholesterol.

TG: triglyceride.

Chapter 1 General introduction

1.1 Banana peel

Banana including dessert banana and cooking banana, is the second largest produced fruit after citrus (approximately 16% of total fruit yield in the world). In 2018, banana production in the world was 115.74 million metric tons, with a cultivated area of 5.73 million hectares (El Barnossi, *et al*., 2020). Banana peel which is about 30% - 40% of the total fresh weight is by-product of banana processing (Albarelli, *et al*., 2011). Therefore, approximately 34.72 - 46.30 million metric tons of banana peels are generated in 2018 and this is a potential material for further utilization. Currently, most of the banana peel usually used as organic fertilizer or simply discarded as waste (Schieber, *et al*., 2001). This leads to not only the waste of banana peel resource but also the pollution of our environment.

However, banana peel is an important resource. Numerous studies have shown that there are many kinds of nutrients and active components in banana peel. Banana peels are full of nutrients, such as dietary fiber (40% - 50%), crude protein (8% - 11%), crude fat (2.2% - 10.9%), starch (3%), vitamins and high potassium (55.23 - 63.52 mg/kg) (Emaga, *et al*., 2007). Banana peels also contain many active components, such as phenolic compounds, flavonoids, phytosterols, carotenoids (Pereira & Maraschin, 2015; Singh, *et al*., 2016). These active components have been reported to exert multiple activities, such as antioxidant (González-Montelongo, *et al*., 2010; Rebello, *et al*., 2014), antibacterial (Rattanavichai & Cheng, 2014), antihyperglycemic (Navghare & Dhawale, 2017) and antihypertensive (Qian, *et al*., 2007; Liu, *et al*., 2009). Some attempts at the practical utilization of banana peels have been made, including the production of protein, ethanol, methane, pectin and enzymes (Schieber, *et al*., 2001; Clarke, *et al*., 2008; Emaga, *et al*., 2008; Essien, *et al*., 2005; Oberoi, *et al*., 2011), as food for livestock (Onwuka, *et al*., 1997), and as a bio-sorbent for heavy metals, water purification and the removal of phenolic compounds (Anwar, *et al*., 2010; Albarelli, *et al*., 2011; Annadurai, *et al.*, 2002; Achak, *et al*., 2009). In order to make full use of banana peel resource, we need to find a more promising approach of the integral use of banana peel.

1.2 Triterpenes in banana peel

Triterpenes, especially tetracyclic and pentacyclic types, represent secondary metabolites that are widely distributed in the plant kingdom and found in leaves, stem bark, fruits and roots (Jäger, *et al*., 2009). In banana peel, phytosterols represent majority of the lipophilic extract (49%-87.5%) with cycloeucalenone, 31-norcyclolaudenone, stigmasterol, β-sitosterol, and campesterol as the major components (Oliveira, *et al*., 2008; Villaverde, *et al*., 2013). Many studies showed that cycloeucalenone and 31-norcyclolaudenone are identified in many parts of banana, such as peel, pulp, flowers, petioles/midrib, leaf sheaths, floral stalk and rachis (Akihisa, *et al*., 1998; Oliveira, *et al*., 2006). In fact, cycloeucalenone is the main component identified in the unripe peel of 10 banana species, with abundances ranging from 806 to 9453 mg/kg of dry unripe peels (Villaverde, *et al*., 2013). Some research indicated that cycloeucalenone showed analgesic and anti-inflammatory effects (Lopes, *et al*., 2014), mild cardiotonic effects (Kongkathip, *et al*., 2002). It can also contribute to hair restoration (Najima, *et al*., 2016) and inhibition of prostate gland enlargement (Akamine, *et al*., 2009). Moreover, cycloeucalenone and 31-norcyclolaudenone have low toxicity (Silva, *et al*., 2014; Lopes, *et al*., 2014). These studies provide the possibility to make more use of major triterpenes in banana peel. But until now, very few studies about the bioactivity of them have been done.

1.3 The bioactivities of triterpenes in other materials

Tetracyclic and pentacyclic triterpenes are the two major categories in triterpenes. A large number of studies have shown that triterpenes have a wide range of pharmacological effects and important biological activities. Numerous reports have revealed many properties of triterpenes *in vitro* and *in vivo*: such as anti-cancer (Laszczyk, 2009; Li, *et al*., 2018), antioxidant (Ramachandran & Prasad, 2008), anti-inflammatory (Yasukawa, *et al*., 1996), wound healing (Agra, *et al*., 2015), antidiabetic (Deutschländer, *et al*., 2011), liver protection (Hu, *et al*., 2013), antiviral (Baltina, *et al*., 2003) and anti-atherosclerotic (Sudhahar, *et al*., 2007).

Many natural triterpenes seem to have promising antidiabetic properties. Some reports have shown that triterpenes can exert several antidiabetic mechanisms. They can inhibit enzymes involved in carbohydrate metabolism, prevent the development of insulin resistance

and normalize plasma glucose and insulin levels (Nazaruk & Borzym-Kluczyk, 2015). Until now, no reports have been done about the antihyperglycemic activity of cycloeucalenone, the main triterpene in banana peel. But as one of the triterpenes, it maybe has high potential possibility of possessing antihyperglycemic effect.

1.4 Diabetes mellitus

Diabetes mellitus (DM), which mainly includes type 1 (5% - 10%) and type 2 (90% - 95%), is a globally prevalent chronic disease (Neeland & Patel, 2019). According to the reports of World Health Organization (2020), approximately 422 million people suffer from DM worldwide and 1.6 million deaths are directly attributed to DM annually. After cancer and cardiovascular diseases, diabetes has become pandemic to human being due to its high prevalence, morbidity, and mortality (Egede & Ellis, 2010). Numerous therapeutic drugs (such as insulin, sulfonylureas, metformin, and acarbose) are available for DM with different mechanisms of action, including regulation of insulin secretion, glucose absorption, carbohydrate metabolism, and antioxidant activity (Samarakoon, *et al*., 2020).

However, the current antidiabetic medicines are responsible for various side effects such as liver problems, lactic acidosis, diarrhea and high rates of secondary failures (Inzucchi, 2002). Therefore, traditional medicinal plants and natural food materials have been necessary resources in several countries to control various DM complications, as they are considered as less toxicity and side effects compared to synthetic drugs (Balaraman, *et al*., 2010).

Many studies have shown that the extracts of some fruits (such as citrus, pomegranate, berries, and Prunus species), vegetables (such as onions, peppers, eggplants, bitter melon, and brassicas) and mushrooms can inhibit α-amylase and α-glucosidase. The bioactive compounds include flavonoids, phenolic acids, tannins, carotenoids, sugars, proteins, vitamin C, fatty acids, alkaloids, saponins, and terpenes (Papoutsis, *et al*., 2021). Among the numerous plants that provide medicinal properties, banana is one of the highest produced fruits. Several studies have reported that the extracts of different parts of banana plant, such as banana fruits, peels, flowers, leaves, stems, and roots, have exerted antidiabetic activities (Shodehinde, *et al*., 2015; Navghare & Dhawale, 2017; Vilhena, *et al*., 2020; Lakshmi, *et al*., 2014). Cycloeucalenone, as one of the

natural triterpenes with low toxicity, maybe have the potential possibility to be used as an antidiabetic agent. The antihyperglycemic activity and mechanisms of major triterpenes in banana peel need to be investigated.

1.5 Research purpose and contents

By investigating the antihyperglycemic activity and mechanism of major triterpenes in banana peel, we will provide the potential possibility of using them as antidiabetic agents. This will not only provide the new bioactivity of triterpenes in banana peel, but also provide an alternative way to recycle banana peel resource.

The present research mainly includes five parts:

Chapter 2 Isolation and purification of major triterpenes from banana peel.

Chapter 3 Identification of four purified triterpenes by 1D and 2D NMR.

Chapter 4 Carbohydrate-digesting enzymes inhibitory activities of four purified triterpenes *in vitro*.

Chapter 5 Antihyperglycemic activity of cycloeucalenone *in vivo*.

Chapter 6 Stability of cycloeucalenone *in vitro* and *in vivo*.

Chapter 2 Isolation and purification of major triterpenes from banana peel

2.1 Introduction

In this chapter, the major triterpenes in banana peel, cycloeucalenone and 31-norcyclolaudenone will be isolated from banana peel powder. In addition, the similar structure compounds, cycloeucalenol and its isomer will also be isolated. Based on the literatures, there are some special signals of proton nuclear magnetic resonance (NMR) of cycloeucalenone and cycloeucalenol. The special proton NMR signals of cycloeucalenone are shown as below: the cyclopropyl protons are represented by two doublets at δ 0.40 and δ 0.60 ppm (Khuong-Huu, *et al*., 1975; Silva, *et al*., 2014). The special proton NMR signals of cycloeucalenol are shown as below: the cyclopropyl protons are represented by two doublets at δ 0.15 and δ 0.38 ppm; a proton at C-3 is represented by a multiplet at δ 3.20 ppm (Liu, *et al*., 2011; Ragasa, *et al*., 2013). If the proton NMR of fraction contains the special proton NMR signals of target compound, it means this fraction maybe contain this compound. Otherwise, it doesn't contain. So, the proton NMR guided fractionation was used to obtain target compounds.

2.2 Materials and methods

2.2.1 Chemicals

Analytical grade ethyl acetate (EtOAc), hexane (Hex) and methanol (MeOH), and HPLC grade MeOH and EtOAc were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Chloroform-*d* ($CDCl_3$) was obtained from EMD Millipore Corporation (Darmstadt, Germany). Silica gel 60 (0.063–0.200 mm) was obtained from Merck KGaA (Darmstadt, Germany).

2.2.2 Isolation and purification of triterpenes from banana peel

Bananas (*Musa acuminata*) were purchased as a general commercially available from a supermarket in Tokyo (Japan). Bananas, yellow-colored ripe products from Ecuador, were

washed with tap water, followed by distilled water. The peels were manually removed from the pulp and immediately frozen at -20˚C. After freeze-drying, the peels were ground into powder. The banana peel powder was sealed and stored at room temperature (25˚C) until further use.

Banana peel powder was extracted with 100% EtOAc (1 : 10 (w/v)) for 24 h under room temperature for 3 times. After filtration, the filtrate was evaporated to nearly dry at 30˚C. The banana peel EtOAc extract was obtained after freeze drying. To separate target triterpenes, the EtOAc extract was partitioned with Hex and 90% aqueous MeOH (1 : 1 (v/v)) at a ratio of 1 : 50 (w/v) for 3 times. The Hex layer was collected as mixture containing target triterpenes.

After concentrated and freeze dried, the Hex layer prepared from banana peel was further separated by column chromatography techniques using silica open column and high-performance liquid chromatography (HPLC) systems (JASCO Corporation, Tokyo, Japan) guided by ^{1}H NMR spectra in $CDCl_3$ on a Bruker AV-600 spectrometer (Bruker, Tokyo, Japan). The special signals on ^{1}H NMR spectra were used as indicators of target triterpenes as below: two doublet signals at δ_H 0.40 ppm and δ_H 0.60 ppm derived from a cyclopropane ring (at C-19) for cycloeucalenone type, and two doublet signals at δ_H 0.15 ppm and δ_H 0.38 ppm derived from a cyclopropane ring (at C-19) with a multiplet signal at δ_H 3.20 ppm derived from the proton at C-3 for cycloeucalenol type (Khuong-Huu, *et al*., 1975; Silva, *et al*., 2014; Liu, *et al*., 2011; Ragasa, *et al*., 2013). The detailed conditions and yield of each fraction were described in the results part.

2.3 Results

2.3.1 Results of isolation and purification of cycloeucalenone and its isomer

The hexane layer prepared from banana peel was separated on silica open column (1 : 20, w/W) to separate triterpenes. Three fractions (Fr. 1, Fr. 2 and Fr. 3) were collected after eluted with EtOAc : Hex at different ratio including 2 : 98, 5 : 95 and 100 : 0 (v/v), using 3 times column volume. Then Fr. 2 was further separated by a HPLC system consisted of PU-1586 pump and UV-2075 detector (JASCO Corporation, Tokyo, Japan), using the ODS-HG-5 (20 × 250 mm) column with isocratic elution of MeOH : EtOAc = 1 : 1 (v/v) at 5 mL/min monitoring at 254 nm with PREP UV cell (range 0.001). After separation, Fr. 2 was divided into 3 fractions,

Fr. 2-1: 0 - 16.5 min, Fr. 2-2: 16.5 - 20.5 min and Fr. 2-3: 20.5 - 50 min. Then Fr. 2-2 was further isolated using a recycling preparative HPLC system comprised of PU-1586 pump with RCY-2088 recycling model equipped with UV-2075 detector (JASCO Corporation, Japan). The chromatographic separation was performed on ODS-HG-5 (20 × 250 mm) with a flow rate of 8 mL/min using 100% MeOH by UV detection at 254 nm with TP cell (range 0.0005) to obtain two main compounds, compound **1** and **2** (The separation scheme is shown as Fig. 2-1). All fractions were checked with proton NMR to get the target fraction.

Based on the proton NMR of Fr. 1, Fr. 2 and Fr. 3, we found that Fr. 2 (0.95%) contains the special signals of cycloeucalenone, two doublet signals at δ 0.42 ppm and δ 0.64 ppm derived from a cyclopropane ring, Fr. 2 maybe contain target compounds (Fig. 2-3). After Fr. 2 was separated by HPLC 1, we collected three fractions (Fr. 2-1, Fr. 2-2 and Fr. 2-3) based on the retention time and peaks (Fig. 2-4). After checked with proton NMR, Fr. 2-2 showed contain the special signals of cycloeucalenone (Fig. 2-5). Fr. 2-2 was further separated by HPLC with recycle mode and collected two fractions, Fr. 2-2-1 and Fr. 2-2-2 (Fig. 2-6). Based on the proton NMR, Fr. 2-2-2 (compound **2**) maybe cycloeucalenone with recovery rate of 0.52%, Fr. 2-2-1 (compound **1**) maybe its isomer, 31-norcyclolaudenone with recovery rate of 0.17% (Fig. 2-7).

2.3.2 Results of isolation and purification of cycloeucalenol and its isomer

The Fr. 3 (0.53%) given from silica column with 100% EtOAc as mentioned above was further separated by using silica column again (1 : 20, w/W). Six fractions were collected by eluted with EtOAc : Hex at different ratio, namely 0 : 100, 5 : 95, 10 : 90, 15 : 85, 20 : 80 and 100 : 0 (v/v), using 3 times column volume. The fraction eluted with EtOAc : Hex = 10 : 90 (v/v) was further separated by HPLC 1 and got 6 fractions based on the retention time and peaks. HPLC 1 conditions were as follows: column: ODS-HG-5 (20 × 250 mm); flow rate: 5 mL/min; range: 0.001 (PREP cell); wavelength: 254 nm; mobile phase: MeOH : EtOAc = 1 : 1 (v/v). The target fraction 1 was further separated by HPLC 2 and got 4 fractions. HPLC 2 conditions were as follows: range: 0.0005 (TP cell); flow rate: 8 mL/min; mobile phase: 100% MeOH; other parameters are same with HPLC 1. The target fraction 2 was further separated by HPLC 3 with recycle mode and two main compounds (**3** and **4**) were obtained (The separation scheme

is shown as Fig. 2-2). HPLC 3 conditions are same with HPLC 2. All fractions were checked with proton NMR to get the target fraction.

Based on proton NMR of Fr. 1, Fr. 2 and Fr. 3, we found Fr. 3 contains the special signals of cycloeucalenol, two doublet signals at δ 0.16 ppm and δ 0.40 ppm derived from a cyclopropane ring, and a multiplet signal at δ 3.24 ppm derived from the proton at C-3, it maybe contains cycloeucalenol (Fig. 2-8). Fr. 3 was used for further separation with silica column and collected six fractions. After checked with proton NMR of six fractions, we found Fr. 3-3 contains the special signals of cycloeucalenol, other 5 fractions do not contain cycloeucalenol (Fig. 2-9). Fr. 3-3 was further separated by HPLC 1 and collected 6 fractions based on the retention time and peaks (Fig. 2-10). After checked with proton NMR, Fr. 3-3-3 was found mainly contains cycloeucalenol, Fr. 3-3-4 maybe contains very little amount, and other fractions do not contain (Fig. 2-11). Fr. 3-3-3 was further separated by HPLC 2 and collected four fractions based on the retention time and peaks (Fig. 2-12). After checked with proton NMR, Fr. 3-3-3-2 contains mainly cycloeucalenol, Fr. 3-3-3-3 contains little amount, other fractions do not contain (Fig. 2-13). Fr. 3-3-3-2 was further separated with HPLC 3 with recycle mode and collected two fractions, Fr. 3-3-3-2-1 and Fr. 3-3-3-2-2 (Fig. 2-14). Based on the proton NMR, Fr. 3-3-3-2-2 (compound **4**) maybe cycloeucalenol with recovery rate of 0.014%, Fr. 3-3-3-2-1 (compound **3**) maybe its isomer with recovery rate of 0.005% (Fig. 2-15).

2.4 Discussion

In banana peel, sterols represent majority of the lipophilic extract (49% - 87.5%) with cycloeucalenone, 31-norcyclolaudenone, stigmasterol, β-sitosterol and campesterol as the major components whereas in banana pulp lipophilic fraction mainly consists of fatty acids, especially unsaturated fatty acids (Oliveira, *et al*., 2008; Villaverde, *et al*., 2013). In this chapter, four purified compounds were obtained from banana peel. Based on the proton NMR, Fr. 2-2-1 (compound **1**) maybe 31-norcyclolaudenone and its recovery rate is 0.17%. Fr. 2-2-2 (compound **2**) maybe cycloeucalenone and its recovery rate is 0.52%. This is in accordance with literatures that cycloeucalenone and 31-norcyclolaudenone are two major components in the unripe banana peel (Oliveira, *et al*., 2008). The content of cycloeucalenone in present study

(5200 mg/kg) accorded with the abundances ranging from 806 to 9453 mg/kg of dry unripe peels in other study (Villaverde, *et al*., 2013). Based on our results and literatures, cycloeucalenone and 31-norcyclolaudenone are two high content compounds in banana peel. More research should be done to fully utilize these two compounds for human beings.

Based on the proton NMR, Fr. 3-3-3-2-1 (compound **3**) maybe the isomer of cycloeucalenol and its recovery rate is 0.005%. Fr. 3-3-3-2-2 (compound **4**) maybe cycloeucalenol and its recovery rate is 0.014%. Compared with the recovery rate of Fr. 2-2-1 and Fr. 2-2-2, Fr. 3-3-3-2-1 and Fr. 3-3-3-2-1 showed lower recovery rate, this indicated that the content of them in banana peel is very low. Our results indicated that Fr. 3-3-3-2-1 and Fr. 3-3-3-2-1 have higher polarity than Fr. 2-2-1 and Fr. 2-2-2 and they are more difficult to get the pure compounds. In order to get pure compounds, all these four compounds need to use the recycle mode of HPLC to separate from their isomers. Cycloeucalenol had been identified in different part of banana and the content usually is very low (Oliveira, *et al*., 2006). This is the first time to isolate the pure isomer of cycloeucalenol from banana peel. In addition, cycloeucalenol and its isomer, with low toxicity, were also found in the plant *Boophone disticha* (Adewusi, *et al*., 2013).

2.5 Conclusion

Four purified compounds were obtained from banana peel EtOAc extract. Fr. 2-2-1 (compound **1**) with 0.17% recovery rate maybe 31-norcyclolaudenone. Fr. 2-2-2 (compound **2**) with 0.52% recovery rate maybe cycloeucalenone. Fr. 3-3-3-2-1 (compound **3**) with 0.005% recovery rate maybe the isomer cycloeucalenol. Fr. 3-3-3-2-2 (compound **4**) with 0.014% recovery rate maybe cycloeucalenol. The structures of these four purified compounds need to be analyzed and confirmed with 1D and 2D NMR in the next chapter.

2.6 Figures and tables

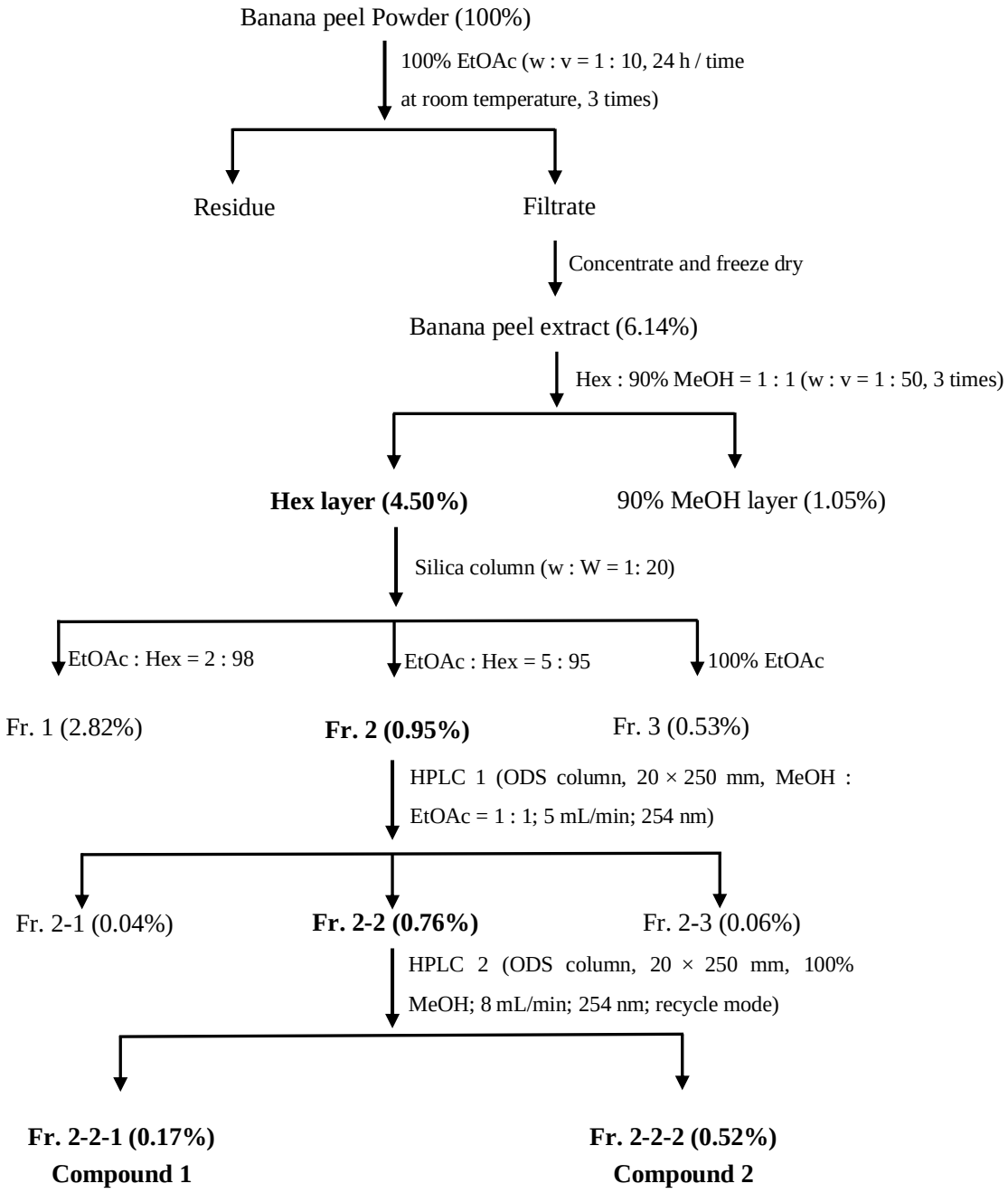

Fig. 2-1 Separation scheme of cycloeucalenone and its isomer from banana peel.

Note: w: weight of sample (g), v: volume of solvent (mL), W: weight of silica gel (g). Values in parenthesis are recovery rate from banana peel powder.

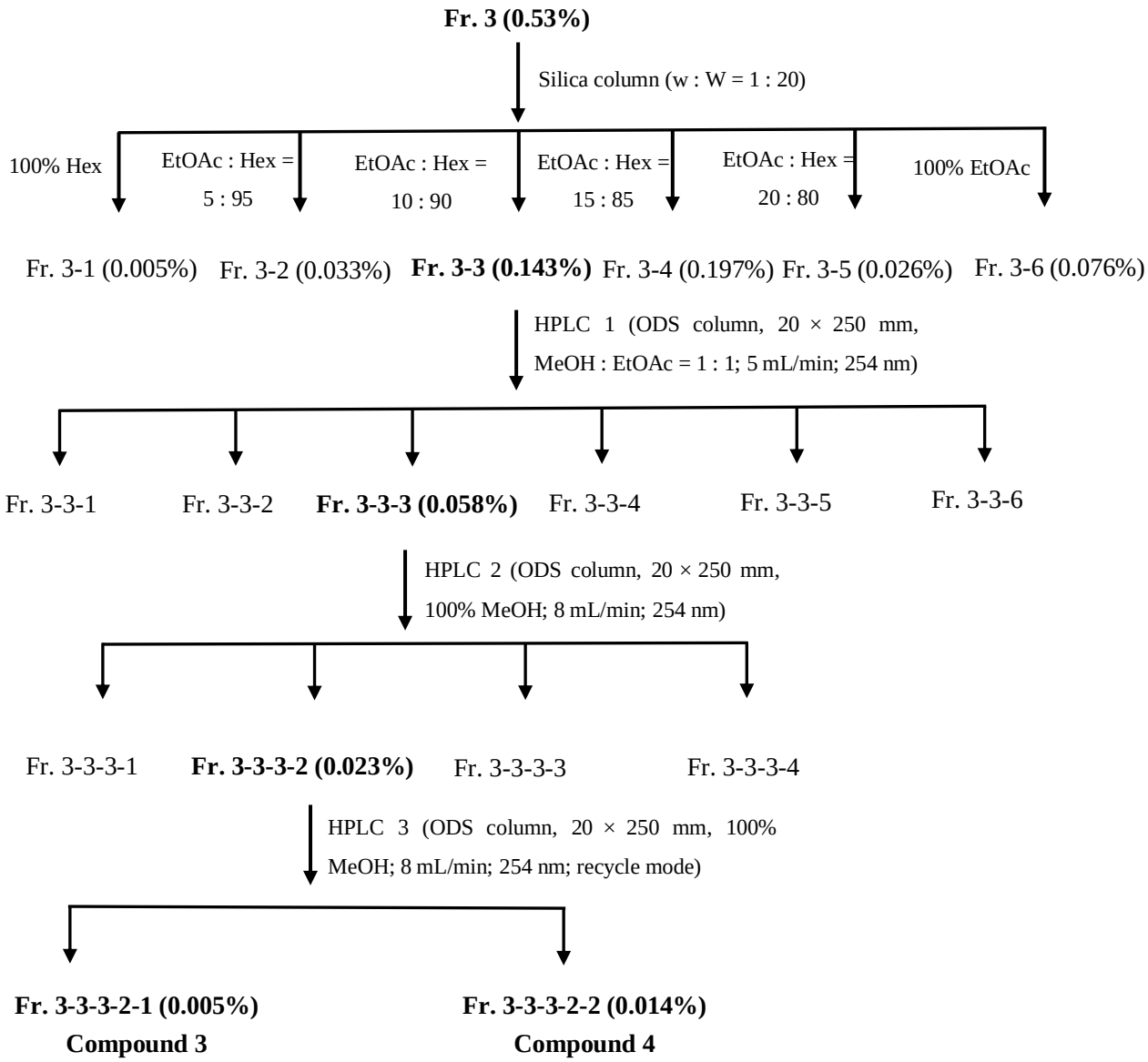

Fig. 2-2 Separation scheme of cycloeucalenol and its isomer from banana peel.

Note: w: weight of sample (g), W: weight of silica gel (g). Values in parenthesis are recovery rate from banana peel powder.

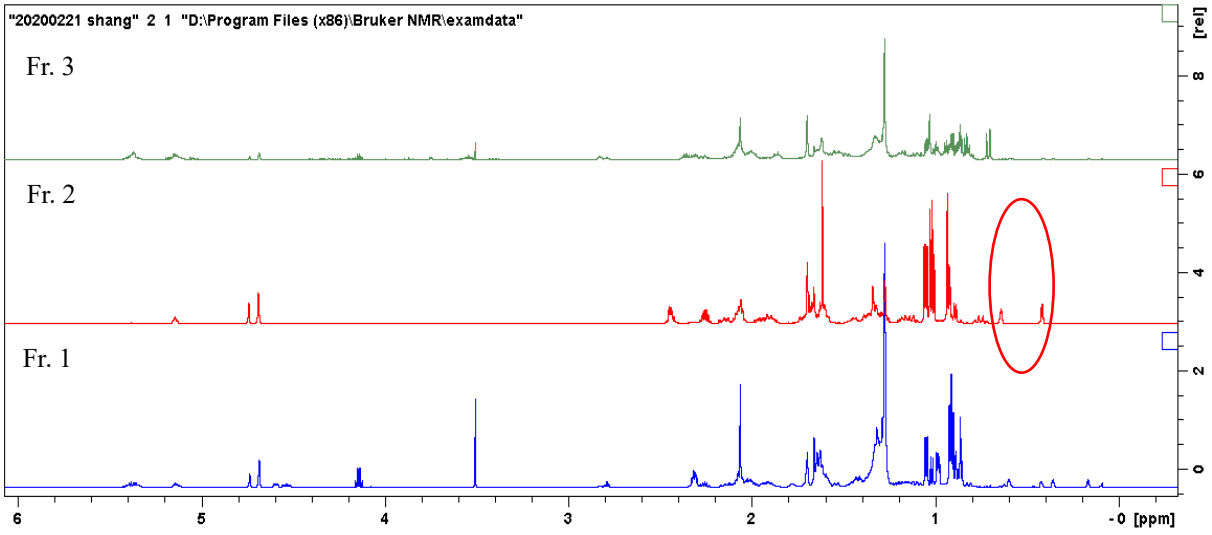

Fig. 2-3 The proton NMR spectra of Fr. 1, Fr. 2 and Fr. 3.

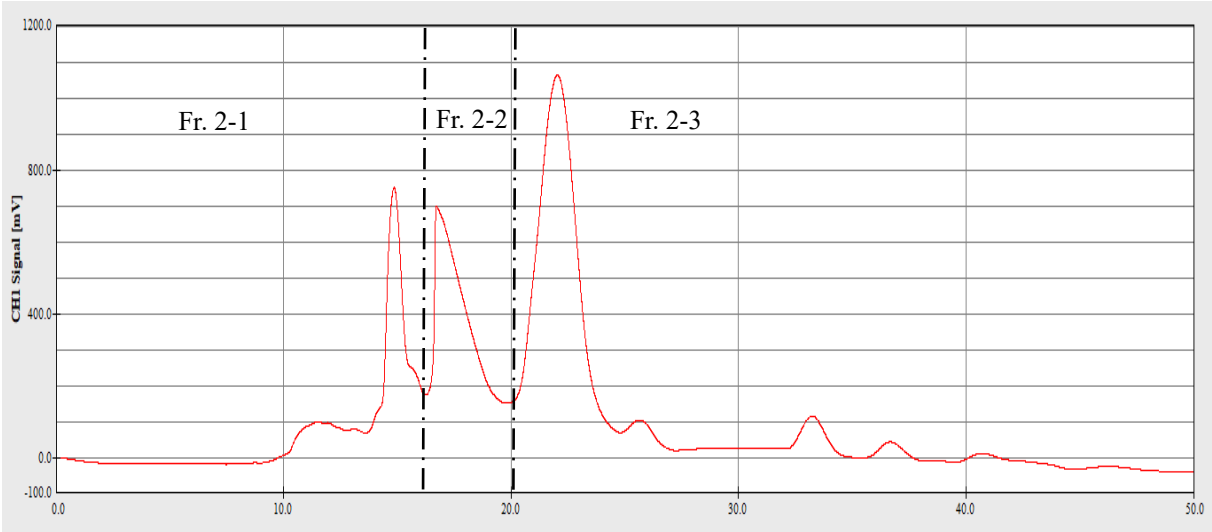

Fig. 2-4 HPLC chromatogram of Fr. 2.

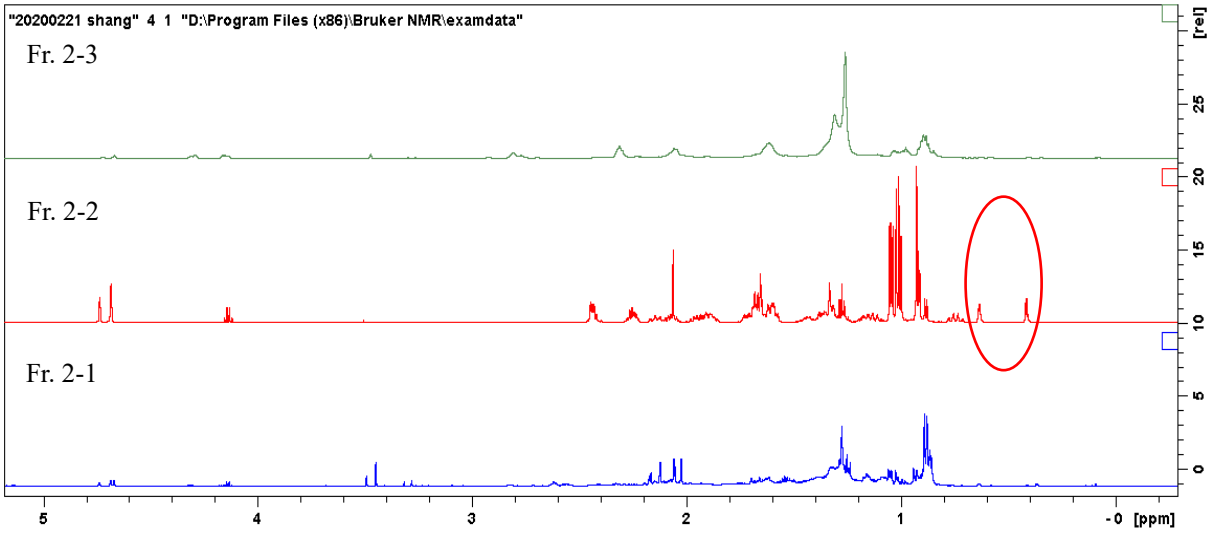

Fig. 2-5 The proton NMR spectra of Fr. 2-1, Fr. 2-2 and Fr. 2-3

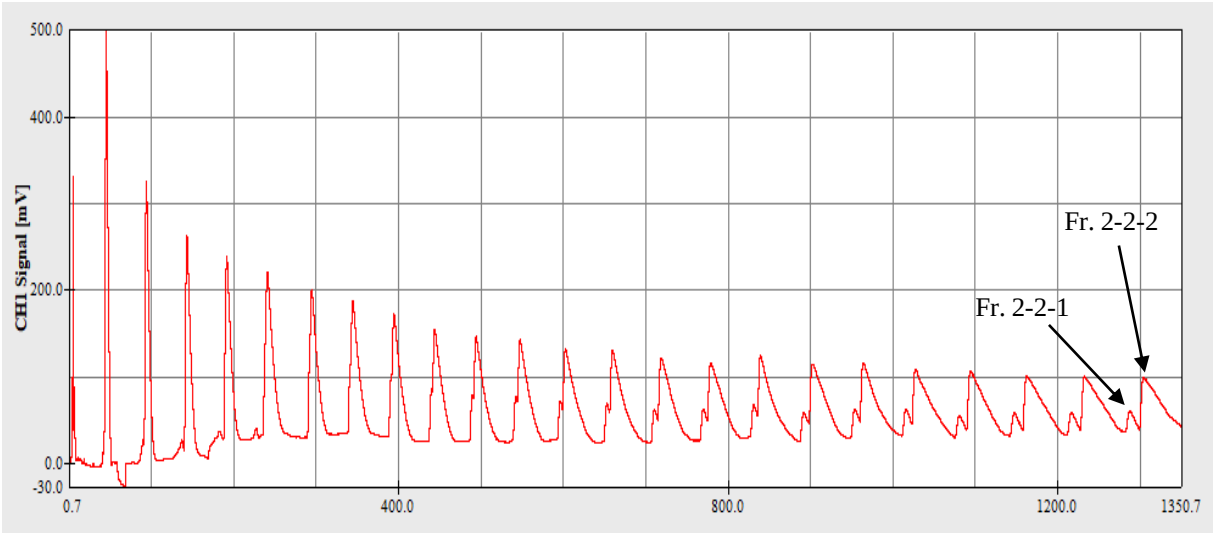

Fig. 2-6 HPLC chromatogram (recycle mode) of Fr. 2-2

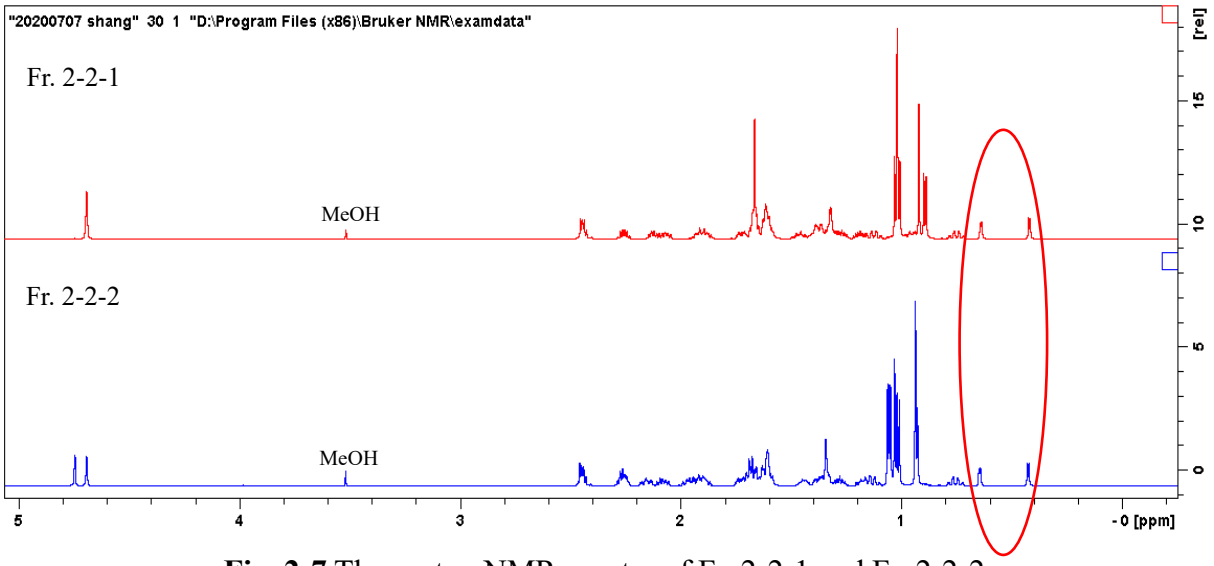

Fig. 2-7 The proton NMR spectra of Fr. 2-2-1 and Fr. 2-2-2.

Note: Very little MeOH still remains in the sample.

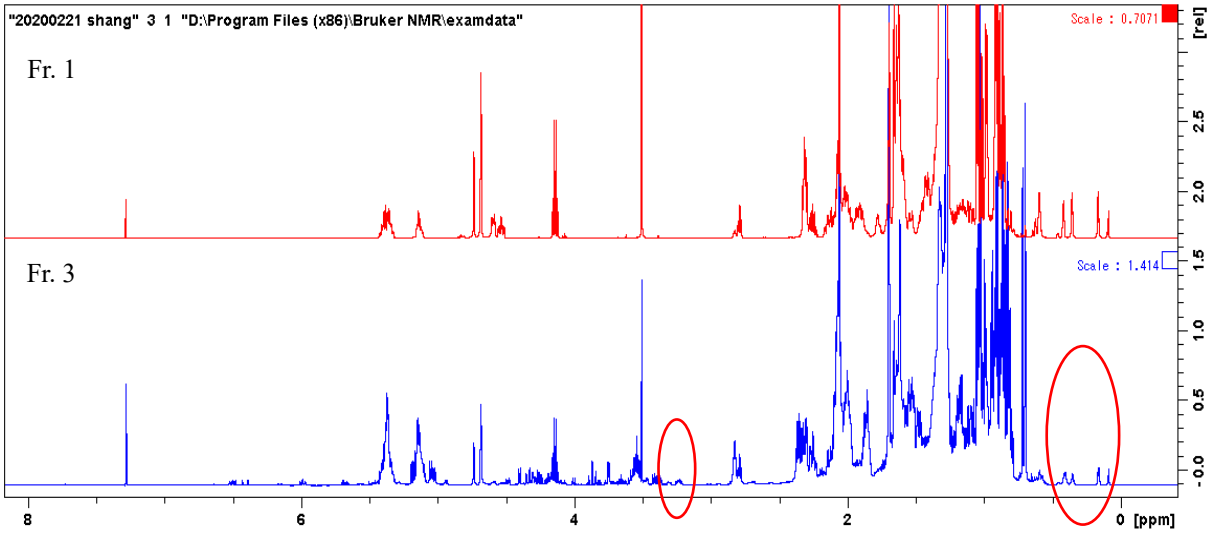

Fig. 2-8 The proton NMR spectra of Fr. 1 and Fr. 3.

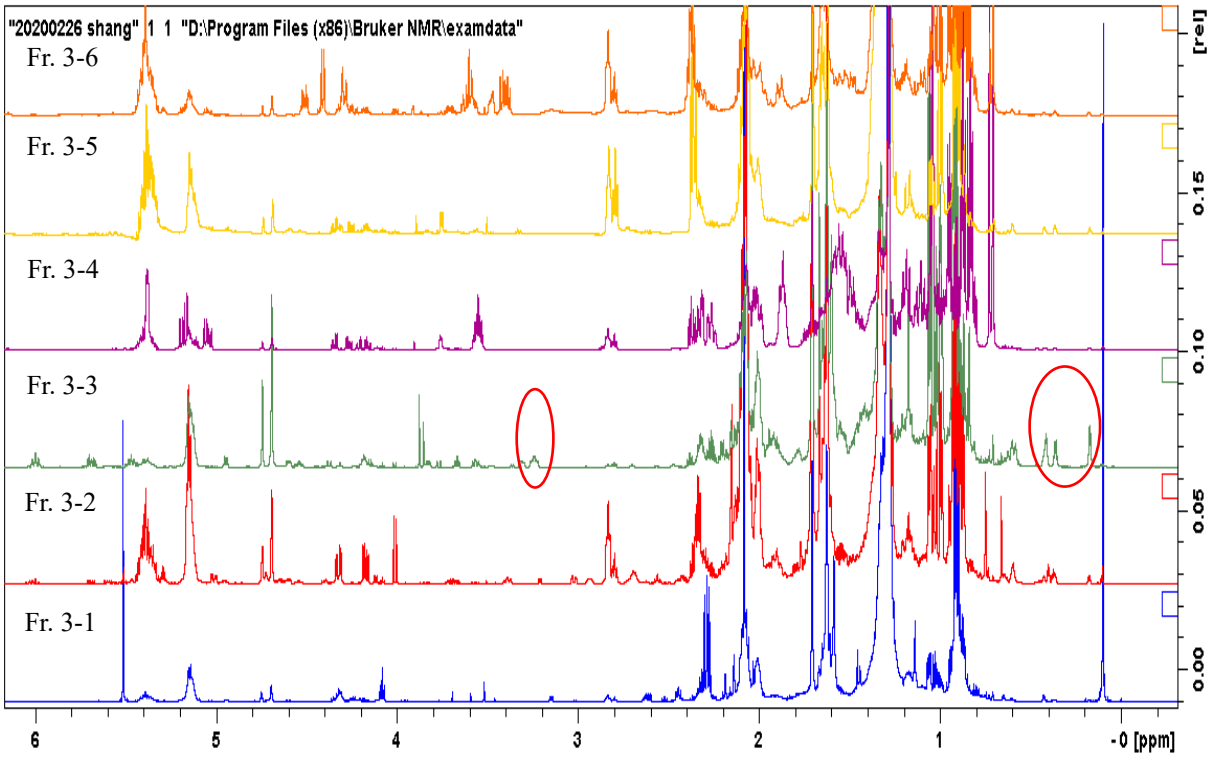

Fig. 2-9 The proton NMR spectra of Fr. 3-1 to Fr. 3-6.

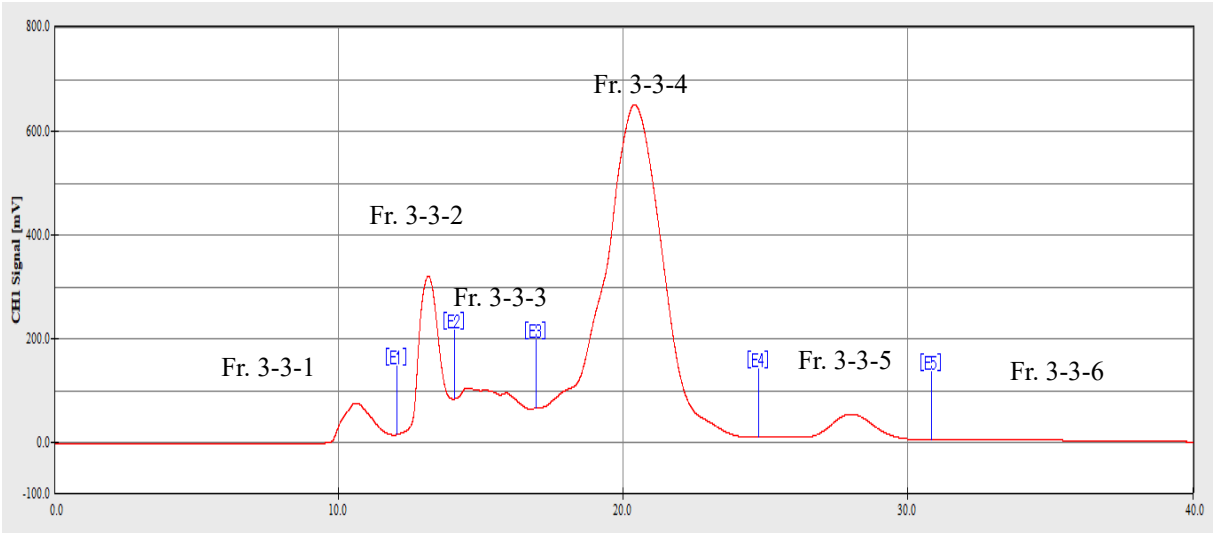

Fig. 2-10 HPLC chromatogram of Fr. 3-3

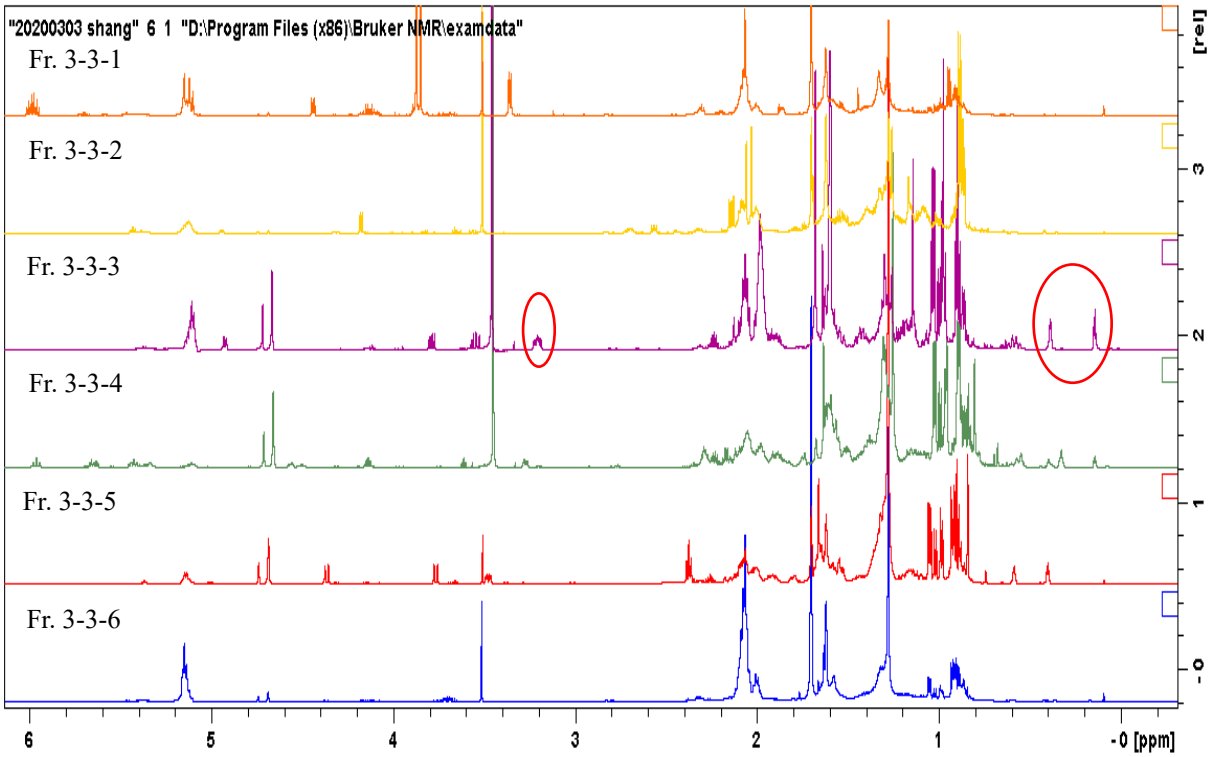

Fig. 2-11 The proton NMR spectra of Fr. 3-3-1 to Fr. 3-3-6.

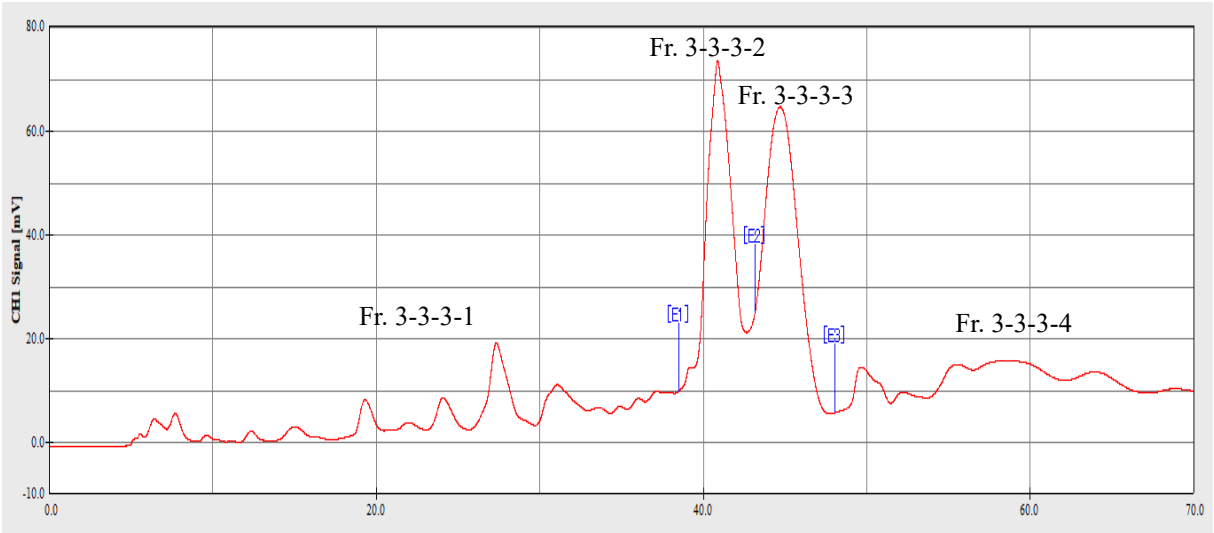

Fig. 2-12 HPLC chromatogram of Fr. 3-3-3.

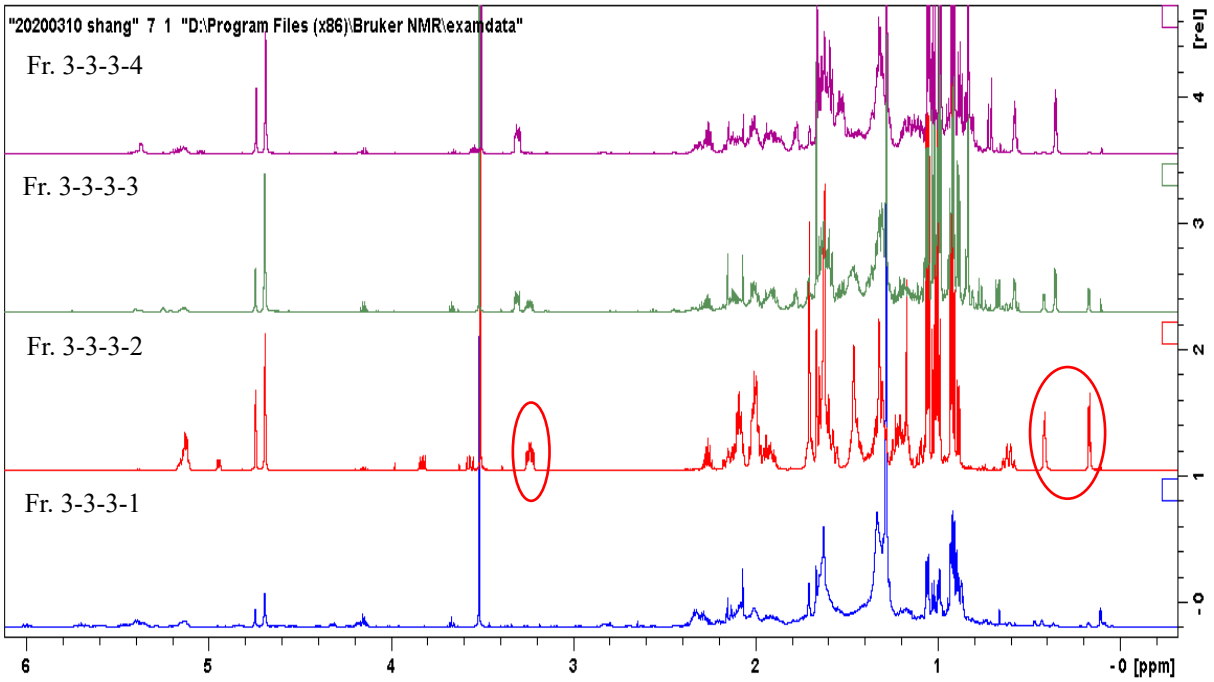

Fig. 2-13 The proton NMR spectra of Fr. 3-3-3-1, Fr. 3-3-3-2, Fr. 3-3-3-3, Fr. 3-3-3-4.

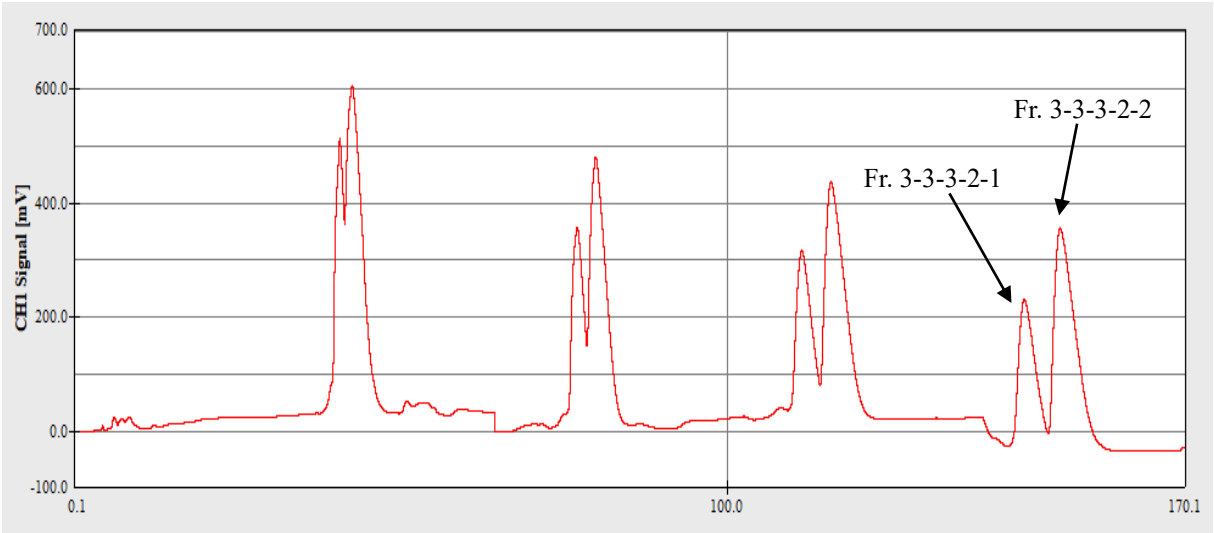

Fig. 2-14 HPLC chromatogram (recycle mode) of Fr. 3-3-3-2.

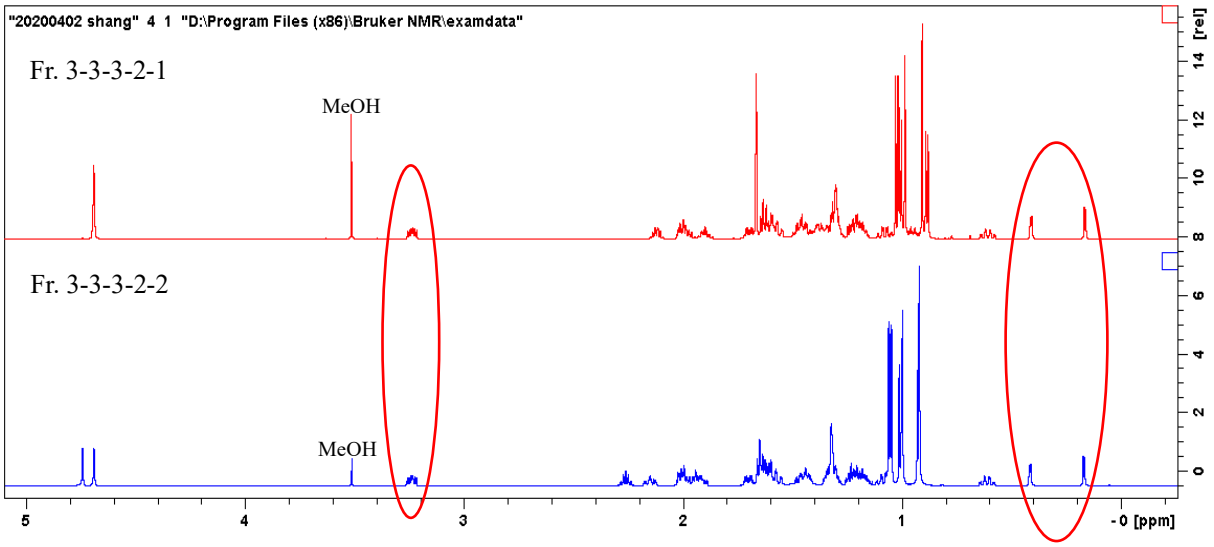

Fig. 2-15 The proton NMR spectra of Fr. 3-3-3-2-1 and Fr. 3-3-3-2-2.

Note: Very little MeOH still remains in the sample.

Chapter 3 Identification of four purified triterpenes by 1D and 2D NMR

3.1 Introduction

In last chapter, four purified compounds were obtained from banana peel EtOAc extract. In order to confirm the structures of these four compounds, 1D and 2D NMR of them were analyzed and identified. The results were also compared with literatures.

3.2 Materials and methods

The NMR spectra of four purified compounds were recorded on a Bruker AVANCE III 600 MHz with ^{1}H (600 MHz) and ^{13}C (150 MHz) in $CDCl_3$ (^{1}H: δ 7.29 ppm and ^{13}C: δ 77.03 ppm) along with COSY, HMQC, HMBC, DEPT 90 and DEPT 135 to characterize these compounds. And the results were also compared with literature data.

3.3 Results

The specific ^{1}H NMR spectrum of compound **1** showed a broad singlet signal at δ 4.69 ppm (2H) characteristic of a terminal double bond. Two doublet signals at δ 0.42 ppm (1H, J = 4.02 Hz) and δ 0.64 ppm (1H, J = 3.78 Hz) corresponds to a cyclopropane ring present in cycloartane-type triterpene. The ^{13}C NMR spectrum showed 30 carbon signals including a double bond between C-25 and C-27 (Δ25(27)) and a carbonyl group at C-3. These data of compound **1** corresponded to the data of 31-norcyclolaudenone $C_{30}H_{48}O$ (syn: cyclomusalenone) in the previous reports (Ragasa, *et al.*, 2007; Silva, *et al.*, 2014). As this triterpene possessing Δ25(27) double bond is an isomer of cycloeucalenone (CE-one) which possessing Δ24(28) double bond, we describe 31-norcyclolaudenone as isomer of CE-one (isoCE-one) in the current report.

The specific ^{1}H NMR spectrum of compound **2** showed two broad singlet signals at δ 4.69 ppm (1H) and δ 4.74 ppm (1H) characteristic of a terminal double bond. Two doublet signals at δ 0.42 ppm (1H, J = 4.02 Hz) and δ 0.64 ppm (1H, J = 3.84 Hz) corresponds to a cyclopropane ring present in cycloartane-type triterpene. The ^{13}C NMR spectrum showed 30 carbon signals

including a double bond between C-24 and C-28 (Δ24(28)) and a carbonyl group at C-3. These data of compound **2** corresponded to the data of cycloeucalenone $C_{30}H_{48}O$ (CE-one) in the previous reports (Khuong-Huu, *et al*., 1975; Silva, *et al*., 2014).

The specific ^{1}H NMR spectrum of compound **3** showed a broad singlet signal at δ 4.69 ppm (2H) characteristic of a terminal double bond. Two doublet signals at δ 0.16 ppm (1H, J = 4.02 Hz) and δ 0.40 ppm (1H, J = 3.72 Hz) corresponds to a cyclopropane ring present in cycloartane-type triterpene. And a multiplet signal at δ 3.24 ppm corresponds to the proton at C-3. The ^{13}C NMR spectrum showed 30 carbon signals including a double bond between C-25 and C-27 and a hydroxyl group at C-3. These data are in agreement with previous reports (Akihisa, *et al.*, 1998; Adewusi, 2012) characterize compound **3** as positional-isomer of cycloeucalenol $C_{30}H_{50}O$. As this triterpene possessing Δ25(27) double bond is an isomer of cycloeucalenol (CE-ol) which possessing Δ24(28) double bond, we describe it as isomer of CE-ol (isoCE-ol) in the current report.

The specific ^{1}H NMR spectrum of compound **4** showed two broad singlet signals at δ 4.69 ppm (1H) and δ 4.74 ppm (1H) characteristic of a terminal double bond. Two doublet signals at δ 0.16 ppm (1H, J = 4.02 Hz) and δ 0.40 ppm (1H, J = 3.78 Hz) corresponds to a cyclopropane ring present in cycloartane-type triterpene. And a multiplet signal at δ 3.24 ppm corresponds to the proton at C-3. The ^{13}C NMR spectrum showed 30 carbon signals including a double bond between C-24 and C-28 and a hydroxyl group at C-3. These data are in agreement with previous reports (Liu, *et al*., 2011; Ragasa, *et al*., 2013) characterize compound **4** as cycloeucalenol $C_{30}H_{50}O$ (CE-ol).

The ^{1}H and ^{13}C NMR data of four purified triterpenes were shown in Table 3-1 and Table 3-2. The spectra of 1D and 2D NMR of four compounds were shown in Appendix. After analyzed with ^{1}H and ^{13}C along with COSY, HMQC, HMBC, DEPT 90, DEPT 135 and compared with literature data, the chemical structures of four compounds were obtained and shown in Fig. 3-1. The structural differences of them are the carbonyl group or hydroxy group at C-3 and the position of double bond (Δ25(27) or Δ24(28)) in the side chain.

3.4 Discussion

CE-one and isoCE-one are two isomeric ketones previously identified in different parts of banana plant (Oliveira, *et al*., 2006). These two compounds have been detected in the unripe peel of banana fruit '*Dwarf Cavendish*' as the major triterpenes (Oliveira, *et al*., 2008). And CE-one is the main component identified in the lipophilic extractives of unripe peel of ten *Musa* species, with an abundance ranging from 806 to 9453 mg/kg of dry unripe peels (Villaverde, *et al*., 2013). The major triterpene, CE-one had shown mild cardiotonic effects (Kongkathip, *et al*., 2002), hair restoration effects (Najima, *et al*., 2016), analgesic and anti-inflammatory effects (Lopes, *et al*., 2014). Antimicrobial tests of isoCE-one indicated that it has weak activity against *C. albicans; E. coli, P. aeruginosa*, and *T.mentagrophytes*; and is inactive against *B. subtilis, S. aureus*, and *A. niger* (Ragasa, *et al*., 2007). More importantly, CE-one and isoCE-one have low cytotoxicity (Silva, *et al*., 2014).

CE-ol and isoCE-ol were also identified in traditional medicine plant *Boophone disticha*. A dose-dependent decrease in cell viability of the mixture compound of two positional-isomers was observed using both MTT and neutral red assays. The IC_{50} values of MTT and neutral red assays were 173.0 ± 5.1 μM and 223.0 ± 6.4 μM, respectively, indicating that the low toxicity of the compound (Adewusi, *et al*., 2013). Some other studies also indicated that CE-ol may have potential anti-inflammatory, antihyperglycemic and cardiotonic effects (Akihisa, *et al*., 2000; Ragasa, *et al*., 2013; Kongkathip, *et al*., 2002).

3.5 Conclusion

Based on the data of 1D and 2D NMR and the literatures, compound **1** is 31-norcyclolaudenone (isoCE-one), compound **2** is cycloeucalenone (CE-one), compound **3** is isomer of cycloeucalenol (isoCE-ol), compound **4** is cycloeucalenol (CE-ol). These four compounds are similar compounds. The structural differences of them are the carbonyl group or hydroxy group at C-3 and the position of double bond in the side chain.

3.6 Figures and tables

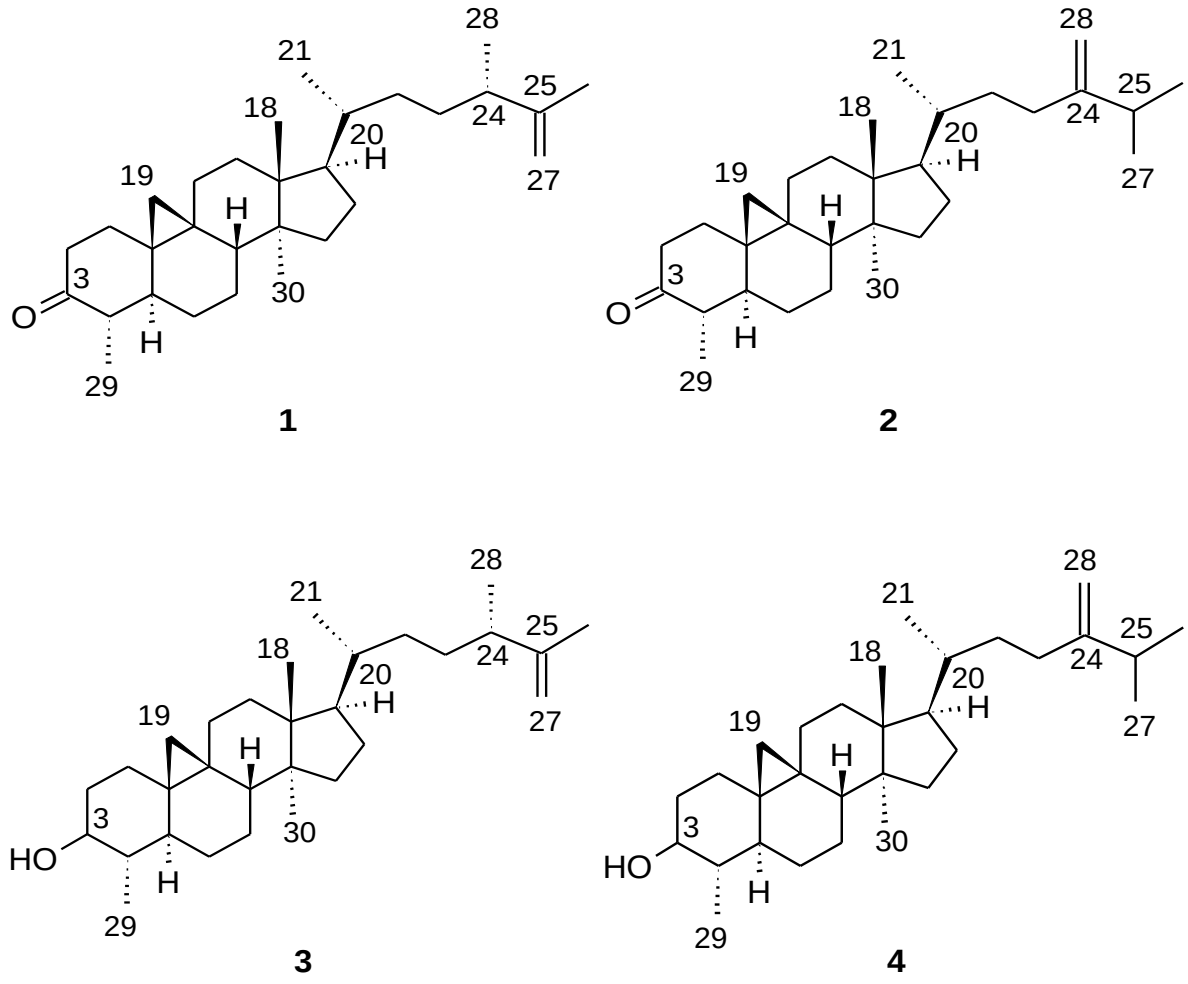

Fig. 3-1 Chemical structures of four triterpenes in banana peel extract.

Note: 31-norcyclolaudenone (isoCE-one, **1**), cycloeucalenone (CE-one, **2**), isomer of cycloeucalenol (isoCE-ol, **3**) and cycloeucalenol (CE-ol, **4**).

Table 3-1 ^{1}H NMR of isoCE-one, CE-one, isoCE-ol and CE-ol

Position	δ_H ppm			
	isoCE-one	CE-one	isoCE-ol	CE-ol
C-1	1.61, 1.89	1.59, 1.90	1.30, 1.57	1.30, 1.57
C-2	2.45 (2H)	2.45 (2H)	1.43, 2.01	1.44, 2.01
C-3	-	-	3.24	3.24
C-4	2.26	2.26	1.19	1.20
C-5	1.60	1.60	1.20	1.23
C-6	0.76, 1.72	0.77, 1.73	0.60, 1.70	0.61, 1.70
C-7	1.12, 1.38	1.14, 1.38	1.30, 1.90	1.32, 1.95
C-8	1.66	1.67	1.60	1.60
C-9	-	-	-	-
C-10	-	-	-	-
C-11	1.27, 2.06	1.28, 2.06	1.07, 1.33	1.09, 1.33
C-12	1.68 (2H)	1.69 (2H)	1.63 (2H)	1.65 (2H)
C-13	-	-	-	-
C-14	-	-	-	-
C-15	1.32 (2H)	1.34 (2H)	1.30 (2H)	1.32 (2H)
C-16	1.32, 1.93	1.34, 1.97	1.22, 2.00	1.23, 2.01
C-17	1.61	1.65	1.59	1.62
C-18	1.02 (3H)	1.03 (3H)	0.98 (3H)	0.99 (3H)
C-19	0.42, 0.64	0.42, 0.64	0.16, 0.40	0.17, 0.41
C-20	1.32	1.44	1.30	1.42
C-21	0.89 (3H)	0.93 (3H)	0.88 (3H)	0.92 (3H)
C-22	0.95, 1.36	1.19, 1.60	0.95, 1.36	1.17, 1.60
C-23	1.19, 1.48	1.92, 2.15	1.18, 1.45	1.91, 2.15
C-24	2.13	-	2.12	-
C-25	-	2.26	-	2.26
C-26	1.66 (3H)	1.05 (3H)	1.66 (3H)	1.05 (3H)
C-27	4.69 (2H)	1.06 (3H)	4.69 (2H)	1.06 (3H)
C-28	1.03 (3H)	4.69, 4.74	1.02 (3H)	4.69, 4.74
C-29	1.01 (3H)	1.01 (3H)	1.00 (3H)	1.01 (3H)
C-30	0.92 (3H)	0.94 (3H)	0.90 (3H)	0.92 (3H)

Note: The symbol "-" represents no proton.

Table 3-2 ^{13}C NMR of four compounds and comparison with references

Position	δ_C ppm							
	isoCE-one	31-norcyclolaudenone[a]	CE-one	cycloeucalenone[b]	isoCE-ol	isomer of cycloeucalenol[c]	CE-ol	cycloeucalenol[d]
C-1	32.8	32.8	32.8	32.8	30.8	31.0	30.8	30.8
C-2	41.0	41.0	41.0	40.8	34.8	35.0	34.8	34.8
C-3	213.5	213.3	213.5	212.2	76.6	76.8	76.6	76.6
C-4	50.0	50.0	50.0	49.8	44.6	44.8	44.6	44.6
C-5	46.1	46.1	46.1	45.9	43.3	43.6	43.3	43.3
C-6	25.9	25.9	25.9	25.8	24.7	24.9	24.7	24.7
C-7	25.2	25.2	25.2	25.1	28.1	28.3	28.1	28.1
C-8	47.1	47.1	47.1	46.9	46.9	47.1	46.9	46.9
C-9	25.0	25.0	25.0	24.9	23.6	23.8	23.5	23.5
C-10	29.3	29.7	29.3	29.3	29.5	29.8	29.5	29.5
C-11	27.2	27.2	27.2	26.9	25.2	25.4	25.2	25.2
C-12	32.9	32.9	32.9	32.8	35.4	35.6	35.4	35.3
C-13	45.4	45.3	45.4	45.2	45.3	45.6	45.3	45.3
C-14	48.8	48.8	48.8	48.7	48.9	49.1	48.9	48.9
C-15	35.5	35.6	35.4	35.3	32.9	33.1	32.9	32.9
C-16	28.1	28.0	28.1	28.0	27.0	27.2	27.0	27.0
C-17	52.2	52.2	52.2	52.1	52.2	52.4	52.2	52.2
C-18	18.0	17.9	18.0	17.9	17.8	18.0	17.8	17.8
C-19	27.0	26.9	27.0	27.1	27.3	27.5	27.3	27.2
C-20	36.1	36.0	36.1	36.0	36.1	36.4	36.1	36.1
C-21	18.4	18.3	18.3	18.3	18.4	18.6	18.3	18.3
C-22	33.9	33.9	34.9	35.0	34.0	34.0	35.0	35.0
C-23	31.5	31.5	31.3	31.3	31.5	31.7	31.3	31.3
C-24	41.7	41.6	156.9	156.1	41.7	41.8	156.9	156.9
C-25	150.5	150.2	33.8	33.7	150.5	150.5	33.8	33.8
C-26	18.7	18.6	22.0	21.8	18.6	18.9	22.0	22.0
C-27	109.5	109.4	21.9	21.8	109.4	109.6	21.9	21.9
C-28	20.2	20.2	106.0	105.6	20.2	20.1	105.9	105.9
C-29	10.8	10.7	10.8	10.7	14.4	14.6	14.4	14.4
C-30	19.2	19.2	19.2	19.1	19.1	19.4	19.1	19.1

Note: The reference data of four compounds were from literatures (a: Ragasa, *et al*., 2007; b: Khuonghuu, *et al*., 1975; c: Adewusi, 2012; d: Liu, *et al*., 2011).

Chapter 4 Carbohydrate-digesting enzymes inhibitory activities of four purified triterpenes *in vitro*

4.1 Introduction

Controlling plasma glucose level is essential to delay or prevent DM. One possible way to decrease the blood sugar absorption rate from the small intestine is to slow or interrupt the digestion of carbohydrate, the major dietary source of glucose (Josse, *et al*., 2003). The inhibition of enzymes that digest carbohydrate, such as α-glucosidase and α-amylase have been studied as a method to control blood sugar levels (Ali, *et al*., 2006; Svensson, *et al*., 2004).

α-Glucosidase is a membrane-bound enzyme located at the epithelium of the small intestine, which catalyzes the cleavage of glucose from disaccharides and oligosaccharides (Hamid, *et al*., 2015). α-Amylase catalyzes the hydrolysis of α - (1, 4) - glycosidic linkages to produce maltose and glucose, whereas α-glucosidase releases glucose from maltose and/or sucrose (Sogaard-Andersen & Valentin-Hansen, 1993; Teeri, 1991). So, absorption of glucose into the bloodstream can be delayed by inhibiting these enzymes, and then ameliorating DM symptoms such as hyperglycemia.

The final concentration of sample required to inhibit 50% of the α-glucosidase and α-amylase activity under the specified condition was described as IC_{50}. The IC_{50} value of four triterpenes were measured and compared. Then the structure-activity relationships of four triterpenes were investigated. In addition, the kinetic analysis of CE-one in α-glucosidase inhibitory assay had been elucidated.

4.2 Materials and methods

4.2.1 Chemicals

Acarbose hydrate (>98.0%) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Soluble starch was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). *p*-Nitrophenyl-α-D-glucopyranose (*p*-NPG), α-glucosidase from yeast, and α-

amylase from porcine pancreas were purchased from Sigma-Aldrich (Darmstadt, Germany).

4.2.2 Inhibitory assay against α-glucosidase

The α-glucosidase inhibition assay was conducted according to previous report (Asghari, *et al*., 2015) with slight modifications. Potassium phosphate buffer (100 mM, pH 7.0) (50 μL) and samples (10 μL) with varying concentrations (four purified compounds: 0, 40, 80, 120, 160, 200, 240, 300 μg/mL; acarbose: 0, 400, 800, 1200, 1600, 2000, 2400, 3000 μg/mL) dissolved in 50% MeOH were mixed prior to the addition of 20 μL of yeast α-glucosidase (0.1 U/mL) (As). The sample replaced by 50% MeOH was used as control (Ac). The enzyme replaced by potassium phosphate buffer (100 mM, pH 7.0) was used as blank of samples (Bs) or blank of control (Bc). The mixture was pre-incubated at 37˚C for 15 min. Addition of 20 μL (1.0 mM) *p*-NPG, as the substrate marked the initiation of the reaction. The reaction was carried out with further incubation at 37˚C for 15 min and terminated by adding 50 μL of sodium carbonate (200 mM). Enzyme activity was determined by measuring the absorbance at 405 nm of the *p*-nitrophenol liberated from *p*-NPG with a microplate reader (Thermomax; Molecular Devices, Sunnyvale, USA). Acarbose was used as positive control. The α-glucosidase inhibitory activity was calculated using the following formula: Inhibitory rate (%) =100 × [(Ac-Bc)-(As-Bs)] / (Ac-Bc).

4.2.3 Inhibitory assay against α-amylase

The α-amylase inhibition assay was conducted using the previous method (Xiao, *et al*., 2006) with modifications. After 30 μL of sodium phosphate buffer (20 mM, pH 6.9) and 10 μL of diverse concentrations (four purified compounds: 0, 40, 80, 120, 160, 200, 240, 300 μg/mL; acarbose: 0, 10, 30, 50, 70, 100, 150 μg/mL) of test samples dissolved in 50% aqueous MeOH were mixed (As) and the 50% MeOH used as control (Ac), 30 μL of 0.1 U/mL α-amylase dissolved in sodium phosphate buffer was added and incubated at 37˚C for 10 min. The enzyme replaced by sodium phosphate buffer was used as blank of sample (Bs) or blank of control (Bc). Then, 30 μL of 0.5 mg/mL starch solution dissolved in sodium phosphate buffer was added and incubated at 37˚C for 10 min. The reaction was stopped by adding 50 μL of 5 mM iodine

contained 1 mM HCl solution. The absorbance was measured at 590 nm to detect remaining starch by a microplate reader (Thermomax; Molecular Devices, Sunnyvale, USA). Acarbose was used as positive control. The α-amylase inhibitory activity was calculated using the following formula: Inhibitory rate (%) =100 × [(Bc-Ac)-(Bs-As)] / (Bc-Ac).

4.2.4 Kinetic analysis of CE-one in α-glucosidase inhibitory assay

To describe the inhibition type of CE-one in α-glucosidase inhibitory assay, the Lineweaver-Burk equation in double reciprocal form was expressed as followed equations (1) and (2) (Yan, *et al*., 2014):

$$\frac{1}{v} = \frac{K_m}{V_{max}}\left[1 + \frac{[I]}{K_i}\right]\frac{1}{[S]} + \frac{1}{V_{max}}\left[1 + \frac{[I]}{\alpha K_i}\right] \quad (1)$$

The secondary plot for calculating K_i can be written as:

$$\text{slope} = \frac{K_m}{V_{max}} + \frac{K_m[I]}{V_{max}K_i} \quad (2)$$

Where v is the enzyme reaction rate in the absence and presence of sample. K_i and K_m are the inhibition constant and Michaelis-Menten constant, respectively. α is the ratio of the uncompetitive inhibition constant to competitive inhibition constant. [I] and [S] are the concentrations of inhibitor and substrate, respectively.

However, these equations are only valid for the linear relationship, K_i cannot be determined directly from the usual equations when the parabolic relationship between slope and [I]. Thus, a modified equation (3) was applied with the modification of usual equations (Zeng, *et al*., 2012):

$$\ln(\text{slope}) = \left(\frac{K_m}{V_{max}}\right)\frac{1}{K_i}[I] + \left(\frac{K_m}{V_{max}}\right) \quad (3)$$

Where the ln(slope) is plotted versus the corresponding inhibitor. The plot has a slope of (K_m/V_{max}) × 1/K_i when the plot is produced linearly. The value of K_i can be obtained from the above equation.

All data are expressed as mean ± standard deviation (M ± SD, n = 3). The data were analyzed by independent-samples Student's t-test with SPSS 22.0 software (IBM Corporation, New York, USA).

4.3 Results

4.3.1 α-Glucosidase inhibitory activity of four triterpenes

It was found that CE-one and isoCE-one inhibited α-glucosidase activity in a concentration-dependent manner. With the increasing concentration of acarbose, CE-one and isoCE-one, α-glucosidase inhibitory activity increased remarkably (Fig. 4-1A, 4-1B and 4-1C). In contrast, the results of both CE-ol and isoCE-ol showed no concentration-dependent manner in α-glucosidase inhibitory activity (Fig. 4-1D and 4-1E). The IC_{50} values of acarbose, CE-one and isoCE-one were estimated to be 295.43 ± 4.13 μM, 31.83 ± 2.46 μM, and 38.85 ± 1.54 μM, respectively (Table 4-1). CE-ol and isoCE-ol cannot get the IC_{50} value under the concentration from 4 to 30 μg/mL (below 10%). The IC_{50} value of CE-ol has been reported to be more than 1000 μM while the acarbose is 884.6 μM in α-glucosidase inhibitory assay (Srisurichan & Pornpakakul, 2015). Compared with acarbose, IC_{50} values of CE-one and isoCE-one showed significant difference in α-glucosidase inhibitory activity ($p < 0.05$) (Table 4-1). CE-one and isoCE-one exhibited higher inhibitory activity than acarbose. Compared with isoCE-one, IC_{50} value of CE-one showed the significant difference ($p < 0.05$) (Table 4-1). CE-one exhibited higher inhibitory activity than isoCE-one in α-glucosidase.

4.3.2 α-Amylase inhibitory activity of four triterpenes

It was also found that CE-one and isoCE-one inhibited α-amylase activity in a concentration-dependent manner. With the increasing concentration of acarbose, CE-one and isoCE-one, α-amylase inhibitory activity increased prominently (Fig. 4-2A, 4-2B and 4-2C). On the contrary, the results of both CE-ol and isoCE-ol also showed no concentration-dependent manner in α-amylase inhibitory activity (Fig. 4-2D and 4-2E). The IC_{50} values of acarbose, CE-one and isoCE-one were estimated to be 10.27 ± 1.16 μM, 20.33 ± 0.59 μM and 27.63 ± 0.83 μM, respectively (Table 4-2). CE-ol and isoCE-ol cannot get the IC_{50} value under the concentration from 4 to 30 μg/mL (below 10%). Compared with acarbose, IC_{50} values of CE-one and isoCE-one showed significant difference in α-amylase inhibitory activity ($p < 0.05$) (Table 4-2). CE-one and isoCE-one showed lower inhibitory activity than acarbose. Compared

with isoCE-one, IC_{50} value of CE-one also showed significant difference ($p < 0.05$) (Table 4-2). CE-one exhibited higher inhibitory activity than isoCE-one in α-amylase.

4.3.3 Results of kinetic analysis of CE-one in α-glucosidase inhibitory assay

Lineweaver-Burk plots of CE-one in the α-glucosidase inhibitory activity were constructed, as displayed in Fig. 4-3. The apparent V_{max} and K_m both appeared to change concurrently by various concentrations of CE-one indicating that CE-one induced mixed-type inhibition of α-glucosidase (Fig. 4-3A). The secondary plot of slope versus concentration of the inhibitor [CE-one] got a parabolic curve (Fig. 4-3B) indicating that CE-one induced a parabolic mixed-type inhibition. By using equation (2), the K_i value was calculated as 73.86 μM (Fig. 4-3C).

4.4 Discussion

Based on the structure and activity of these four triterpenes, our results indicated that the carbonyl ketone at C-3 is crucial for their α-glucosidase and α-amylase inhibitory activities. And the position of double bond in the side chain can also affect these inhibitory activities.

There are several reports on plant-derived triterpenes inhibitory activity against carbohydrate digestive enzymes (Nazaruk & Borzym-Kluczyk, 2015). Regarding lupane-type triterpenes in other studies, α-glucosidase and α-amylase inhibitory activities of lupenone, which possesses a carbonyl ketone at C-3 (IC_{50}: 88.62 μM and 108.61 μM, respectively) were stronger than those of lupeol, which possesses a hydroxy group at C-3 (IC_{50}: 90.86 μM and 116.19 μM, respectively), with acarbose IC_{50} values of 44.88 μM and 42.67 μM, respectively (Ochieng, *et al*., 2020). Our results agree with those of other studies, showing that the carbonyl ketone at C-3 is important for α-glucosidase and α-amylase inhibitory activities (Pujirahayu, *et al*., 2019; Yonemoto, *et al*., 2014). More interestingly, based on the comparison of IC_{50} values, lupenone has a marginally stronger (less than two times) α-glucosidase inhibitory activity than lupeol, while CE-one showed a significantly stronger inhibitory activity (more than 10 times) than CE-ol. It has been reported that lupenone in banana peel is a candidate possessing potent antidiabetic activity in alloxan-induced diabetic mice (Wu, *et al*., 2015). However, our bioassay-guided fractionation did not identify lupenone in banana peel extract as an inhibitor

of α-glucosidase and α-amylase. Our results provide no information on the content of lupenone in our material, however, CE-one and isoCE-one are the major inhibitors in banana peel based on these results.

In addition, our results revealed the contribution of double bond in the side chain (C21-C28) of the triterpenes to their inhibitory properties. CE-one Δ24(28) exhibited higher inhibitory activities against both α-glucosidase and α-amylase than isoCE-one Δ25(27). Our results are consistent with those of cycloartane-type triterpene acids, in which the position of the double bond in the side chain is also important for α-glucosidase inhibitory activity (Pujirahayu, *et al*., 2019). The effects of the double bond position were not elucidated in this study. However, the proton NMR data of the two purified triterpenes showed different signal patterns for paired geminal protons on each double bond. Regarding the double bond of CE-one, two separated signals at δ 4.69 ppm (1H) and δ 4.74 ppm (1H) were identified. However, one overlapped signal at δ 4.69 ppm (2H) were identified from isoCE-one. If the overlapped signals of isoCE-one are derived from two exchangeable protons on the terminal double bond in the chemical environment, the conformation of the side chain will be more flexible. These data suggest that moderately restricted flexibility of the side chain is effective in enhancing triterpene inhibitory activity against carbohydrate digestive enzymes. Further experiments, with three-dimensional analysis, are required to elucidate the role of terminal double bond in the triterpene inhibition mechanism against carbohydrate digestive enzymes.

In the previous reports on α-glucosidase inhibitors, several triterpene acids, such as ursolic acid, corosolic acid and oleanolic acid, are known to show uncompetitive or non-competitive modes of inhibition (Salah, *et al*., 2014; Hou, *et al*., 2009; Zhang, *et al*., 2017). The inhibition mode of lupenol against α-glucosidase is non-competitive (Ramu, *et al*., 2014), and that of lupenone against α-amylase is a mixed type (Yonemoto, *et al*., 2014). Our results using CE-one possessing a carbonyl ketone at C-3 demonstrated mixed-type inhibition against α-glucosidase. This suggests that the C-3 ketonic triterpenes have more complex properties than triterpene acids in carbohydrate digestive enzyme inhibition.

To the best of our knowledge, the present study is the first report about the inhibitory activity of and active sites for CE-one on α-glucosidase and α-amylase. This study indicates a potential application of banana peel-derived triterpenes as antidiabetic agents.

4.5 Conclusion

CE-one showed higher α-glucosidase and α-amylase inhibitory activities than that of isoCE-one. In contrast, CE-ol and isoCE-ol had insufficient inhibitory activity against either enzyme. The IC_{50} values of CE-one and isoCE-one on α-glucosidase were 31.83 ± 2.46 μM and 38.85 ± 1.54 μM, respectively. And their IC_{50} values on α-amylase were 20.33 ± 0.59 μM and 27.63 ± 0.83 μM, respectively. The main active sites of CE-one and isoCE-one are the carbonyl group at C-3 and double bond in the side chain. CE-one induced a parabolic mixed-type inhibition with the K_i value of 73.86 μM in α-glucosidase inhibitory assay.

4.6 Figures and tables

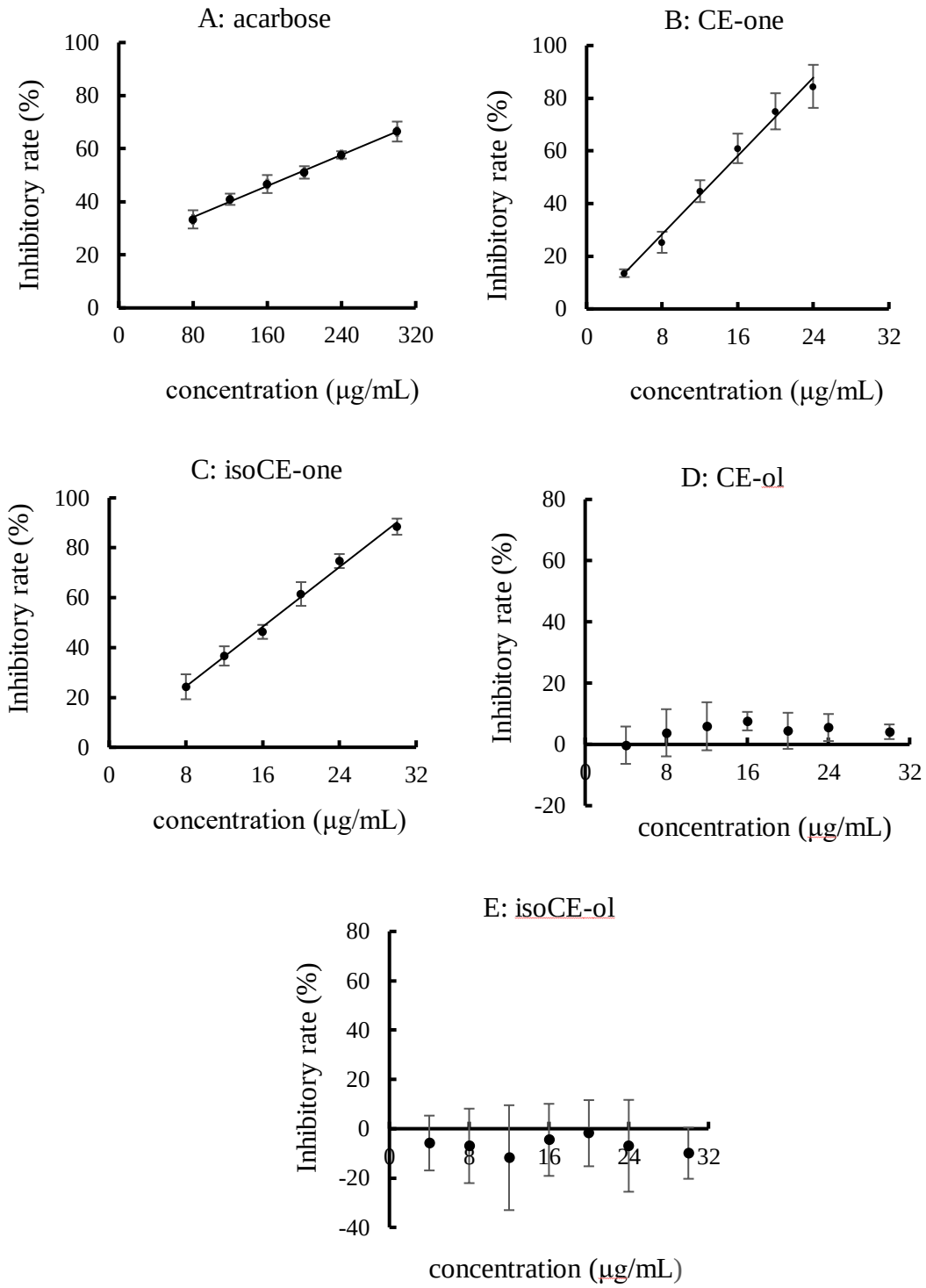

Fig. 4-1 Inhibitory effects of acarbose and four purified triterpenes on α-glucosidase.

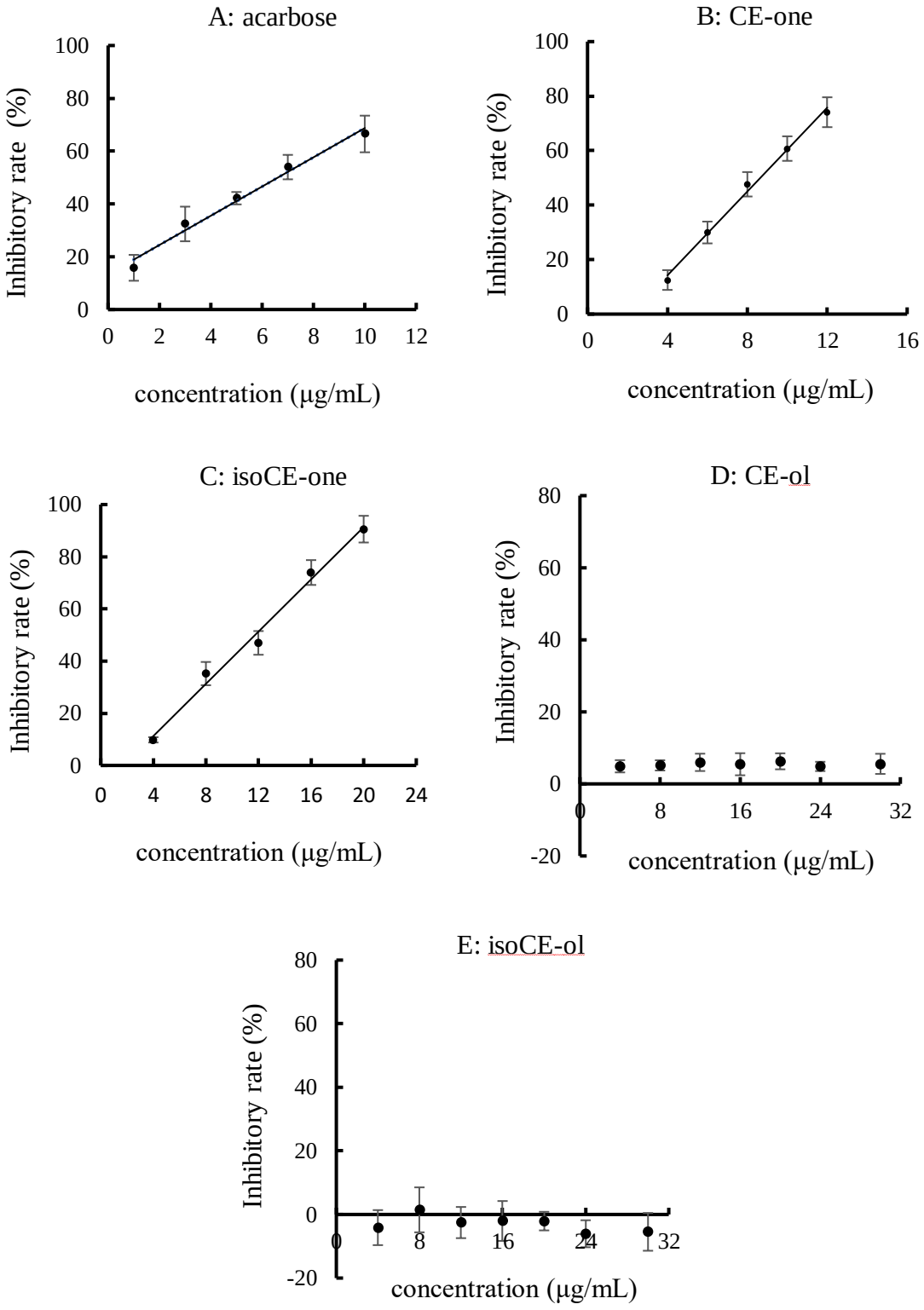

Fig. 4-2 Inhibitory effects of acarbose and four purified triterpenes on α-amylase.

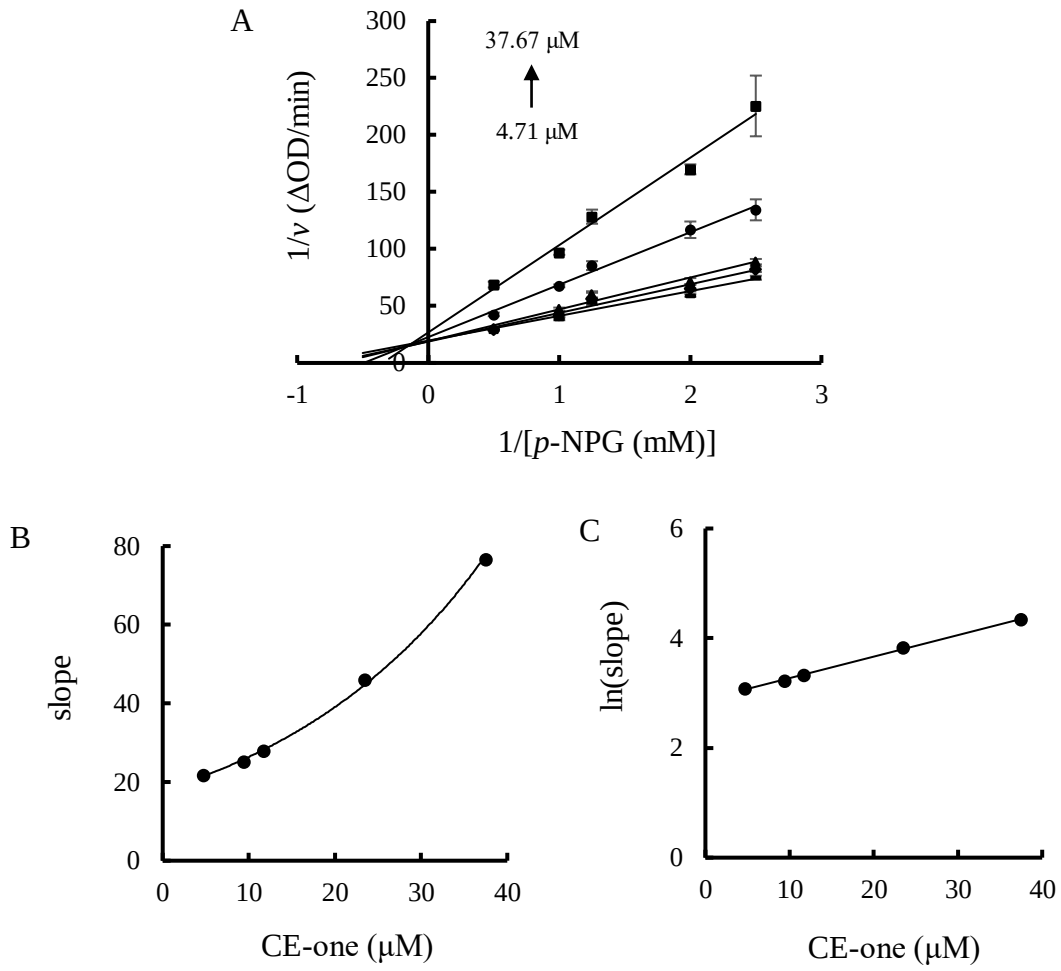

Fig. 4-3 Kinetic analysis of α-glucosidase inhibition by CE-one.

Lineweaver-Burk plots for α-glucosidase (0.1 U/mL) with various concentrations of CE-one with 4.71 (▂), 9.42 (◆), 11.77 (▲), 23.55 (●), and 37.67 (■) μM (Panel A). The secondary plot of slope versus [CE-one] (Panel B). The secondary plot of parabolic type of inhibition applied with Eq. (2) in the Materials and methods (Panel C).

Table 4-1 The IC_{50} of four compounds in α-glucosidase inhibition assay.

Compound	α-glucosidase (μM)
cycloeucalenone (CE-one)	$31.83\pm2.46^{**,\#\#}$
31-norcyclolaudenone (isoCE-one)	$38.85\pm1.54^{**}$
cycloeucalenol (CE-ol)	-
isomer of cycloeucalenol (isoCE-ol)	-
acarbose	295.43±4.13

Note: All data are expressed as mean ± standard deviation (M ± SD, n = 3). The symbol "-" represents cannot estimate IC_{50}. The symbol "*" represents the difference between samples (CE-one and isoCE-one) and positive control (acarbose) (*: $p < 0.05$; **: $p < 0.01$). The symbol "#" represents the difference between CE-one and isoCE-one (#: $p < 0.05$; ##: $p < 0.01$).

Table 4-2 The IC_{50} of four compounds in α-amylase inhibition assay.

Compound	α-amylase (μM)
cycloeucalenone (CE-one)	$20.33\pm0.59^{**,\#\#}$
31-norcyclolaudenone (isoCE-one)	$27.63\pm0.83^{**}$
cycloeucalenol (CE-ol)	-
isomer of cycloeucalenol (isoCE-ol)	-
acarbose	10.27±1.16

Note: All data are expressed as mean ± standard deviation (M ± SD, n = 3). The symbol "-" represents cannot estimate IC_{50}. The symbol "*" represents the difference between samples (CE-one and isoCE-one) and positive control (acarbose) (*: $p < 0.05$; **: $p < 0.01$). The symbol "#" represents the difference between CE-one and isoCE-one (#: $p < 0.05$; ##: $p < 0.01$).

Chapter 5 Antihyperglycemic activity of cycloeucalenone *in vivo*

5.1 Introduction

In the previous chapter, CE-one had shown the highest α-glucosidase and α-amylase inhibitory activities *in vitro*. But the effect of CE-one *in vivo* still unclear. In this chapter, the carbohydrate-loading tests of CE-one in normal mice and the antihyperglycemic activity of CE-one in STZ-induced diabetic mice were investigated.

5.2 Materials and methods

5.2.1 Chemicals

Maltose monohydrate, D (+)-glucose and DMSO were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Acarbose hydrate was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Soluble starch, streptozotocin (STZ) and carboxymethyl cellulose sodium salt (CMC) were bought from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Metformin was bought from Sanwa Kagaku Kenkyusho Co., Ltd. (Nagoya, Japan).

5.2.2 Animals

The male ddY mice (6 weeks old) and male ICR mice (7 weeks old) were purchased from SLC, Inc. (Hamamatsu, Japan). They were raised at 24 ± 1˚C, 50 ± 10% humidity and a 12 h light-dark cycle condition (lights on from 8:00 A.M. to 8:00 P.M.). The mice had free access to water and standard food and acclimated for one week. The animal experiments were done according to the guidelines for animal experiment of Tokyo University of Marine Science and Technology with the approval of the animal care and use committee of Tokyo University of Marine Science and Technology.

5.2.3 Carbohydrate-loading tests of CE-one in normal mice

5.2.3.1 Oral maltose and starch tolerance tests in normal mice

After fasting for 20 h, three groups (n = 5) of ddY mice were given different dose of CE-one (20, 40, and 80 mg/kg body weight) by oral administration, 30 min later, maltose or starch (2 g/kg body weight) was given to mice with the same method. Before given CE-one, collect blood from tail as 0 min, after given maltose or starch collect blood in small tubes with heparin sodium at 30, 60, 90, and 120 min, respectively. One present of CMC was used for control group (n = 5). Acarbose (40 mg/kg body weight) was used for positive control group (n = 5). After blood was centrifuged at 8000 rpm for 3 min (Centrifuge MCD-2000, AS ONE, Japan), the plasma was obtained. The blood glucose level was measured by commercial assay kit (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) with a microplate reader (Thermomax; Molecular Devices, Sunnyvale, USA). The area-under-the-concentration-curve (AUC) as difference from initial value of each group was calculated with the trapezoidal rule. And the AUC value (% of control) was obtained.

5.2.3.2 Oral glucose tolerance test in normal mice

After fasting for 20 h, two groups of ddY mice (n = 6) were given CE-one at dose of 40 and 80 mg/kg body weight, respectively, together with glucose (2 g/kg body weight) by oral administration. Before given CE-one and glucose, collect blood from tail as 0 min, after given collect blood in small tubes with heparin sodium at 30, 60, 90, and 120 min, respectively. Control group (n = 6) was given 1% CMC together with glucose. The blood glucose level of each group was measured with the same method as above. After given CE-one and glucose, collect the feces of each group (0 - 2 h and 2 - 6 h). After feces were dried and the feces total weight of each group was obtained. The feces were first dissolved in 2 mL water, then extracted with same volume of EtOAc for 3 times. After collecting the EtOAc layer, it was concentrated to dry, then the CE-one in the feces was analyzed by proton NMR.

5.2.4 Antihyperglycemic activity of CE-one in STZ-induced diabetic mice

After fasting for 20 h, ICR mice were injected with 100 mg/kg STZ by single intraperitoneal injection (STZ dissolved in citrate buffer, pH = 4.3, prepare freshly and inject within 20 min). Normal control group was injected with citrate buffer (Group 1, NC, n = 5). Two weeks later, measure the fasting blood glucose (more than 200 mg/dL means diabetes).

The diabetic mice were divided into 4 groups randomly (n = 5). Group 2: Diabetes control (DC); Group 3: Low dose of CE-one, 20 mg/kg body weight (CL); Group 4: High dose of CE-one, 40 mg/kg body weight (CH); Group 5: Positive control group, metformin, 250 mg/kg body weight (PC). The samples were given to mice by oral administration every day (Normal control group and diabetes control group were given 1% CMC). The experiment continued for 5 weeks. The fasting blood glucose (FBG), body weight, food intake and water consumption were measured every week.

Finally, after fasting for 12 h, the mice were sacrificed. The blood was harvested and centrifuged at 8000 rpm for 3 min to obtain plasma (stored at -60˚C for further biochemical analysis). The weight of liver and kidneys were measured, and small part of liver was used to measure glycogen according to previous method (Hideo, *et al*., 2012). Fasting plasma insulin (FIN) was determined by using an ELISA kit (Morinaga, Yokohama, Japan). Total cholesterol (TC), triglyceride (TG), alanine transaminase (ALT) and aspartate transaminase (AST) were determined by commercial assay kit (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan). All the above indicators were operated according to the guidelines of the manufacturer. The kidney index and liver index were calculated as below:

Kidney, liver index (%) = 100 × weight of kidneys, liver (g) / body weight (g).

All data are expressed as mean ± standard error (M ± SE, n = 5 - 6). The experimental data were analyzed by homogeneity of variance and Duncan multiple comparisons of analysis of variance (ANOVA) with SPSS 22.0 software (IBM Corporation, New York, USA).

5.3 Results

5.3.1 Results of carbohydrate-loading tests of CE-one in normal mice

5.3.1.1 Oral maltose tolerance test (OMTT)

As shown in Fig. 5-1A, CE-one can decrease the blood glucose level in dose dependence manner. The dose of CE-one at 40 mg/kg showed 17.8% decrease of blood glucose compared with control group at 30 min. The dose of CE-one at 80 mg/kg showed 26.7% decrease of blood glucose which is similar with positive control, acarbose, with 23.3% decrease. The results of

AUC value showed that there is no significant difference between the low and medium dose group of CE-one (20 and 40 mg/kg) and control group. But high dose group of CE-one (80 mg/kg) and positive control (acarbose) both showed significant difference with control group (Fig. 5-1B). These results indicated that CE-one can inhibit the α-glucosidase activity in normal mice and suppress the blood glucose level.

5.3.1.2 Oral starch tolerance test (OSTT)

As shown in Fig. 5-2A, CE-one can also decrease the blood glucose level in dose dependence manner. The dose of CE-one at 40 and 80 mg/kg showed 33.4% and 39.2% decrease of blood glucose compared with control group at 30 min, respectively. The dose of CE-one at 80 mg/kg showed similar effect with positive control, acarbose which is 40.4% decrease. The results of AUC value showed that there is no significant difference between the low dose of CE-one (20 mg/kg) and control group. But medium and high dose group of CE-one (40 and 80 mg/kg) and positive control (acarbose) showed significant difference with control group (Fig. 5-2B). These results indicated that CE-one can inhibit the α-amylase activity in normal mice and reduce the blood glucose level.

5.3.1.3 Oral glucose tolerance test (OGTT)

As shown in Fig. 5-3A, compared with control, CE-one showed no suppression on the blood glucose at dose of 40 mg/kg body weight. In contrast, CE-one can reduce the blood glucose level at dose of 80 mg/kg, with a 13.6% decrease at 30 min and a 19.9% decrease at 60 min compared with control. The results of AUC value also showed that there is no significant difference at 40 mg/kg, but a significant difference at 80 mg/kg group when compared with control group (Fig. 5-3B). These results indicated that CE-one maybe can also inhibit the absorption of glucose in normal mice and lower the blood glucose level. More interestingly, after given CE-one to mice, the feces weight of 40 mg/kg group and 80 mg/kg group increased compared with control (Table 5-1). And CE-one in the feces cannot be detected within 2 h but can be detected during 2 - 6 h (Table 5-1). These results indicated that CE-one maybe can stimulate the feces excretion and it needs more than 2 h for metabolism *in vivo*. The detailed mechanisms are not elucidated in this study because of the limited time. Further studies about the mechanisms need to be carried on in the future.

5.3.2 Results of antihyperglycemic activity of CE-one in STZ-induced diabetic mice

At the end of the experiment, the body weight of NC group was 47.92 ± 0.82 g while DC group showed a significant weight loss (Table 5-2). Compared with DC group, the body weight of CL, CH and PC groups showed no difference. These results indicated that CE-one and metformin cannot improve the condition of body weight. The food intake and water consumption of diabetes control group showed significant increase when compared with NC group with 6.41 ± 0.32 g and 5.62 ± 0.28 g, respectively. Compared with DC group, the food intake and water consumption of CL, CH, and PC groups showed significant decrease (Table 5-2). The fasting blood glucose of NC group was 66.04 ± 3.67 mg/dL while the DC group was 405.08 ± 66.87 mg/dL. The blood glucose level of PC group showed significant decrease. After given CE-one, the blood glucose level of CL, CH groups also significantly decreased (Table 5-2). These results indicated that CE-one can improve the diabetes condition with less food intake and water consumption.

As shown in Table 5-3, the kidney index and liver index of DC group showed significant increase compared with NC group. After given CE-one, the liver index decreased while the kidney index showed no difference compared with DC group. However, both kidney index and liver index of PC group showed no difference compared with DC group. This result indicated that the positive control, metformin maybe cannot improve the liver and kidney conditions. But CE-one can improve liver condition while it has no effect on kidneys.

As shown in Table 5-4, compared with NC group, the insulin level and glycogen content of DC group were decreased significantly. CE-one and metformin had no effect on the insulin level but can increase the glycogen content in diabetic mice. Compared with NC group, both ASL level and ALT level of DC group were increased significantly. After given CE-one, the ASL level decreased significantly while ALT level had no change compared with DC group (Table 5-4). The PC group showed the similar results. These results indicated that CE-one can improve AST level of liver and increase the content of liver glycogen while have no effect on insulin level.

As shown in Table 5-5, the TC level of DC and PC groups showed no difference with NC group. Compared with DC group, the TC level of CL and CH groups showed significant

decrease. The TG level of DC group showed significant decrease compared with NC group. After given CE-one, the TG levels of CL and CH groups showed no difference with DC group. In addition, the TG level of PC group also showed no difference compared with DC group. These results indicate that CE-one can reduce the TC level but has no effect on TG level in diabetic mice.

5.4 Discussion

α-Glucosidase and α-amylase are enzymes that can hydrolyze maltose and starch, releasing glucose. Therefore, blood glucose level after oral administration of maltose or starch indirectly reflects the activities of α-glucosidase or α-amylase. Our results of OMTT and OSTT suggested that oral administration of CE-one significantly restrained postprandial hyperglycemia in normal mice. These are in accordance with our *in vitro* results in chapter 4. One report showed that the similar compound, isoCE-one can inhibit the α-glucosidase and α-amylase activities and inhibit the formation of advanced glycation end products in a BSA-fructose model (Sheng, *et al*., 2017). The results of OGTT also showed CE-one can suppress the blood glucose level at dose of 80 mg/kg. This indicated that CE-one maybe can also inhibit the glucose absorption in normal mice. Regulation of glucose uptake is critical for treatment of metabolic syndrome as well as diabetes. Some reports showed that ursane-type triterpenoids, such as corosolic acid, ilekudinol B, ursolic acid and pomolic acid, from *Weigela subsessilis*, might enhance glucose uptake by acting as insulin mimics and as insulin sensitizers (Lee & Thuong, 2010). Besides, regulation of glucose transporter gene expression is an important mechanism in the regulation of glucose uptake in animal cells. Glucose transport in adipocytes is regulated by facilitative glucose transporters, GLUT1 and GLUT4 (Scheepers, *et al*., 2004). Pachymic acid, one of the lanostane-type triterpenoids, can stimulate glucose uptake, GLUT4 gene expression and translocation (Huang, *et al*., 2010). More interestingly, CE-one can increase the amount of feces. The mechanism remains unclear. Maybe it can stimulate the intestinal cells movement or inhibit the lipid absorption then increase the frequency of defecation. More experiments need to be done to clarify the detailed mechanisms in the future.

During treatment of samples for 5 weeks, FBG, food intake and water consumption of

STZ-induced diabetic mice were significantly higher than those of normal mice, while body weight was prominently lower than that of normal mice. These characteristics were similar to the characteristics of hyperglycemia, weight reduction, polydipsia and polyphagia in patients with diabetes (Ashcroft & Rorsman, 2012). After five weeks treatment of CE-one, FBG, food intake and water consumption significantly decreased compared with DC group while body weight remains no change. These results indicated the diabetes condition was improved by CE-one.

Liver plays a crucial role in energy balance because it is one of the most important places to regulate glucose metabolism and maintain a dynamic balance of the blood glucose concentration (Maleki, *et al*., 2019). It is the main site for regulating glucose absorption, glycogen synthesis, and gluconeogenesis and makes a momentous role in maintaining blood glucose metabolism and energy balance (Han, *et al*., 2019). Consequently, the degree of liver injury and the amount of liver glycogen synthesis indirectly reflect the insulin activity and glucose metabolism ability of the body (Ren, *et al*., 2019). Generally, the levels of AST and ALT are detected together to evaluate the liver damage (Hanley, *et al*., 2004). In this study, the contents of AST and ALT in plasma and liver index of diabetic mice were significantly increased, indicating that hyperglycemia led to severe liver injury, and the content of liver glycogen was significantly reduced in comparison to that of normal mice. However, five weeks of CE-one treatment significantly improved liver index, prominently reduced the AST level in plasma and significantly increased the content of liver glycogen in diabetic mice, thereby indicating that CE-one regulated the blood glucose level by improving the condition of liver injury and promoting the production of glycogen in diabetic mice (Li, *et al.*, 2019). The research of Mosa and Khalil indicated that consumption of fresh and dried banana peels may modify the risk of acute liver failure of rats (Mosa & Khalil, 2015). Some other reports also showed that triterpenoids can restrain the high levels of ALT and AST and exhibit hepatoprotective effect in mice, which related to its effect of anti-oxidation and anti-inflammation. (Yin, *et al*., 2019; Liu, *et al*., 2020).

The level of glucose is also regulated by hepatic glycogen phosphorylase (GP) catalyzing glycogenolysis leading to an increased hepatic glucose output and glycogen synthase, which stimulates gluconeogenesis (Tahrani, *et al*., 2011). It has been stated that inhibition of hepatic

GP has the potential to be an effective therapy for attenuating hyperglycemia associated with type 2 diabetes (Baker, *et al*., 2005). Among triterpenes, oleanane derivatives were mostly reported as potential GP inhibitors, such as oleanolic acid, hederagenin, tormentic acid, asiatic acid, corosolic acid and maslinic acid (Luo, *et al*., 2008; Yang, *et al*., 2010; Wen, *et al*., 2005). It was also reported that liver had the highest bioavailability for triterpene compared with other tissues and it is the major organ for triterpene storage and/or metabolism (Yin, *et al*., 2012). This is in accord with our results that CE-one mainly exert the effect on liver. Moreover, the kidney index and the level of insulin in diabetic mice remained no change indicated that CE-one maybe cannot improve kidneys condition and insulin secretion *in vivo*.

In addition, our results also showed that CE-one can reduce the level of cholesterol in plasma. This is consistent with the reports that triterpenes have anti-hyperlipidemic activity (Machaba, *et al*., 2014; Musa, *et al*., 2019). Triterpene treatment possesses therapeutic effects on diet-induced hyperlipidemia by inhibiting the intestinal absorption and storage of cholesterol. The reason is their structural similarity with cholesterol, these compounds can interfere with the solubilization of cholesterol in the gut, reducing its absorption (Liu, *et al*., 2007).

5.5 Conclusion

CE-one can exert the antihyperglycemic activity both in normal mice and STZ-induced diabetic mice. It can inhibit α-glucosidase and α-amylase activity and the glucose absorption in normal mice. The underlying mechanism of antihyperglycemic activity in diabetic mice maybe because of its improvement effect of liver injury and increasement of glycogen content. In addition, CE-one can also reduce the level of cholesterol in plasma.

5.6 Figures and tables

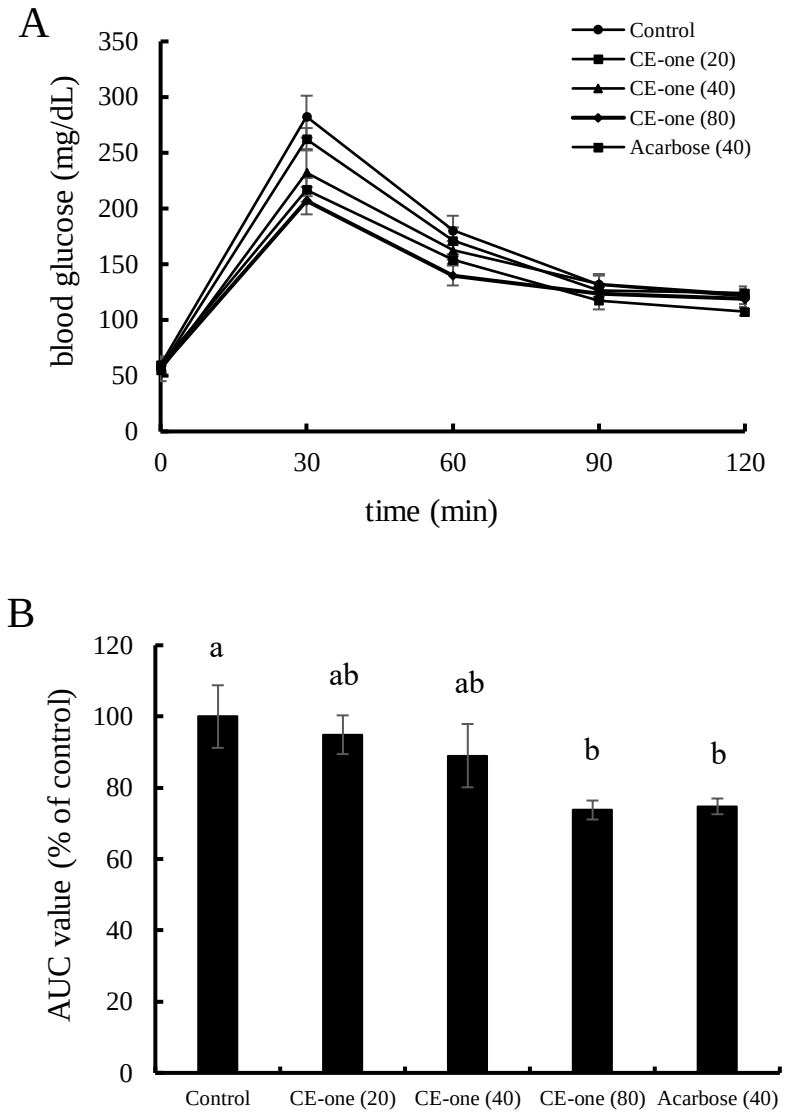

Fig. 5-1 The results of OMTT of CE-one. A: The time course of blood glucose level. B: The results of AUC value. Different letters on each column indicate a significant difference at $p < 0.05$. The numbers in parenthesis are the dose of samples (mg/kg body weight). The data are expressed as mean ± standard error (M ± SE, n = 5).

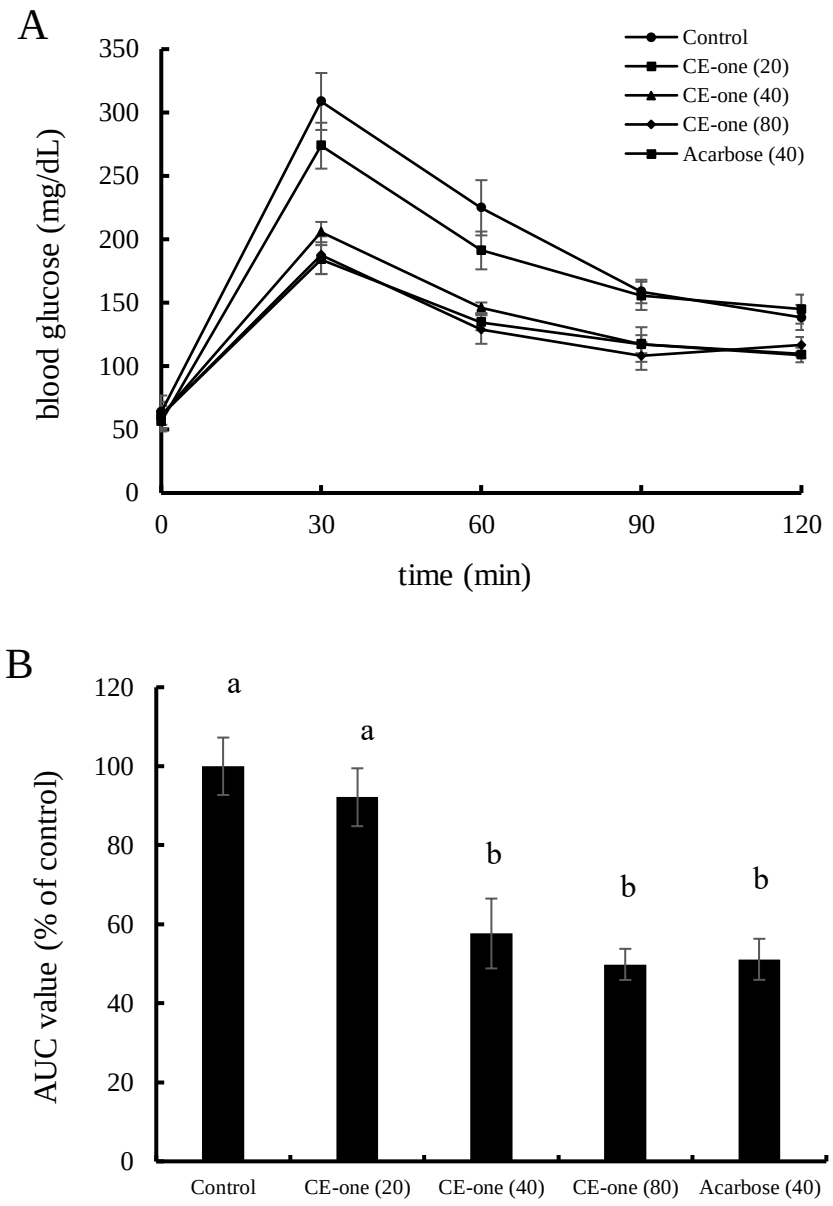

Fig. 5-2 The results of OSTT of CE-one. A: The time course of blood glucose level. B: The results of AUC value. Different letters on each column indicate a significant difference at $p < 0.05$. The numbers in parenthesis are the dose of samples (mg/kg body weight). The data are expressed as mean ± standard error (M ± SE, n = 5).

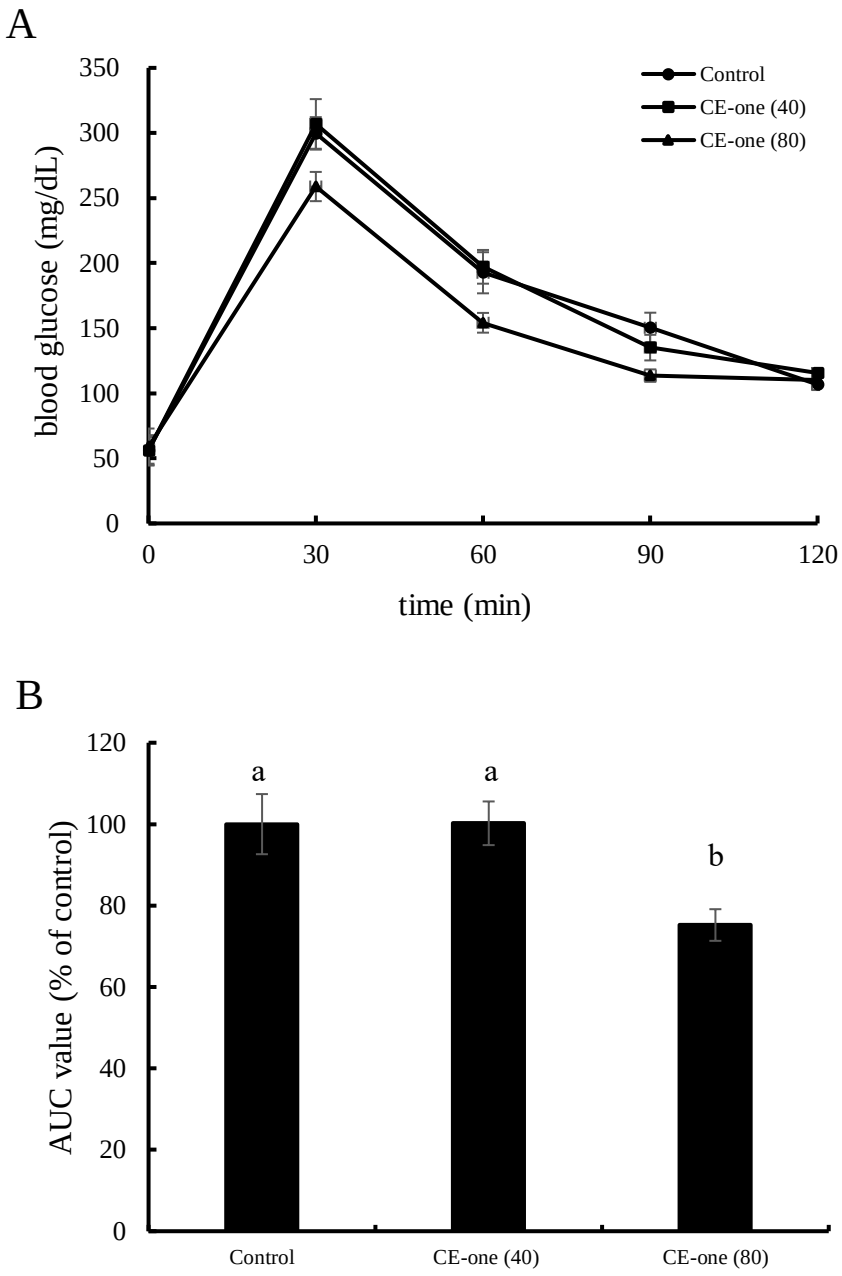

Fig. 5-3 The results of OGTT of CE-one. A: The time course of blood glucose level. B: The results of AUC value. Different letters on each column indicate a significant difference at $p < 0.05$. The numbers in parenthesis are the dose of samples (mg/kg body weight). The data are expressed as mean ± standard error (M ± SE, n = 6).

Table 5-1 The results of feces weight and CE-one in feces

Group	Feces weight (mg)	CE-one in feces (0-2 h)	CE-one in feces (2-6 h)
Control	31.6±5.0	-	-
CE-one (40)	44.6±6.6	-	+
CE-one (80)	49.6±5.8	-	+

Note: The symbol "-" means cannot detect CE-one. The symbol "+" means can detect CE-one. The data are expressed as mean ± standard error (M ± SE, n = 6).

Table 5-2 Body weight, food intake, water consumption and blood glucose of mice groups

Group	Body weight (g)	Food intake (g)	Water consumption (g)	Blood glucose (mg/dL)
NC	47.92 ± 0.82^{b}	6.41 ± 0.32^{a}	5.62 ± 0.28^{a}	66.04 ± 3.67^{a}
DC	38.84 ± 0.90^{a}	15.31 ± 0.76^{d}	56.79 ± 1.32^{d}	405.08 ± 66.87^{c}
CL	36.08 ± 1.36^{a}	12.54 ± 0.61^{c}	46.23 ± 1.98^{c}	256.98 ± 21.63^{b}
CH	37.69 ± 1.56^{a}	$11.18\pm0.72b^{c}$	46.99 ± 2.00^{c}	203.54 ± 28.08^{b}
PC	35.56 ± 1.11^{a}	9.40 ± 0.86^{b}	35.93 ± 3.96^{b}	249.25 ± 43.92^{b}

Note: Different letters in each column indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard error (M ± SE, n = 5).

Table 5-3 Kidney and liver index of mice groups

Group	Kidney index (%)	Liver index (%)
NC	1.38 ± 0.06^{a}	3.67 ± 0.08^{a}
DC	1.97 ± 0.06^{b}	4.42 ± 0.20^{b}
CL	1.95 ± 0.20^{b}	3.83 ± 0.23^{a}
CH	1.81 ± 0.07^{b}	4.17 ± 0.12^{ab}
PC	1.90 ± 0.08^{b}	4.49 ± 0.19^{b}

Note: Different letters in each column indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard error (M ± SE, n = 5).

Table 5-4 Fasting plasma insulin, liver glycogen content, AST and ALT levels of mice groups

Group	Insulin (ng/mL)	Glycogen (mg/g)	AST (Karmen units/mL)	ALT (Karmen units/mL)
NC	0.25±0.05^{b}	31.51±5.17^{c}	271.00±46.74^{a}	20.34±1.08^{a}
DC	0.06±0.02^{a}	3.07±0.49^{a}	752.77±62.24^{c}	55.88±11.86^{b}
CL	0.06±0.01^{a}	5.85±1.47^{a}	557.45±74.29^{bc}	54.04±4.66^{b}
CH	0.07±0.04^{a}	10.97±3.72^{ab}	427.55±95.23^{ab}	46.34±7.95^{b}
PC	0.06±0.02^{a}	19.17±6.11^{bc}	609.15±53.57^{bc}	56.63±10.97^{b}

Note: Different letters in each column indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard error (M ± SE, n = 5).

Table 5-5 TC and TG levels of mice groups

Group	TC (mg/dL)	TG (mg/dL)
NC	156.9±7.9^{b}	96.9±8.0^{b}
DC	154.0±8.6^{b}	44.3±12.0^{a}
CL	107.4±9.3^{ab}	43.2±12.9^{a}
CH	93.6±11.0^{a}	45.1±14.1^{a}
PC	155.9±15.6^{b}	60.2±11.5^{a}

Note: Different letters in each column indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard error (M ± SE, n = 5).

Chapter 6 Stability of cycloeucalenone *in vitro* and *in vivo*

6.1 Introduction

The stability of triterpenes is very important for its bioavailability and application. However, triterpenoids are usually unstable and can be converted into other triterpenoids during the processing (Tai, *et al.*, 2019). In order to develop more utilization of CE-one in different industry and investigate its bioavailability in body, the stability of CE-one had been investigated *in vitro* and *in vivo* in this chapter.

6.2 Materials and methods

6.2.1 Stability of CE-one *in vitro*

6.2.1.1 HPLC conditions and standard cure of CE-one

HPLC conditions are as follows: column: ODS-HG-5 (4.6 × 250 mm); column temperature: 40˚C; mobile phase: 100% MeOH; flow rate: 1 mL/min; time: 20 min; sample volume: 50 μL; PDA detector: 204 nm; pressure: 7.8 MPa.

Around 1 mg of CE-one was dissolved in 100% MeOH to prepare 1000 μg/mL CE-one. It was diluted to 1, 2, 4, 8, 16, 32, 64, 128, and 256 μg/mL. The standard samples were analyzed by HPLC.

6.2.1.2 Thermal stability of CE-one

To simulate different processing conditions, 37˚C (24 h), 60˚C (12 h and 24 h), 100˚C (1 h), 200˚C (0.5 h) were chosen to investigate the thermal stability. Around 1 mg of CE-one was weighed in each dry bottle, after kept under these conditions, they were dissolved in 1 mL 100% MeOH. After dilution by 10 times, samples were analyzed by HPLC (each condition was performed in triplicate).

6.2.1.3 Stability of CE-one in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF)

a. Preparation of SGF (without pepsin)

SGF was prepared as described in pervious report (Fu, *et al.*, 2002), with slight modification. Dissolve 100 mg of sodium chloride (NaCl) in moderate water. Add 1 M

hydrochloric acid (HCl) and sufficient water to make 50 mL. The final pH of this solution is about 1.2.

b. Preparation of SIF (without pancreatin)

SIF was prepared as described in pervious report (Fu, *et al*., 2002), with slight modification. Dissolve 340 mg of monopotassium phosphate (KH_2PO_4) in moderate water. Add 1 M sodium hydroxide (NaOH) and sufficient water to make 50 mL. The final pH of this solution is about 6.8.

c. Preparation of samples

First, 200 μg/mL of CE-one in 100% DMSO was prepared. Then 25 μL CE-one solution was taken and mixed with 475 μL SGF, SIF or water and kept at 37˚C for 0 h, 2 h, 4 h (Water was used as control). After extracted with EtOAc for 3 times, the EtOAc layer was collected and evaporated to dry. After dissolved in 200 μL 100% MeOH, samples were analyzed by HPLC (each condition was performed in triplicate).

The remaining rate (%) = 100 × Actual concentration / Initial concentration

6.2.2 Stability of CE-one in normal mice

After fasting for 20 h, four groups (n = 3) of ddY mice were used. Group 1: after the mice were sacrificed, the blood was collected and the part from the top of stomach to the bottom of small intestine was taken, then 40 mg/kg body weight CE-one was injected from the top of this part directly. Group 2, 3, 4: after oral administration of 40 mg/kg body weight CE-one for 0.5 h, 1.0 h, 2.0 h, respectively, then the mice were sacrificed and the blood and same part from the top of stomach to the bottom of small intestine were taken.

The taken part was washed with 20 mL normal saline, and the content inside the stomach and small intestine was collected. The content was extracted with 20 mL EtOAc for 2 times. After EtOAc layer was evaporated to dry, it was dissolved with 0.5 mL 100% MeOH. After centrifuge, the supernatant was collected and evaporated to dry again. After dissolved in 0.70 mL $CDCl_3$, samples were analyzed by proton NMR. In addition, the plasma of each mouse was extracted with EtOAc for 3 times. After the supernatants were collected and evaporated to dry, they were also analyzed by proton NMR. For each sample, the solvent $CDCl_3$ was used as

reference. And the specific proton signal at 4.69 ppm was used to define CE-one. The relative amount of CE-one was calculated as below: CE-one (%) = 100 × integrate of proton signal at 4.69 ppm / integrate of residual proton signal of $CDCl_3$. The Group 1 was defined as control group, which means the content of CE-one is 100% in stomach and small intestine, 0% in plasma.

All data are expressed as mean ± standard deviation (M ± SD, n = 3). The experimental data were analyzed by homogeneity of variance and Duncan multiple comparisons of analysis of variance (ANOVA) with SPSS 22.0 software (IBM Corporation, New York, USA).

6.3 Results

6.3.1 Stability of CE-one in different temperatures, SGF and SIF

The various concentrations of CE-one as standard samples were analyzed by HPLC for the following quantitative analysis. All different concentrations of CE-one showed the retention time with range from 12.330 to 12.370 min. The minimum concentration of CE-one for detection was 1 μg/mL. The linearity was confirmed at the concentrations below 256 μg/mL under these conditions. The typical series of concentrations for standard curve of CE-one (1 μg/mL - 256 μg/mL, n=1) were shown in Fig. 6-1 as chromatograph and in Fig. 6-2 as standard curve. The peak area of CE-one showed in a concentration-dependent manner. The R^2 of standard cure is 0.9993 which demonstrates its high reliability. The retention time and peak area of different concentrations of CE-one standard samples were shown in Table 6-1.

The HPLC results of CE-one under different temperatures were shown in Fig. 6-3. For the thermal stability, there are some other peaks appeared at 100˚C, especially 200˚C. In contrast, nearly no other peaks appeared below 60˚C (Fig. 6-3). Under 100˚C for 1 h and 200˚C for 0.5 h, the remaining rate of CE-one are 82.26 ± 2.55% and 26.14 ± 3.44%, respectively (Table 6-2). They have significant differences compared with below 60˚C (Table 6-2). The remaining rate of CE-one was more than 93% below 60˚C and had no significant difference (Table 6-2). These results indicated that CE-one is stable below 60˚C and not stable beyond 100˚C, especially 200˚C.

The HPLC results of CE-one in SGF, SIF and water are shown in Fig 6-4, Fig 6-5, Fig 6-

6, respectively. For SGF, after keep 2 h and 4 h at 37˚C, the relative remaining rate of CE-one are 84.56 ± 2.92% and 75.83 ± 2.82%, respectively (Table 6-3). For SIF, after keep 2 h and 4 h at 37˚C, the relative remaining rate of CE-one are 85.40 ± 0.36% and 76.57 ± 1.58%, respectively (Table 6-3). For water, after keep 2 h and 4 h at 37˚C, the relative remaining rate of CE-one are 90.86 ± 1.30% and 89.04 ± 1.20%, respectively (Table 6-3). The relative remaining rate of CE-one in SGF, SIF and water are decreased as the keeping time increased. There are significant differences between 2 h and 4 h in SGF and SIF. In contrast, there is no difference between 2 h and 4 h in water (Table 6-3). Compared with water, the relative remaining rate of 2 h and 4 h in SGF and SIF showed significant difference (Table 6-3). All these results indicated that CE-one can decrease in SGF and SIF over time, and it is easier to decrease in SGF and SIF than in water. But the stability of CE-one in SGF and SIF is relatively high, around 75% still remains after 4 h.

6.3.2 Stability of CE-one in different parts of normal mice

As shown in Table 6-4, after oral administration of CE-one for 0.5 h, 1.0 h, and 2.0 h in normal mice, the relative amount of CE-one in the content of stomach and small intestine were 89.46 ± 7.62%, 81.83 ± 9.49% and 77.09 ± 9.12%, respectively. It was found that the relative amount of CE-one had a time-dependent manner. With the increasing of time, the amount of CE-one decreased gradually. At 0.5 h and 1.0 h, there is no significant difference. But at 2.0 h, there is a significant difference. This indicated that after 2 h, CE-one will have a significant lose in normal mice. But most part of CE-one (around 77%) still remains in stomach and small intestine after given CE-one for 2 h. In contrast, the CE-one cannot be detected in plasma. This maybe because of the amount of CE-one in plasma lower than limit of detection of proton NMR.

6.4 Discussion

CE-one is one of cycloartane-type triterpenes which is abundant in banana peel. There have been no reports about the stability of CE-one until now. Some reports indicated that the melting point of CE-one is 83 - 84˚C (Kongkathip, *et al*., 2002), and the boiling point is predicted to be over 500˚C. It was reported that 70˚C (or lower) was the best drying temperature

of *Rhizoma Alismatis* to obtain triterpenoids because of the obvious destruction of chemical composition when dried over 80˚C (Tai, *et al*., 2019). This is in accordance with our results that CE-one is stable below 60˚C. One report showed that a pentacyclic triterpene which was extracted from *Centella asiatica* was stable over a period of four months when stored as a dried powder in a well-closed container kept out of light at 4˚C (Puttarak, *et al*., 2016). In this study, the stability of CE-one is high below 60˚C and low beyond 100˚C, especially 200˚C. This indicated that CE-one can be used as ingredient for functional food but cannot used for baked food. Under SGF (pH 1.2) and SIF (pH 6.8) condition, CE-one is relatively stable. There are still around 75% of CE-one remains after keep in SGF and SIF for 4 hours. This indicated that CE-one with intact form is stable in acidic or neutral conditions. The aqueous alcoholic solution of one pentacyclic triterpene was stable at pH values of 5.8 and 7.0 but was not stable at a pH of 8.2 (Puttarak, *et al*., 2016). This report is similar with our results which showed that CE-one is relatively stable in acidic or neutral conditions. All these results indicated that CE-one is relatively stable *in vitro*, the stability *in vivo* also need to be evaluated in next part.

The stability of triterpenes *in vivo* is important to exert their bioactivities. In our results, after oral administration of CE-one for 2 h in normal mice, there are still around 77% of relative amount remains in the content of stomach and small intestine. This indicated that most of CE-one did not change the intact form, its stability is relatively high *in vivo*. CE-one cannot be detected in plasma indicated that with intact form it maybe not easy to absorute from intestine wall. This complied with triterpenes are large molecules and their penetration through cell membranes can be difficult. However, permeability models with Caco-2 cell monolayer indicated that some triterpenes, such as lupeol, oleanolic acid, ursolic acid, asiatic acid and boswellic acid, can pass the intestinal epithelium (J. C. Furtado, *et al*., 2017). Besides, triterpene absorption can be enhanced by high fat meals and triterpene bioavailability can be improved by increasing the poor solubility in the gastrointestinal fluid and their absorption, or by inhibiting their metabolism (J. C. Furtado, *et al*., 2017). In addition, it has been observed that the bioavailability of triterpenes can be improved by combining them with cyclodextrins (Cerga, *et al*., 2011). CE-one combined with cyclodextrins maybe one option to improve its bioavailability in the future. In the present research, the stability of CE-one is stable both *in vitro* and *in vivo* which indicated that it is maybe one good ingredient for functional food. And the thermal

stability of CE-one also provides the possibility of using oven to dry the banana peel and produce CE-one on a large scale in industry.

6.5 Conclusion

The stability of CE-one is relatively high both *in vitro* and *in vivo*. CE-one is stable below 60˚C and not stable beyond 100˚C, especially 200˚C. CE-one can be used as ingredient for functional food but cannot used for baked food. It is also relatively stable in SGF and SIF condition. There are still around 75% of CE-one remains after keep in SGF and SIF for 4 hours. After oral administration of CE-one for 2 h in normal mice, there are still around 77% of relative amount remains in the content of stomach and small intestine. To the best of our knowledge, the present study is the first report about the stability of CE-one *in vitro* and *in vivo*. The present study provides some evidence of CE-one for food processing and effectivity in intestine as health promoting ingredient. This will provide some valuable information for utilization of CE-one in the future.

6.6 Figures and tables

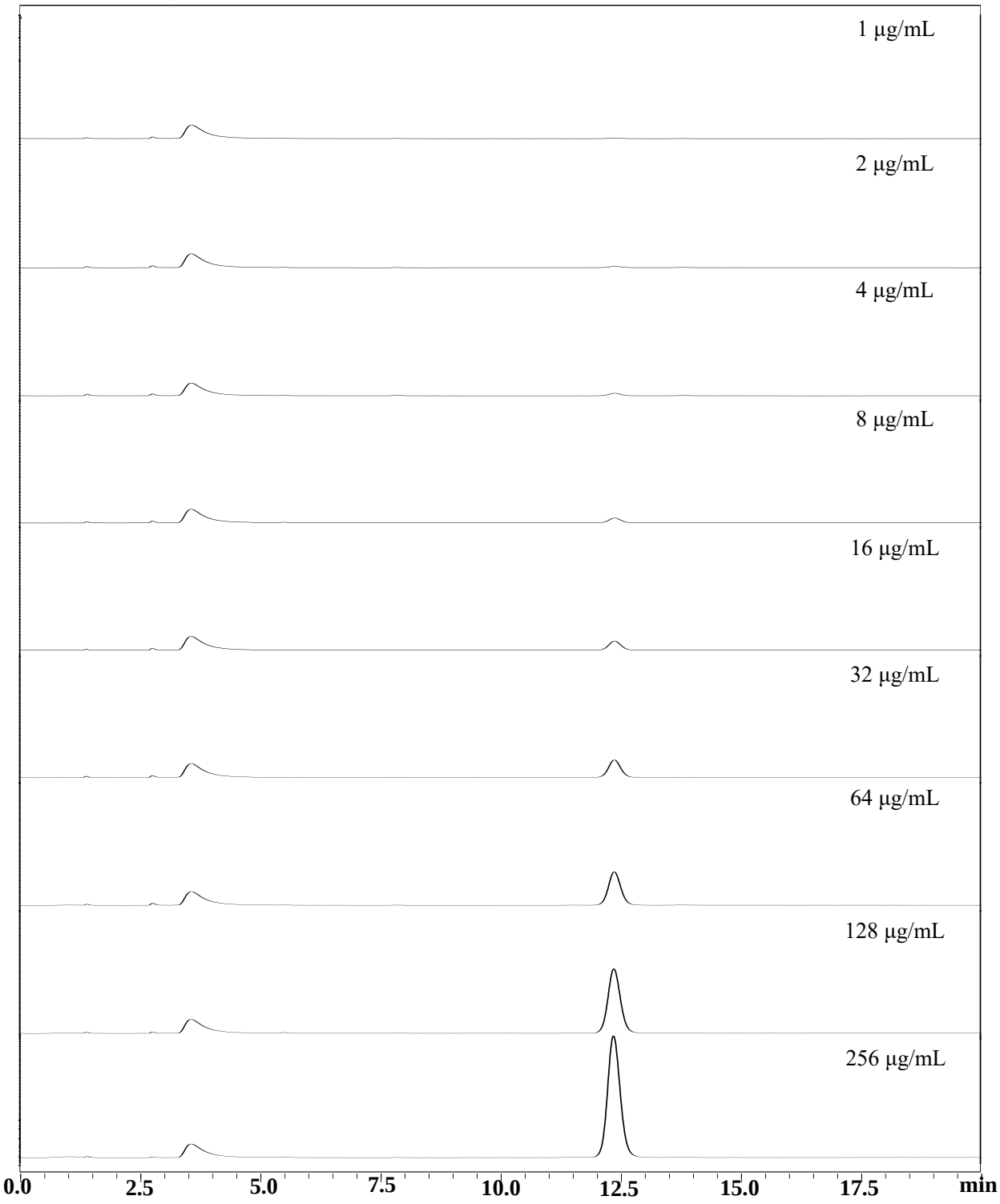

Fig. 6-1 The HPLC results of CE-one standard samples.

The retention time of peaks at around 12.5 min are CE-one with various concentrations. The peak area of each sample was summarized in Table 6-1.

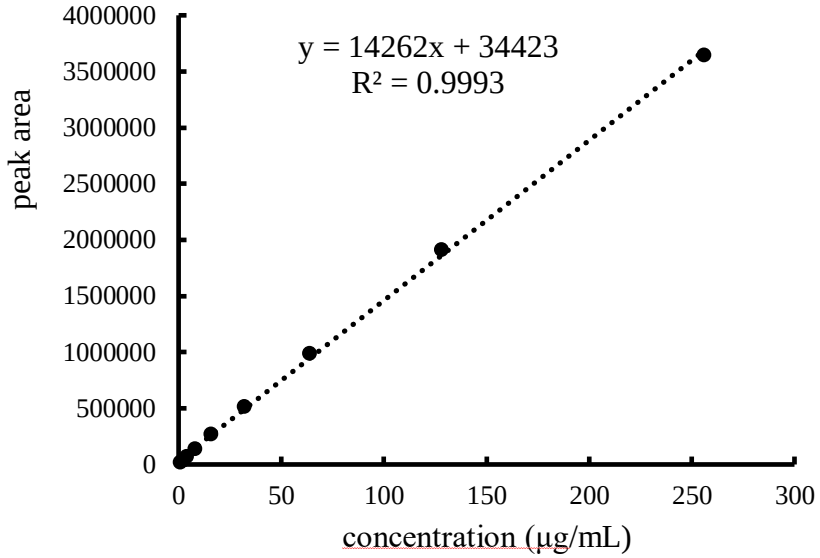

Fig. 6-2 The standard curve of CE-one in HPLC.

The concentrations of CE-one are 1, 2, 4, 8, 16, 32, 64, 128, and 256 μg/mL.

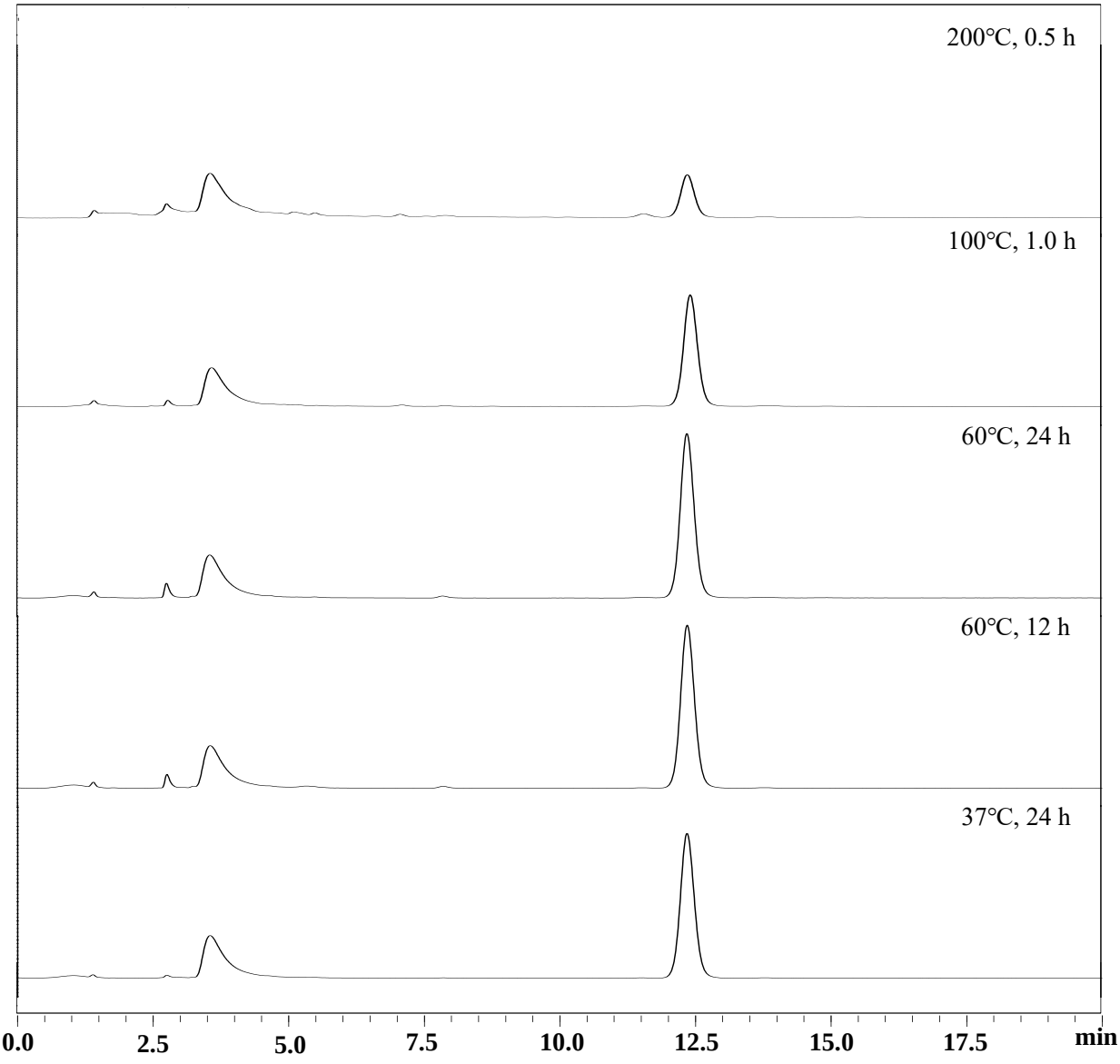

Fig. 6-3 The HPLC results of CE-one under different temperatures (one representative of results in triplicate). The retention time of peaks at around 12.5 min are CE-one. The remaining rate of each condition was summarized in Table 6-2.

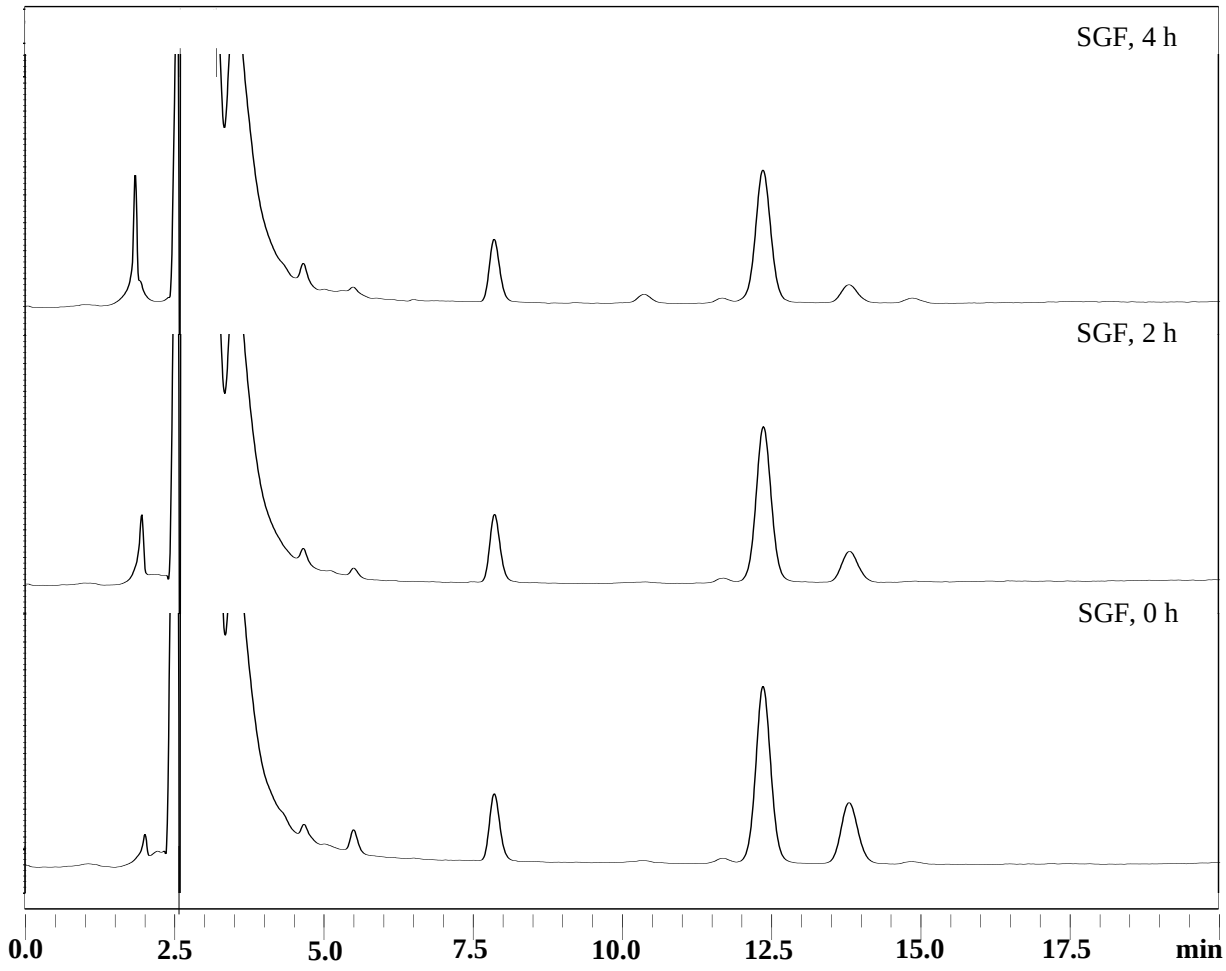

Fig. 6-4 The HPLC results of CE-one in SGF (one representative of results in triplicate). The retention time of peaks at around 12.5 min are CE-one. The relative remaining rate of each condition was summarized in Table 6-3.

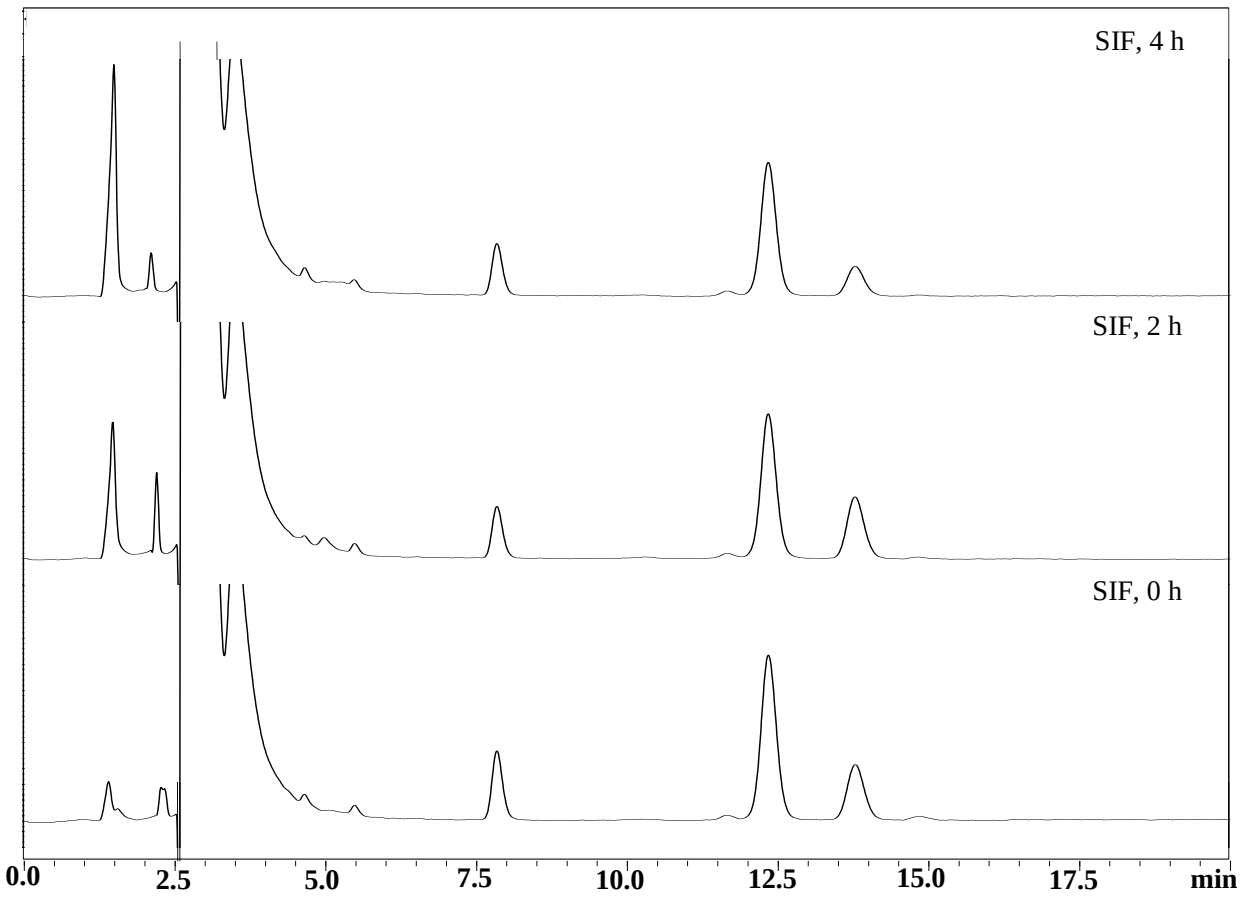

Fig. 6-5 The HPLC results of CE-one in SIF (one representative of results in triplicate). The retention time of peaks at around 12.5 min are CE-one. The relative remaining rate of each condition was summarized in Table 6-3.

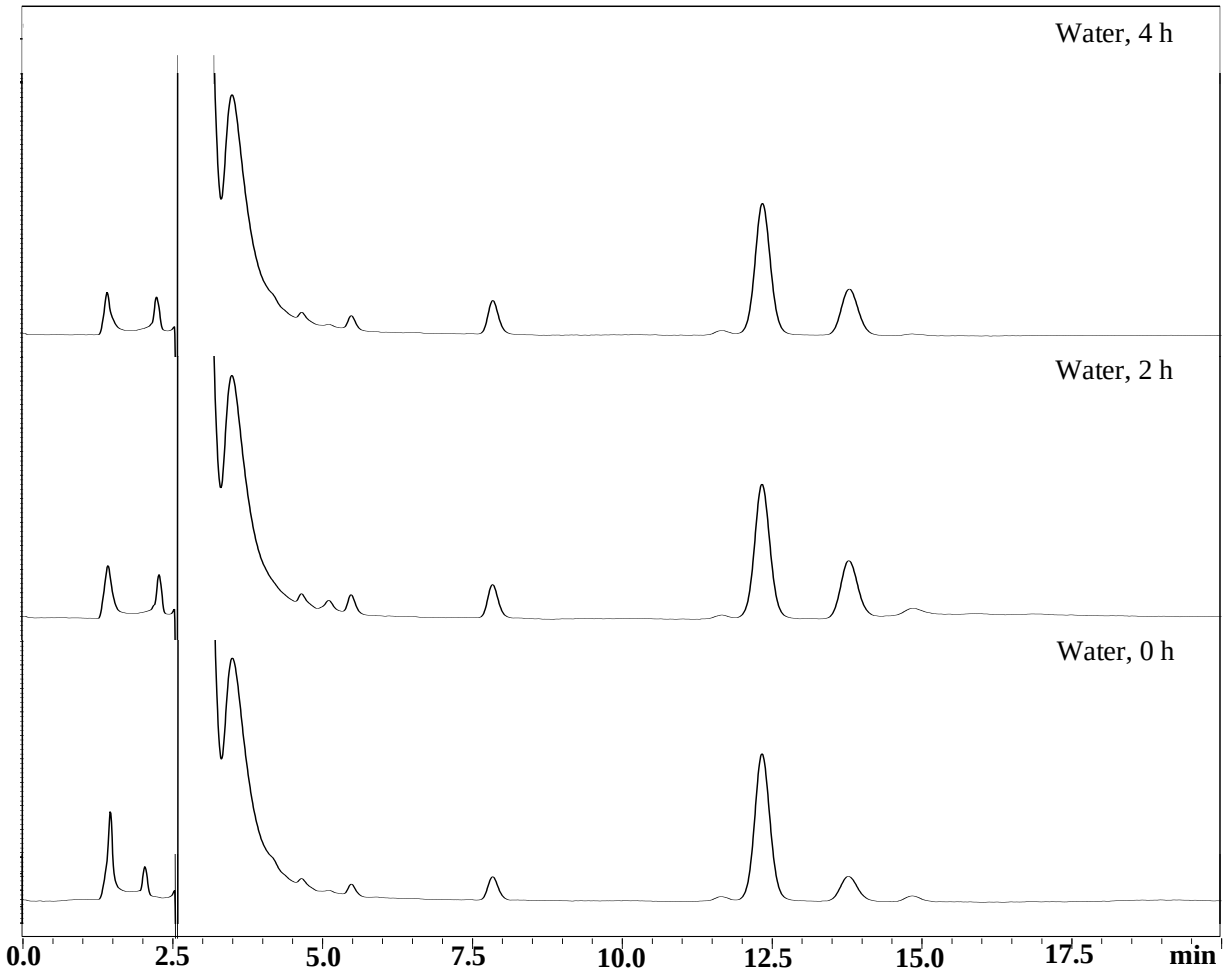

Fig. 6-6 The HPLC results of CE-one in Water (one representative of results in triplicate). The retention time of peaks at around 12.5 min are CE-one. The relative remaining rate of each condition was summarized in Table 6-3.

Table 6-1 The retention time and peak area of different standard samples of CE-one.

Concentration (μg/mL)	Retention time (min)	Peak area
1	12.365	18910
2	12.366	36415
4	12.365	72175
8	12.360	139622
16	12.357	268312
32	12.357	516579
64	12.356	986712
128	12.347	1912048
256	12.332	3646851

Table 6-2 The remaining rate of CE-one under different temperature

Condition	Remaining rate (%)
200˚C, 0.5 h	26.14 ± 3.44^{a}
100˚C, 1 h	82.26 ± 2.55^{b}
60˚C, 24 h	94.07 ± 0.98^{c}
60˚C, 12 h	93.04 ± 2.90^{c}
37˚C, 24 h	95.72 ± 4.61^{c}

Note: Different letters following each value indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard deviation (M ± SD, n = 3).

Table 6-3 The relative remaining rate of CE-one in SGF, SIF and water.

Time	SGF (%)	SIF (%)	Water (%)
0 h	100.00 ± 1.51^{Aa}	100.00 ± 0.86^{Aa}	100.00 ± 1.43^{Aa}
2 h	84.56 ± 2.92^{Ab}	85.40 ± 0.36^{Ab}	90.86 ± 1.30^{Bb}
4 h	75.83 ± 2.82^{Ac}	76.57 ± 1.58^{Ac}	89.04 ± 1.20^{Bb}

Note: Different letters in the same column (a, b, c) and same row (A, B) indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard deviation (M ± SD, n = 3).

Table 6-4 The relative amount of CE-one in different parts of normal mice

Group	Content of stomach and small intestine (%)	Plasma (%)
1 (0 h)	100.00 ± 10.68^{a}	-
2 (0.5 h)	89.46 ± 7.62^{ab}	-
3 (1 h)	81.83 ± 9.49^{ab}	-
4 (2 h)	77.09 ± 9.12^{b}	-

Note: The symbol "-" means cannot detect CE-one. Different letters following each value indicate a significant difference at $p < 0.05$. The data are expressed as mean ± standard deviation (M ± SD, n = 3).

Summary

Banana including dessert banana and cooking banana, is the second largest produced fruit after citrus. In 2018, banana production in the world was 115.74 million metric tons, which was about 16% of the world's total fruit output (El Barnossi, *et al*., 2020). Banana peel as by-product, which is about 35% of the total fresh weight, usually used as organic fertilizer or simply discarded as waste (Schieber, *et al*., 2001). Many studies have shown that banana peel is an important resource which contains many kinds of nutrients and active components (Emaga, *et al*., 2007; Pereira & Maraschin, 2015; Singh, *et al*., 2016). Currently, banana peel as an important resource has been severely underestimated. This study focused on the antihyperglycemic activity of triterpenes in banana peel. The purpose of this research is to develop potential antidiabetic activity of natural triterpenes in banana peel and provide an alternative way to take full advantage of banana peel resource.

In this study, four triterpenes, cycloeucalenone (CE-one), 31-norcyclolaudenone (isoCE-one), cycloeucalenol (CE-ol) and its isomer (isoCE-ol) were isolated from EtOAc extract of banana peel (Chapter 2). And their structures were confirmed based on the analysis of 1D and 2D NMR (Chapter 3). The effects of these triterpenes on carbohydrate digestive enzymes were investigated *in vitro* to elucidate their structure-activity relationships and kinetic property of CE-one (Chapter 4). To confirm effects of the triterpenes *in vivo*, the major triterpene, CE-one was evaluated in carbohydrate loaded mice and STZ-treated diabetic model mice (Chapter 5). In addition, the physical and physiological stabilities of CE-one were investigated under the conditions for food processing and the conditions in digestive tract, respectively (Chapter 6).

Banana peel as by-product has been studied for many years. It has been reported that phytosterols represent majority of the lipophilic extract of banana peel with CE-one, isoCE-one, stigmasterol, β-sitosterol, and campesterol as the major components (Oliveira, *et al*., 2008; Villaverde, *et al*., 2013). In this study, CE-one and isoCE-one were also found to be the major triterpenes in banana peel. In addition, CE-ol and isoCE-ol were isolated from banana peel for the first time. The α-glucosidase and α-amylase are important enzymes of dietary carbohydrate digestion in our body. Inhibitors of these enzymes are effective in delaying carbohydrate

digestion and glucose absorption to suppress postprandial hyperglycemia (Tadera, *et al*., 2006). Our results of *in vitro* experiments indicated that CE-one and isoCE-one possess much stronger α-glucosidase and α-amylase inhibitory activities than CE-ol and isoCE-ol and the main active site of them is the carbonyl group at C-3 rather than hydroxy group at C-3. And the double bold in the side chain can also affect these activities. CE-one and isoCE-one exhibited both α-glucosidase and α-amylase inhibitory activities *in vitro*. And CE-one showed higher α-glucosidase and α-amylase inhibitory activities than that of isoCE-one. In contrast, CE-ol and isoCE-ol had insufficient inhibitory activity against either enzyme. The IC_{50} values of CE-one and isoCE-one against α-glucosidase were 31.83 ± 2.46 μM and 38.85 ± 1.54 μM, respectively, and their IC_{50} values against α-amylase were 20.33 ± 0.59 μM and 27.63 ± 0.83 μM, respectively. The main active sites of CE-one and isoCE-one are the carbonyl group at C-3 and double bond in the side chain. And CE-one induced a parabolic mixed-type inhibition with the K_i value of 73.86 μM in α-glucosidase inhibitory assay.

Besides, our results of *in vivo* experiments also indicated that CE-one can exert the antihyperglycemic activity in carbohydrates (starch, maltose, or glucose) loaded mice. It can inhibit α-glucosidase and α-amylase activity and the glucose absorption in normal mice. The results of CE-one in STZ-induced diabetic mice indicated that CE-one can decrease the blood glucose level, food intake, water consumption and liver index. CE-one cannot affect the body weight, kidney index, and insulin level of diabetic mice. In addition, CE-one can also decrease total cholesterol level in plasma while has no effect on triglyceride level. The main effect of CE-one are inhibition of α-glucosidase and α-amylase activities and improvement of liver injury condition. There are some studies have been reported about the antihyperglycemic activity of banana peel. The methanol extract of banana peel can effectively lower blood glucose level of mice in oral glucose tolerance test which showed similar effect with glibenclamide at the highest dose (Hossain, *et al*., 2017). It also exerted more potent in α-amylase inhibitory activity than that of *Citrus limon*, *Punica granatum* (Vasu, *et al*., 2017). The ethyl acetate extract of banana peel also displayed potent antihyperglycemic activity in alloxan-induced diabetic mice and further results indicated that lupenone is the effective ingredient (Wu *et al*., 2015). In addition, umbelliferone and lupeol in banana flower also exerted antihyperglycemic activity *in vitro* and *in vivo* (Ramu, *et al*., 2014; Ramu, *et al*., 2016). The present study indicated that CE-

one, the natural compound with low toxicity in banana peel, maybe can be used as one of candidates for treatment and/or prevention of diabetes in the future.

Moreover, the stability of CE-one was investigated *in vitro* and *in vivo*. It was reported that the chemical composition of triterpenoids usually will be destroyed obviously when dried over 80˚C (Tai, *et al*., 2019). Our results indicated that the thermal stability of CE-one is stable below 60˚C and not stable beyond 100˚C, especially 200˚C. This will provide some important reference values for the industrial production of CE-one in banana peel. For the utilization of by-product, one of the important steps is drying process. The very common and cheap drying method is driven by solar energy or oven. Our results indicated that this common drying method can be used to produce CE-one (below 60˚C). In addition, CE-one can also be used as one of the ingredients for functional food. But it cannot be used as the ingredient for baked food because of its processing temperature usually higher than 200˚C. The stability of CE-one in SGF and SIF indicated that it is relatively stable in acidic or neutral conditions. There are still around 75% of CE-one remains in SGF and SIF after 4 hours. Furthermore, after oral administration of CE-one for 2 h in normal mice, there are still around 77% of relative amount remains in the content of stomach and small intestine. Our results indicated that CE-one is a good candidate as the potential antidiabetic agent.

The present study showed the antihyperglycemic activity and mechanisms, and the stability of CE-one *in vitro* and *in vivo*. It not only provides the possibility to use CE-one as potential antidiabetic agent, but also provides an alternative method to recycle banana peel resource. Nevertheless, further studies about the absorption and metabolism of CE-one *in vivo* and more direct evidence of antihyperglycemic mechanisms *in vivo* still need to be investigated in the future.

References

Achak, M., Hafidi, A., Ouazzani, N., Sayadi, S., & Mandi, L. (2009). Low cost biosorbent "banana peel" for the removal of phenolic compounds from olive mill wastewater: Kinetic and equilibrium studies. *Journal of Hazardous Materials*, 166, 117-125.

Adewusi, E. A. (2012). *In vitro* effect of selected medicinal plants on β-amyloid induced toxicity in neuroblastoma cells. *Doctoral dissertation*, pp. 59-60, Pretoria, University of Pretoria.

Adewusi, E. A., Steenkamp, P., Fouche, G., & Steenkamp, V. (2013). Isolation of cycloeucalenol from *Boophone disticha* and evaluation of its cytotoxicity. *Natural Product Communications*, 8, 1213-1216.

Agra, L. C., Ferro, J. N., Barbosa, F. T., & Barreto, E. (2015). Triterpenes with healing activity: A systematic review. *Journal of Dermatological Treatment*, 26, 465-470.

Akamine, K., Koyama, T., & Yazawa, K. (2009). Banana peel extract suppressed prostate gland enlargement in testosterone-treated mice. *Bioscience, Biotechnology, and Biochemistry*, 73, 1911-1914.

Akihisa, T., Kimura, Y., & Tamura, T. (1998). Cycloartane triterpenes from the fruit peel of *Musa sapientum*. *Phytochemistry*, 47, 1107-1110.

Akihisa, T., Yasukawa, K., Yamaura, M., Ukiya, M., Kimura, Y., Shimizu, N., & Arai, K. (2000). Triterpene alcohol and sterol ferulates from rice bran and their anti-inflammatory effects. *Journal of Agricultural and Food Chemistry*, 48, 2313-2319.

Albarelli, J. Q., Rabelo, R. B., Santos, D. T., Beppu, M. M., & Meireles, M. A. A. (2011). Effects of supercritical carbon dioxide on waste banana peels for heavy metal removal. *The Journal of Supercritical Fluids*, 58, 343-351.

Ali, H., Houghton, P. J., & Soumyanath, A. (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes: With particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, 107, 449-455.

Annadurai, G., Juang, R. S., & Lee, D. J. (2002). Use of cellulose-based wastes for adsorption of dyes from aqueous solutions. *Journal of Hazardous Materials*, 92, 263-274.

Anwar, J., Shafique, U., Salman, M., Dar, A., & Anwar, S. (2010). Removal of Pb (II) and Cd (II) from water by adsorption on peels of banana. *Bioresource Technology*, 101, 1752-1755.

Asghari, B., Salehi, P., Farimani, M. M., & Ebrahimi, S. N. (2015). α-Glucosidase inhibitors from fruits of *Rosa canina* L. *Records of Natural Products*, 9, 276-283.

Ashcroft, F. M., & Rorsman, P. (2012). Diabetes mellitus and the β cell: The last ten years. *Cell*, 148, 1160-1171.

Baker, D. J., Timmons, J. A., & Greenhaff, P. L. (2005). Glycogen phosphorylase inhibition in type 2 diabetes therapy: A systematic evaluation of metabolic and functional effects in rat skeletal muscle. *Diabetes*, 54, 2453-2459.

Balaraman, A. K., Singh, J., Dash, S., & Maity, T. K. (2010). Antihyperglycemic and hypolipidemic effects of *Melothria maderaspatana* and *Coccinia indica* in streptozotocin induced diabetes in rats. *Saudi Pharmaceutical Journal*, 18, 173-178.

Baltina, L. A., Flekhter, O. B., Nigmatullina, L. R., Boreko, E. I., Pavlova, N. I., Nikolaeva, S. N., Savinova, O.V., & Tolstikov, G. A. (2003). Lupane triterpenes and derivatives with antiviral activity. *Bioorganic & Medicinal Chemistry Letters*, 13, 3549-3552.

Cerga, O., Borcan, F., Ambrus, R., & Popovici, I. (2011). Syntheses of new cyclodextrin complexes with oleanolic and ursolic acids. *Journal of Agroalimentary Processes and Technologies*, 17, 405-409.

Clarke, W. P., Radnidge, P., Lai, T. E., Jensen, P. D., & Hardin, M. T. (2008). Digestion of waste bananas to generate energy in Australia. *Waste Management*, 28, 527-533.

Deutschländer, M. S., Lall, N., Van de Venter, M., & Hussein, A. A. (2011). Hypoglycemic evaluation of a new triterpene and other compounds isolated from *Euclea undulata* Thunb. var. *myrtina* (Ebenaceae) root bark. *Journal of Ethnopharmacology*, 133, 1091-1095.

Egede, L. E. & Ellis, C. (2010). Diabetes and depression: Global perspectives. *Diabetes Research and Clinical Practice*, 87, 302-312.

El Barnossi, A., Moussaid, F., & Housseini, A. I. (2020). Tangerine, banana and pomegranate peels valorisation for sustainable environment: A review. *Biotechnology Reports*, e00574.

Emaga, T. H., Andrianaivo, R. H., Wathelet, B., Tchango, J. T., & Paquot, M. (2007). Effects of the stage of maturation and varieties on the chemical composition of banana and plantain peels. *Food Chemistry*, 103, 590-600.

Emaga, T. H., Ronkart, S. N., Robert, C., Wathelet, B., & Paquot, M. (2008). Characterisation of pectins extracted from banana peels (*Musa AAA*) under different conditions using an experimental design. *Food Chemistry*, 108, 463-471.

Essien, J. P., Akpan, E. J., & Essien, E. P. (2005). Studies on mould growth and biomass production using waste banana peel. *Bioresource Technology*, 96, 1451-1456.

Fu, T. J., Abbott, U. R., & Hatzos, C. (2002). Digestibility of food allergens and nonallergenic proteins in simulated gastric fluid and simulated intestinal fluid a comparative study. *Journal of Agricultural and Food Chemistry*, 50, 7154-7160.

González-Montelongo, R., Lobo, M. G., & González, M. (2010). Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds. *Food Chemistry*, 119, 1030-1039.

Hamid, H. A., Yusoff, M. M., Liu, M., & Karim, M. R. (2015). α-Glucosidase and α-amylase inhibitory constituents of *Tinospora crispa*: Isolation and chemical profile confirmation by ultra-high-performance liquid chromatography-quadrupole time-of-flight/mass spectrometry. *Journal of Functional Foods*, 16, 74-80.

Han, P., Cui, Q., Lu, W., Yang, S., Shi, M., Li, Z., Gao, P., Xu, B., & Li, Z. (2019). Hepatocyte growth factor plays a dual role in tendon-derived stem cell proliferation, migration, and differentiation. *Journal of Cellular Physiology*, 234, 17382-17391.

Hanley, A. J., Williams, K., Festa, A., Wagenknecht, L. E., D'Agostino, R. B., Kempf, J., Zinman, B., & Haffner, S. M. (2004). Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes*, 53, 2623-2632.

Hideo, K., Yasuhiro, K., & Masashi, K. (2012). *Experiment of Nutritional Physiology and Biochemistry*, pp. 27-30, Tokyo, Kodansha.

Hossain, I., Akter, S., Shoma, J. F., Hossan, M. S., & Rahmatullah, M. (2017). Antihyperglycemic effect of methanol extract of *Musa sapientum* fruit skins in glucose-challenged mice. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6, 159-166.

Hou, W., Li, Y., Zhang, Q., Wei, X., Peng, A., Chen, L., & Wei, Y. (2009). Triterpene acids isolated from *Lagerstroemia speciosa* leaves as α-glucosidase inhibitors. *Phytotherapy Research*, 23, 614-618.

Hu, J., Zhao, J., Chen, W., Lin, S., Zhang, J., & Hong, Z. (2013). Hepatoprotection of 1 β-

hydroxyeuscaphic acid-the major constituent from *Rubus aleaefolius* against CCl_4-induced injury in hepatocytes cells. *Pharmaceutical Biology*, 51, 686-690.

Huang, Y. C., Chang, W. L., Huang, S. F., Lin, C. Y., Lin, H. C., & Chang, T. C. (2010). Pachymic acid stimulates glucose uptake through enhanced GLUT4 expression and translocation. *European Journal of Pharmacology*, 648, 39-49.

Inzucchi, S. E. (2002). Oral antihyperglycemic therapy for type 2 diabetes: scientific review. *JAMA*, 287, 360-372.

Jäger, S., Trojan, H., Kopp, T., Laszczyk, M. N., & Scheffler, A. (2009). Pentacyclic triterpene distribution in various plants-rich sources for a new group of multi-potent plant extracts. *Molecules*, 14, 2016-2031.

J. C. Furtado, N. A., Pirson, L., Edelberg, H., M Miranda, L., Loira-Pastoriza, C., Preat, V., Larondelle Y., & André, C. M. (2017). Pentacyclic triterpene bioavailability: an overview of *in vitro* and *in vivo* studies. *Molecules*, 22, 400.

Josse, R. G., Chiasson, J. L., Ryan, E. A., Lau, D. C. W., Ross, S. A., Yale, J. F., Maheux, P., Tessier D., Wolever, T. M. S., Gerstein, H., Rodger, N. W., Dornan, J. M., Murphy, L. J., Rabasa-Lhoret, R., & Meneilly, G. S. (2003). Acarbose in the treatment of elderly patients with type 2 diabetes. *Diabetes Research and Clinical Practice*, 59, 37-42.

Khuong-Huu, F., Sangare, M., Chari, V. M., Bekaert, A., Devys, M., Barbier, M., & Lukacs, G. (1975). Carbon-13 nuclear magnetic resonance spectral analysis of cycloartanol and related compounds. *Tetrahedron Letters*, 16, 1787-1790.

Kongkathip, N., Dhumma-upakorn, P., Kongkathip, B., Chawananoraset, K., Sangchomkaeo, P., & Hatthakitpanichakul, S. (2002). Study on cardiac contractility of cycloeucalenol and cycloeucalenone isolated from *Tinospora crispa*. *Journal of Ethnopharmacology*, 83, 95-99.

Lakshmi, V., Agarwal, S. K., Ansari, J. A., Mahdi, A. A., & Srivastava, A. K. (2014). Antidiabetic potential of *Musa paradisiaca* in streptozotocin-induced diabetic rats. *The Journal of Phytopharmacology*, 3, 77-81.

Laszczyk, M. N. (2009). Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. *Planta Medica*, 75, 1549-1560.

Lee, M. S., & Thuong, P. T. (2010). Stimulation of glucose uptake by triterpenoids from *Weigela*

subsessilis. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 24, 49-53.

Li, X., Wang, W., Fan, Y., Wei, Y., Yu, L. Q., Wei, J. F., & Wang, Y. F. (2018). Anticancer efficiency of cycloartane triterpenoid derivatives isolated from *Cimicifuga yunnanensis* Hsiao on triple-negative breast cancer cells. *Cancer Management and Research*, 10, 6715-6725.

Li, Y., Liu, Y., Liang, J., Wang, T., Sun, M., & Zhang, Z. (2019). Gymnemic acid ameliorates hyperglycemia through PI3K/AKT-and AMPK-mediated signaling pathways in type 2 diabetes mellitus rats. *Journal of Agricultural and Food Chemistry*, 67, 13051-13060.

Liu, J., Ren, H., Xu, J., Bai, R., Yan, Q., Huang, W., Wu, X., Fu, J., Wang, Q., Wu, Q., & Fu, R. (2009). Total synthesis and antihypertensive activity of (±) 7, 8-dihydroxy-3-methyl-isochromanone-4. *Bioorganic & Medicinal Chemistry Letters*, 19, 1822-1824.

Liu, J., Sun, H., Wang, X., Mu, D., Liao, H., & Zhang, L. (2007). Effects of oleanolic acid and maslinic acid on hyperlipidemia. *Drug Development Research*, 68, 261-266.

Liu, Y., Cheng, X., Qin, J., Yan, S., Jin, H., & Zhang, W. (2011). Chemical constituents of *Toona ciliata* var. *pubescens*. *Chinese Journal of Natural Medicines*, 9, 115-119.

Liu, Y., Wang, Z., Kong, F., Teng, L., Zheng, X., Liu, X., & Wang, D. (2020). Triterpenoids extracted from *Antrodia cinnamomea* mycelia attenuate acute alcohol-induced liver injury in C57BL/6 mice via suppression inflammatory response. *Frontiers in Microbiology*, 11, 1113.

Lopes, L. C., de Carvalho, J. E., Kakimore, M., Vendramini-Costa, D. B., Medeiros, M. A., Spindola, H. M., Ávila-Román, J., Lourenço, A. M., & Motilva, V. (2014). Pharmacological characterization of *Solanum cernuum* Vell.: 31-norcycloartanones with analgesic and anti-inflammatory properties. *Inflammopharmacology*, 22, 179-185.

Luo, J. G., Liu, J., & Kong, L. Y. (2008). New pentacyclic triterpenes from *Gypsophila oldhamiana* and their biological evaluation as glycogen phosphorylase inhibitors. *Chemistry & Biodiversity*, 5, 751-757.

Machaba, K. E., Cobongela, S. Z., Mosa, R. A., Oladipupo, L. A., Djarova, T. G., & Opoku, A. R. (2014). *In vivo* anti-hyperlipidemic activity of the triterpene from the stem bark of *Protorhus longifolia* (Benrh) Engl. *Lipids in Health and Disease*, 13, 1-7.

Maleki, Z., Jazayeri, S., Eslami, O., Shidfar, F., Hosseini, A. F., Agah, S., & Norouzi, H. (2019). Effect of soy-milk consumption on glycemic status, blood pressure, fibrinogen and malondialdehyde in patients with non-alcoholic fatty liver disease: a randomized controlled trial. *Complementary Therapies in Medicine*, 44, 44-50.

Mosa, Z. M. & Khalil, A. F. (2015). The effect of banana peels supplemented diet on acute liver failure rats. *Annals of Agricultural Sciences*, 60, 373-379.

Musa, W. J., Situmeang, B., & Sianturi, J. (2019). Anti-cholesterol triterpenoid acids from *Saurauia vulcani* Korth (Actinidiaceae). *International Journal of Food Properties*, 22, 1439-1444.

Najima, M., Miyata, A., Kinoshita, K., Horikawa, A., & Kunishige, K. (2016). Hair restoration efficacy of supplements containing banana extract, parthenolide derived from feverfew, and soy isoflavone in healthy Japanese-A randomized, double-blind, placebo-controlled study. *Medical Consultation & New Remedies*, 53, 1028-1036.

Navghare, V. V. & Dhawale, S. C. (2017). *In vitro* antioxidant, hypoglycemic and oral glucose tolerance test of banana peels. *Alexandria Journal of Medicine*, 53, 237-243.

Nazaruk, J. & Borzym-Kluczyk, M. (2015). The role of triterpenes in the management of diabetes mellitus and its complications. *Phytochemistry Reviews*, 14, 675-690.

Neeland, I. J. & Patel, K. V. (2019). Diabetes: key markers of injury and prognosis. *Biomarkers in Cardiovascular Disease*. pp. 41-51. Houston, United States, Elsevier.

Oberoi, H. S., Vadlani, P. V., Saida, L., Bansal, S., & Hughes, J. D. (2011). Ethanol production from banana peels using statistically optimized simultaneous saccharification and fermentation process. *Waste Management*, 31, 1576-1584.

Ochieng, C. O., Nyongesa, D. W., Yamo, K. O., Onyango, J. O., Langat, M. K., & Manguro, L. A. (2020). α-Amylase and α-glucosidase inhibitors from *Zanthoxylum chalybeum* Engl. root bark. *Fitoterapia*, 146, 104719.

Oliveira, L., Freire, C. S. R., Silvestre, A. J. D., Cordeiro, N., Torres, I. C., & Evtuguin, D. (2006). Lipophilic extractives from different morphological parts of banana plant “Dwarf Cavendish”. *Industrial Crops and Products*, 23, 201-211.

Oliveira, L., Freire, C. S., Silvestre, A. J., & Cordeiro, N. (2008). Lipophilic extracts from banana fruit residues: a source of valuable phytosterols. *Journal of Agricultural and Food*

Chemistry, 56, 9520-9524.

Onwuka, C. F. I., Adetiloye, P. O., & Afolami, C. A. (1997). Use of household wastes and crop residues in small ruminant feeding in Nigeria. *Small Ruminant Research*, 24, 233-237.

Papoutsis, K., Zhang, J., Bowyer, M. C., Brunton, N., Gibney, E. R., & Lyng, J. (2021). Fruit, vegetables, and mushrooms for the preparation of extracts with α-amylase and α-glucosidase inhibition properties: A review. *Food Chemistry*, 338, 128119.

Pereira, A. & Maraschin, M. (2015). Banana (*Musa spp*) from peel to pulp: ethnopharmacology, source of bioactive compounds and its relevance for human health. *Journal of Ethnopharmacology*, 160, 149-163.

Pujirahayu, N., Bhattacharjya, D. K., Suzuki, T., & Katayama, T. (2019). α-Glucosidase inhibitory activity of cycloartane-type triterpenes isolated from Indonesian stingless bee propolis and their structure-activity relationship. *Pharmaceuticals*, 12, 102.

Puttarak, P., Brantner, A., & Panichayupakaranant, P. (2016). Biological activities and stability of a standardized pentacyclic triterpene enriched *Centella asiatica* extract. *Natural Product Sciences*, 22, 20-24.

Qian, H., Huang, W. L., Wu, X. M., Zhang, H. B., Zhou, J. P., & Ye, W. C. (2007). A new isochroman-4-one derivative from the peel of *Musa sapientum L.* and its total synthesis. *Chinese Chemical Letters*, 18, 1227-1230.

Ragasa, C. Y., Lim, K. F., Shen, C. C., & Raga, D. D. (2013). Hypoglycemic potential of triterpenes from *Alstonia scholaris*. *Pharmaceutical Chemistry Journal*, 47, 54-57.

Ragasa, C. Y., Martinez, A., Chua, J. E. Y., & Rideout, J. A. (2007). A triterpene from *Musa errans*. *Philippine Journal of Sciences*, 136, 167-171.

Ramachandran, S. & Prasad, N. R. (2008). Effect of ursolic acid, a triterpenoid antioxidant, on ultraviolet-B radiation-induced cytotoxicity, lipid peroxidation and DNA damage in human lymphocytes. *Chemico-Biological Interactions*, 176, 99-107.

Ramu, R., S. Shirahatti, P., Zameer, F., Lakkappa Dhananjaya, B., & MN, N. P. (2016). Assessment of *in vivo* antidiabetic properties of umbelliferone and lupeol constituents of banana (*Musa* sp. var. Nanjangud rasa bale) flower in hyperglycaemic rodent model. *PLoS One*, 11, e0151135.

Ramu, R., Shirahatti, P. S., Zameer, F., Ranganatha, L. V., & Prasad, M. N. (2014). Inhibitory

effect of banana (*Musa* sp. var. *Nanjangud rasa bale*) flower extract and its constituents umbelliferone and lupeol on α-glucosidase, aldose reductase and glycation at multiple stages. *South African Journal of Botany*, 95, 54-63.

Rattanavichai, W. & Cheng, W. (2014). Effects of hot-water extract of banana (*Musa acuminata*) fruit's peel on the antibacterial activity, and anti-hypothermal stress, immune responses and disease resistance of the giant freshwater prawn, *Macrobrachium rosenbegii*. *Fish & Shellfish Immunology*, 39, 326-335.

Rebello, L. P. G., Ramos, A. M., Pertuzatti, P. B., Barcia, M. T., Castillo-Muñoz, N., & Hermosín-Gutiérrez, I. (2014). Flour of banana (*Musa AAA*) peel as a source of antioxidant phenolic compounds. *Food Research International*, 55, 397-403.

Ren, L., Zhou, X., Huang, X., Wang, C., & Li, Y. (2019). The IRS/PI3K/Akt signaling pathway mediates olanzapine-induced hepatic insulin resistance in male rats. *Life Sciences*, 217, 229-236.

Salah El Dine, R., Ma, Q., Kandil, Z. A., & El-Halawany, A. M. (2014). Triterpenes as uncompetitive inhibitors of α-glucosidase from flowers of *Punica granatum L*. *Natural Product Research*, 28, 2191-2194.

Samarakoon, D. N. A. W., Uluwaduge, D. I., & Siriwardhene, M. A. (2020). Mechanisms of action of Sri Lankan herbal medicines used in the treatment of diabetes: A review. *Journal of Integrative Medicine*, 18, 14-20.

Scheepers, A., Joost, H. G., & Schurmann, A. (2004). The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. *Journal of Parenteral and Enteral Nutrition*, 28, 364-371.

Schieber, A., Stintzing, F. C., & Carle, R. (2001). By-products of plant food processing as a source of functional compounds-recent developments. *Trends in Food Science & Technology*, 12, 401-413.

Sheng, Z., Dai, H., Pan, S., Ai, B., Zheng, L., Zheng, X., Prinyawiwatkul, W., & Xu, Z. (2017). Phytosterols in banana (Musa spp.) flower inhibit α-glucosidase and α-amylase hydrolysations and glycation reaction. *International Journal of Food Science & Technology*, 52, 171-179.

Shodehinde, S. A., Ademiluyi, A. O., Oboh, G., & Akindahunsi, A. A. (2015). Contribution of

Musa Paradisiaca in the inhibition of α-amylase, α-glucosidase and angiotensin-I converting enzyme in streptozotocin induced rats. *Life Sciences*, 133, 8-14.

Silva, A. A. S., Morais, S. M., Falcão, M. J. C., Vieira, I. G. P., Ribeiro, L. M., Viana, S. M., Teixeira, M. J., Barreto, F. S., Carvalho, C. A., Cardoso, R. P. A., & Andrade-Junior, H. F. (2014). Activity of cycloartane-type triterpenes and sterols isolated from *Musa paradisiaca* fruit peel against *Leishmania infantum chagasi*. *Phytomedicine*, 21, 1419-1423.

Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2016). Bioactive compounds in banana and their associated health benefits-A review. *Food Chemistry*, 206, 1-11.

Sogaard-Andersen, L., & Valentin-Hansen, P. (1993). Protein-protein interactions in gene regulation: the cAMP-CRP complex sets the specificity of a second DNA-binding protein, the CytR repressor. *Cell*, 75, 557-566.

Srisurichan, S., & Pornpakakul, S. (2015). Triterpenoids from the seedpods of *Holarrhena curtisii* King and Gamble. *Phytochemistry Letters*, 12, 282-286.

Sudhahar, V., Kumar, S. A., Sudharsan, P. T., & Varalakshmi, P. (2007). Protective effect of lupeol and its ester on cardiac abnormalities in experimental hypercholesterolemia. *Vascular Pharmacology*, 46, 412-418.

Svensson, E. I., Kristoffersen, L., Oskarsson, K., & Bensch, S. (2004). Molecular population divergence and sexual selection on morphology in the banded demoiselle (Calopteryx splendens). *Heredity*, 93, 423-433.

Tadera, K., Minami, Y., Takamatsu, K., & Matsuoka, T. (2006). Inhibition of α-glucosidase and α-amylase by flavonoids. *Journal of nutritional science and vitaminology*, 52, 149-153.

Tahrani, A. A., Bailey, C. J., Del Prato, S., & Barnett, A. H. (2011). Management of type 2 diabetes: new and future developments in treatment. *The Lancet*, 378, 182-197.

Tai, Y., Zou, F., Zhang, Q., Wang, J., Rao, R., Xie, R., Wu, S., Chu, K., Xu, W., Li, X., & Huang, M. (2019). Quantitative analysis of eight triterpenoids and two sesquiterpenoids in *Rhizoma Alismatis* by using UPLC-ESI/APCI-MS/MS and Its application to optimisation of best harvest time and crude processing temperature. *Journal of Analytical Methods in Chemistry*, 8320171.

Teeri, T. T. (1991). Engineering of enzymes of carbohydrate metabolism. *Current Opinion in*

Biotechnology, 2, 614-621.

Vasu, P., Khan, N. D., Khan, Z. H., & Mular, S. M. (2017). *In vitro* antidiabetic activity of methanolic extract of *Citrus limon, Punica granatum, Musa acuminata* peel. *International Journal of Applied Research*, 3, 804-806.

Vilhena, R. O., Figueiredo, I. D., Baviera, A. M., Silva, D. B., Marson, B. M., Oliveira, J. A., Peccinini, R.G., Borges, I.K., & Pontarolo, R. (2020). Antidiabetic activity of *Musa x paradisiaca* extracts in streptozotocin-induced diabetic rats and chemical characterization by HPLC-DAD-MS. *Journal of Ethnopharmacology*, 254, 112666.

Villaverde, J. J., Oliveira, L., Vilela, C., Domingues, R. M., Freitas, N., Cordeiro, N., Freire, C. S., & Silvestre, A. J. (2013). High valuable compounds from the unripe peel of several *Musa* species cultivated in Madeira Island (Portugal). *Industrial Crops and Products*, 42, 507-512.

Wen, X., Sun, H., Liu, J., Wu, G., Zhang, L., Wu, X., & Ni, P. (2005). Pentacyclic triterpenes. Part 1: The first examples of naturally occurring pentacyclic triterpenes as a new class of inhibitors of glycogen phosphorylases. *Bioorganic & Medicinal Chemistry Letters*, 15, 4944-4948.

Wu, H., Xu, F., Hao, J., Yang, Y., & Wang, X. (2015). Antihyperglycemic activity of banana (*Musa nana Lour.*) peel and its active ingredients in alloxan-induced diabetic mice. *3rd International Conference on Material, Mechanical and Manufacturing Engineering*, pp. 231-238, Atlantis Press.

Xiao, Z. Z., Storms, R., & Tsang, A. (2006). A quantitative starch-Iodine method for measuring alpha-amylase and glucoamylase activities. *Analytical Biochemistry*, 351, 146-148.

Yan, J., Zhang, G., Pan, J., & Wang, Y. (2014). α-Glucosidase inhibition by luteolin: kinetics, interaction and molecular docking. *International Journal of Biological Macromolecules*, 64, 213-223.

Yang, J., Chen, H., Zhang, L., Wang, Q., & Lai, M. X. (2010). Anti-diabetic effect of standardized extract of *Potentilla discolor* Bunge and identification of its active components. *Drug Development Research*, 71, 127-132.

Yasukawa, K., Akihisa, T., Oinuma, H., Kasahara, Y., Kimura, Y., Yamanouchi, S., Kumaki, K., Tamura, T., & Takido, M. (1996). Inhibitory effect of di-and trihydroxy triterpenes from

the flowers of compositae on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation in mice. *Biological and Pharmaceutical Bulletin*, 19, 1329-1331.

Yin, M. C., Lin, M. C., Mong, M. C., & Lin, C. Y. (2012). Bioavailability, distribution, and antioxidative effects of selected triterpenes in mice. *Journal of Agricultural and Food Chemistry*, 60, 7697-7701.

Yin, Y., Zhang, Y., Li, H., Zhao, Y., Cai, E., Zhu, H., Li, P., & Liu, J. (2019). Triterpenoids from fruits of *Sorbus pohuashanensis* inhibit acetaminophen-induced acute liver injury in mice. *Biomedicine & Pharmacotherapy*, 109, 493-502.

Yonemoto, R., Shimada, M., Gunawan-Puteri, M. D., Kato, E., & Kawabata, J. (2014). α-Amylase inhibitory triterpene from *Abrus precatorius* leaves. *Journal of Agricultural and Food Chemistry*, 62, 8411-8414.

Zeng, Y. F., Lü, Z. R., Yan, L., Oh, S., Yang, J. M., Lee, J., & Ye, Z. M. (2012). Towards alpha-glucosidase folding induced by trifluoroethanol: kinetics and computational prediction. *Process Biochemistry*, 47, 2284-2290.

Zhang, B. W., Xing, Y., Wen, C., Yu, X. X., Sun, W. L., Xiu, Z. L., & Dong, Y. S. (2017). Pentacyclic triterpenes as α-glucosidase and α-amylase inhibitors: structure-activity relationships and the synergism with acarbose. *Bioorganic & Medicinal Chemistry Letters*, 27, 5065-5070.

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Appendix

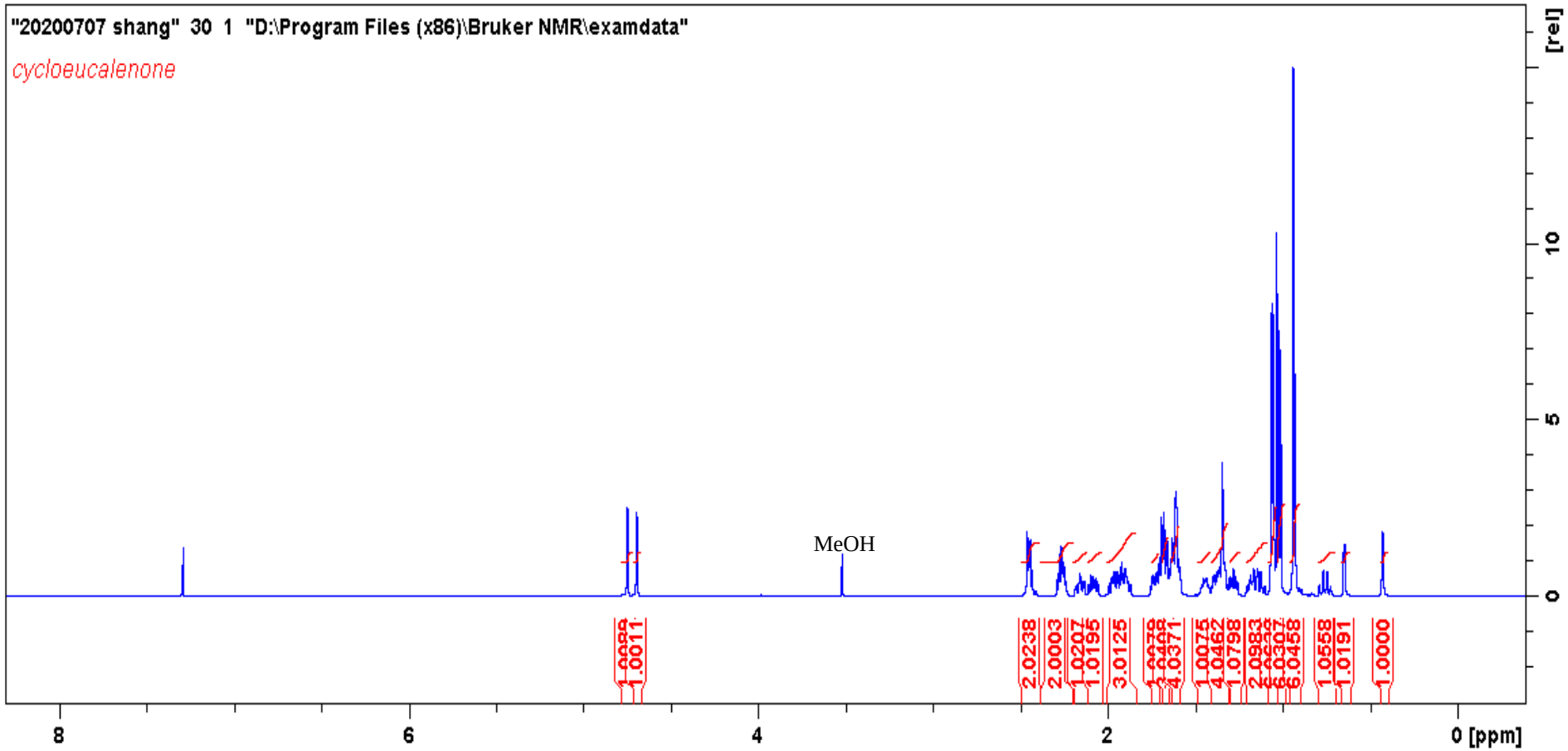

Fig. 1 ^{1}H NMR spectrum for CE-one (600 MHz NMR in $CDCl_3$).

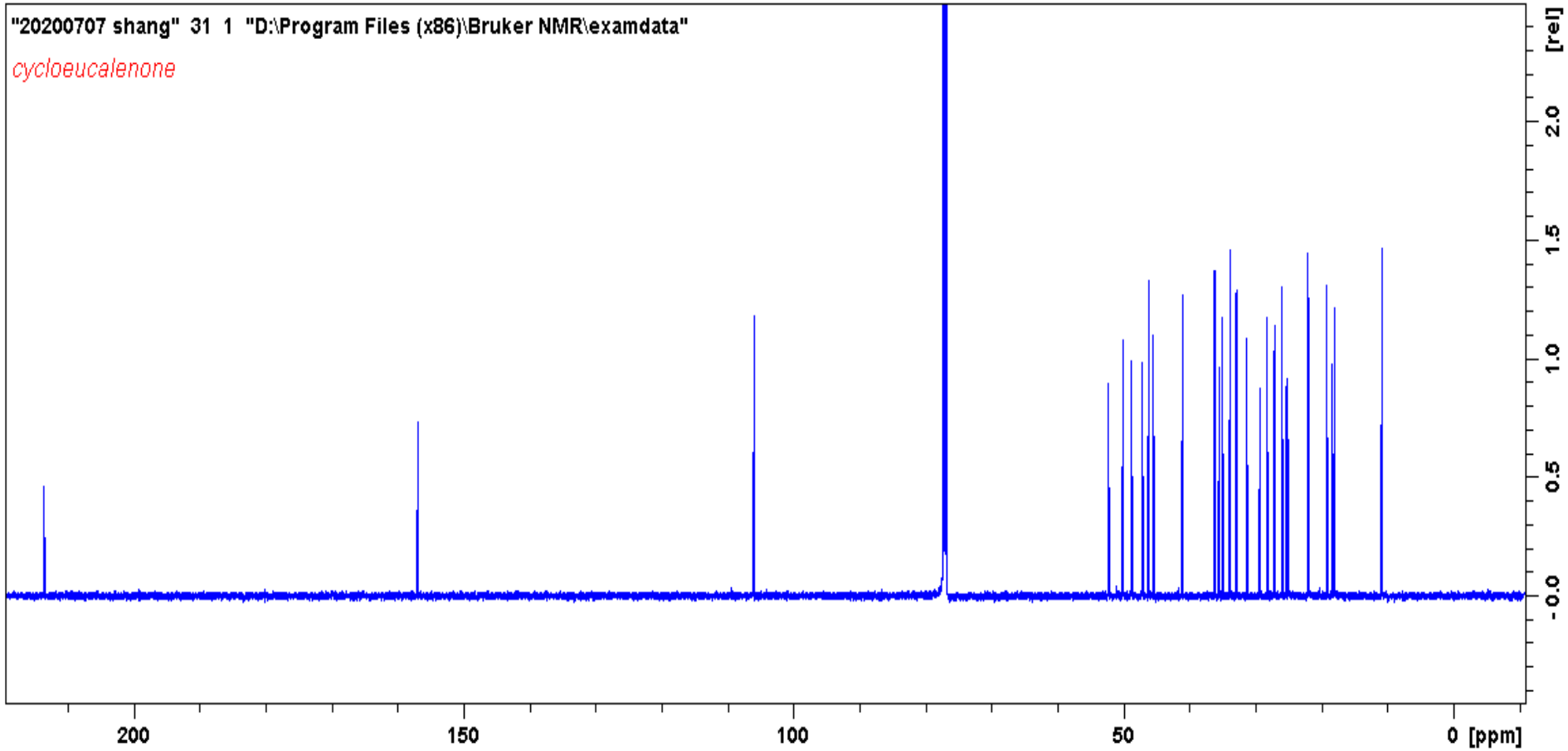

Fig. 2 ^{13}C NMR spectrum for CE-one (150 MHz NMR in $CDCl_3$).

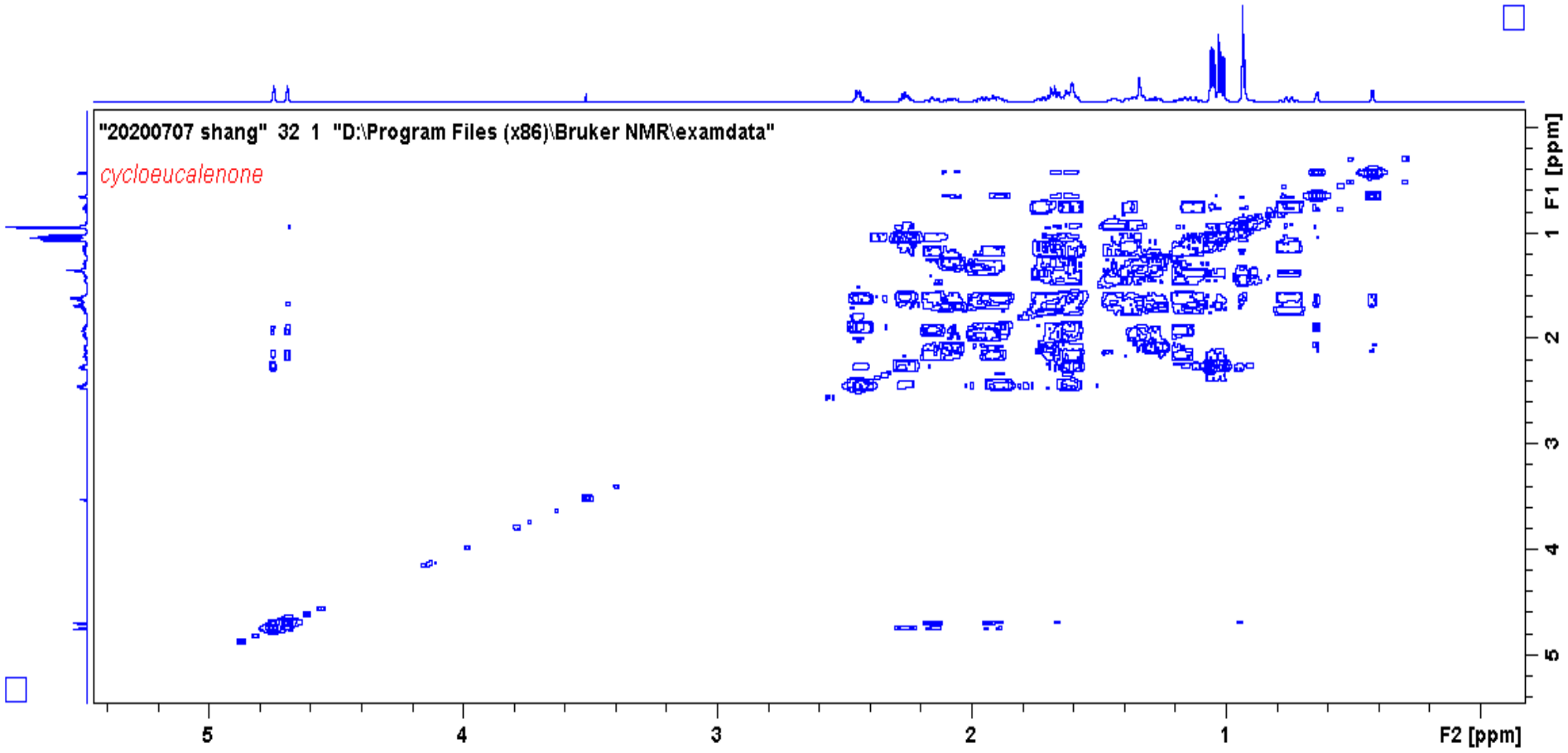

Fig. 3 COSY (2D NMR spectrum) for CE-one (600 MHz NMR in $CDCl_3$).

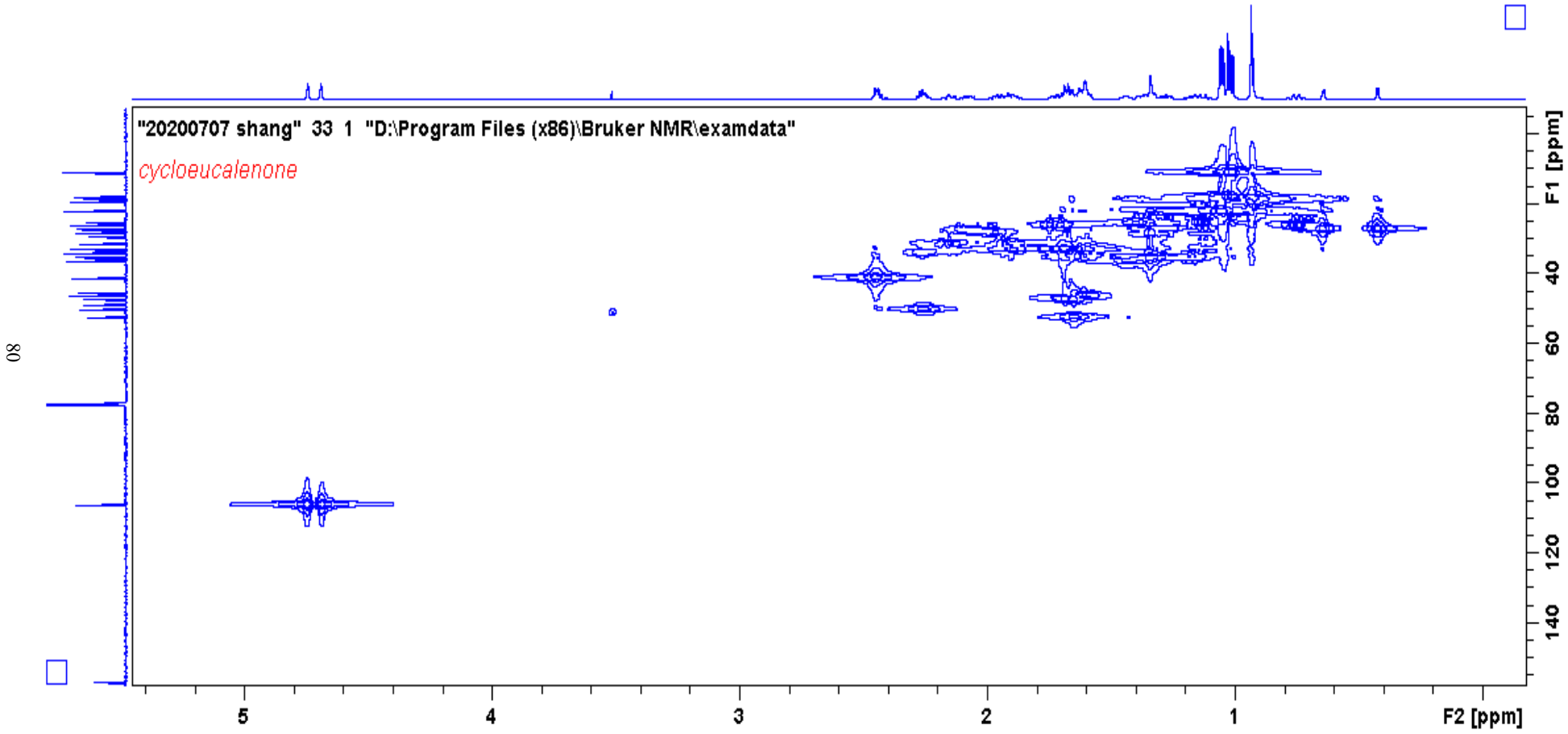

Fig. 4 HMQC (2D NMR spectrum) for CE-one (600 MHz NMR in $CDCl_3$).

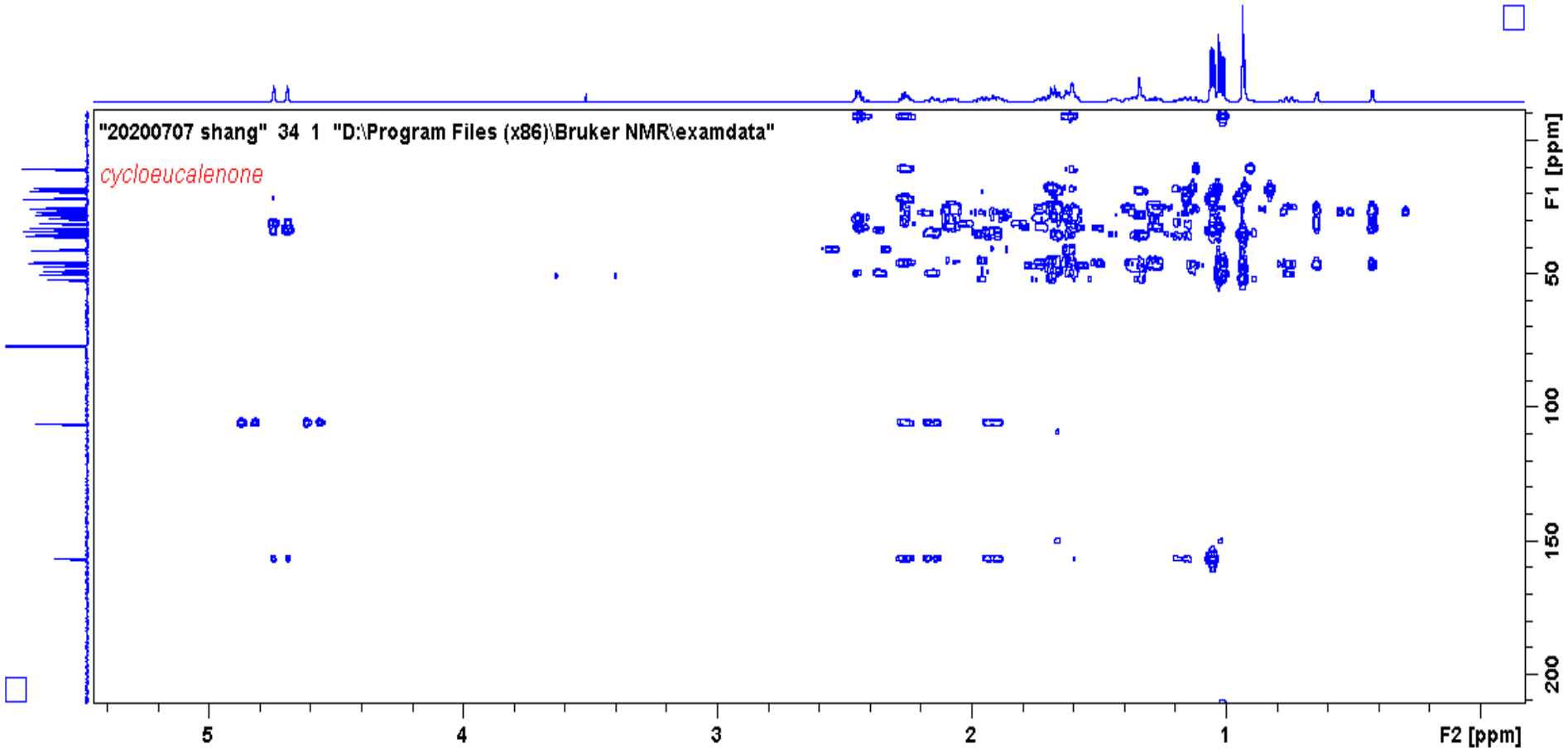

Fig. 5 HMBC (2D NMR spectrum) for CE-one (600 MHz NMR in $CDCl_3$).

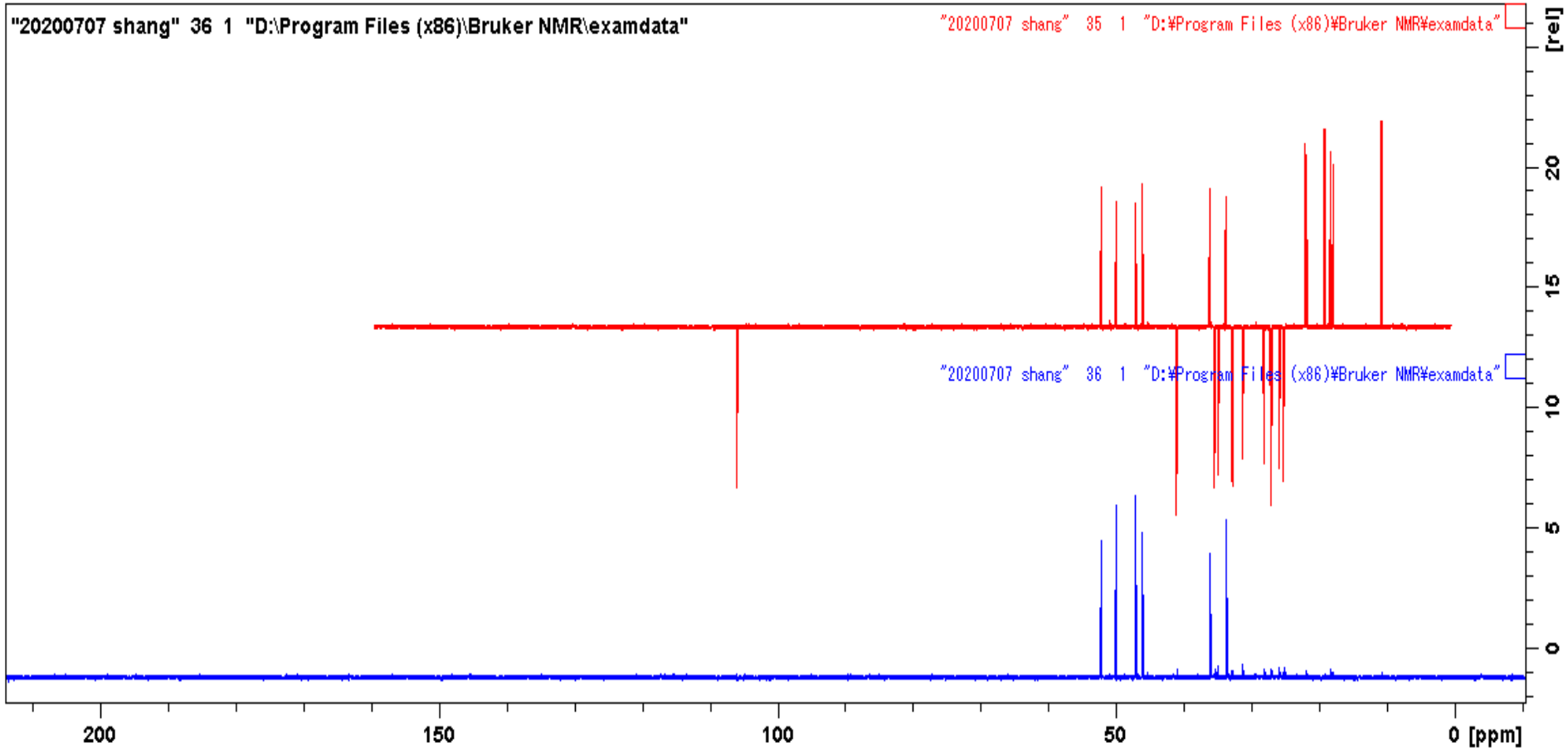

Fig. 6 DEPT 135 (top) and DEPT 90 (bottom) for CE-one (150 MHz NMR in $CDCl_3$).

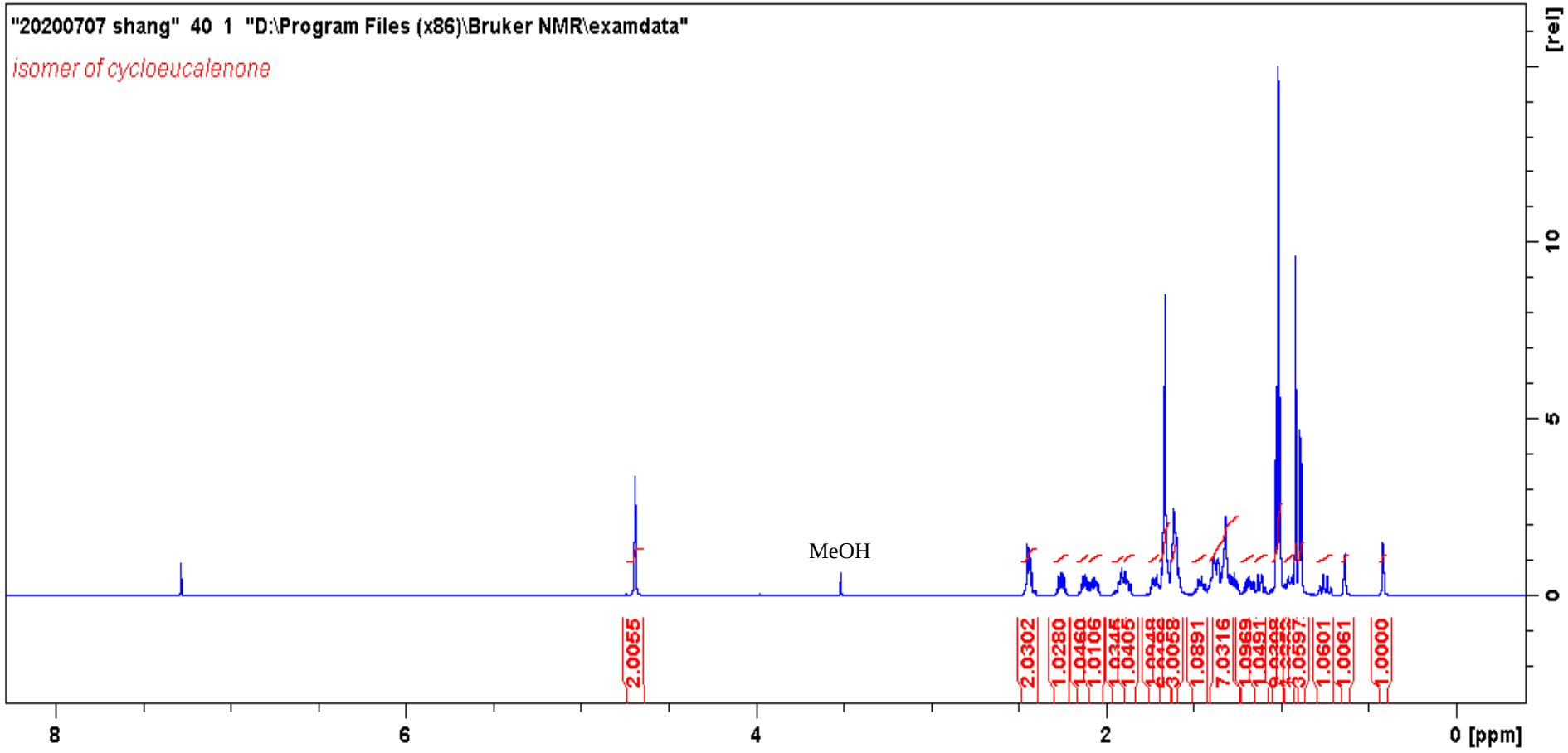

Fig. 7 ^{1}H NMR spectrum for isoCE-one (600 MHz NMR in $CDCl_3$).

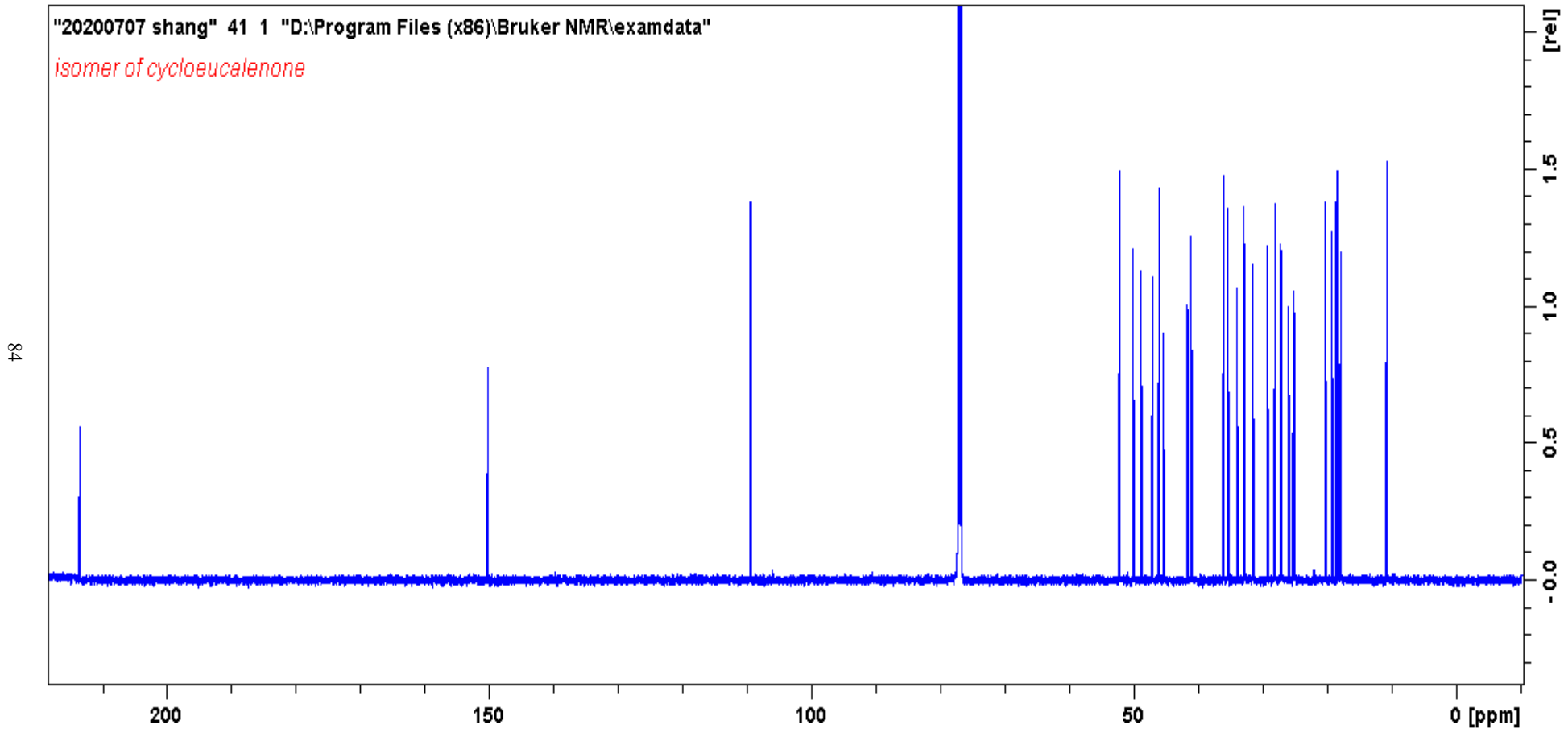

Fig. 8 ^{13}C NMR spectrum for isoCE-one (150 MHz NMR in $CDCl_3$).

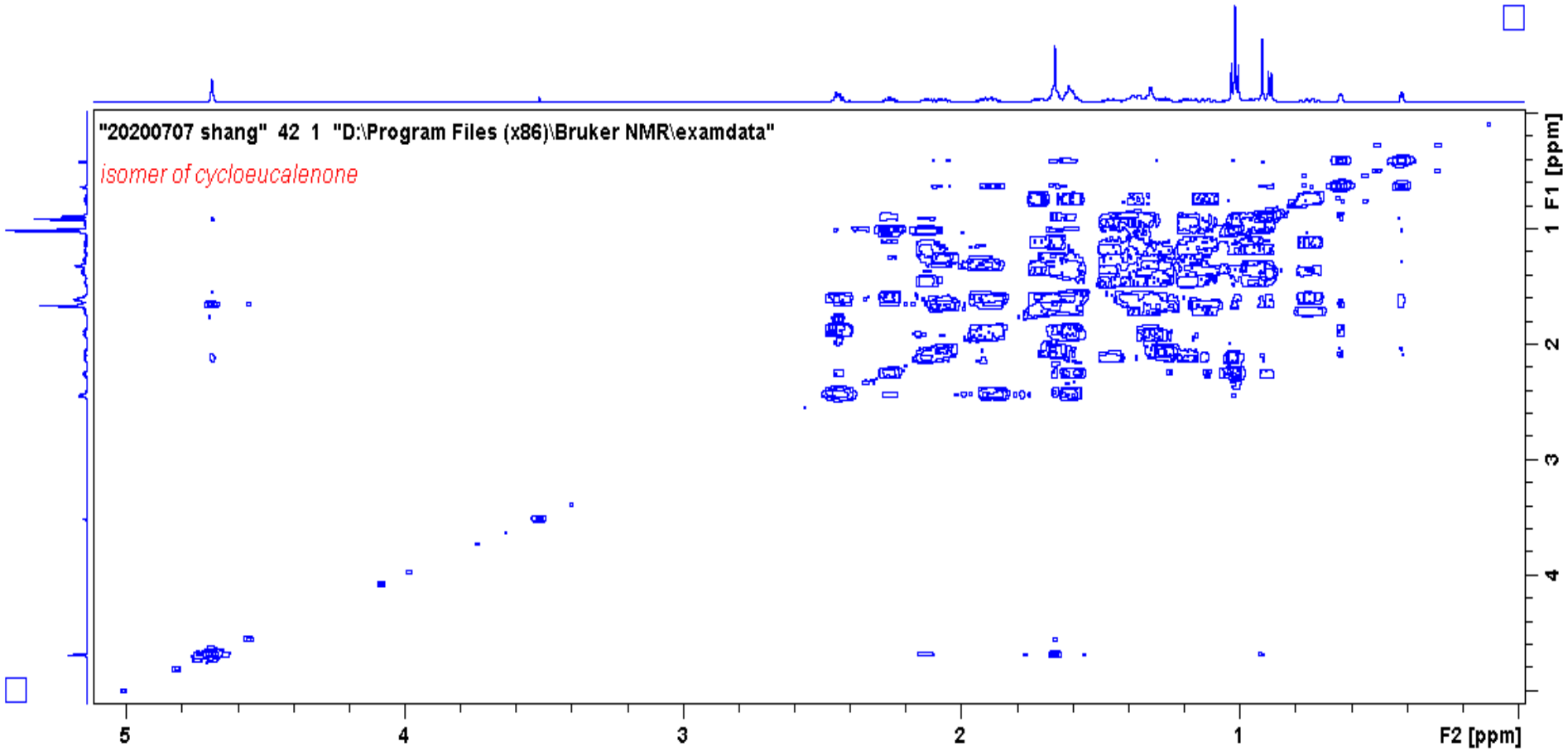

Fig. 9 COSY (2D NMR spectrum) for isoCE-one (600 MHz NMR in $CDCl_3$).

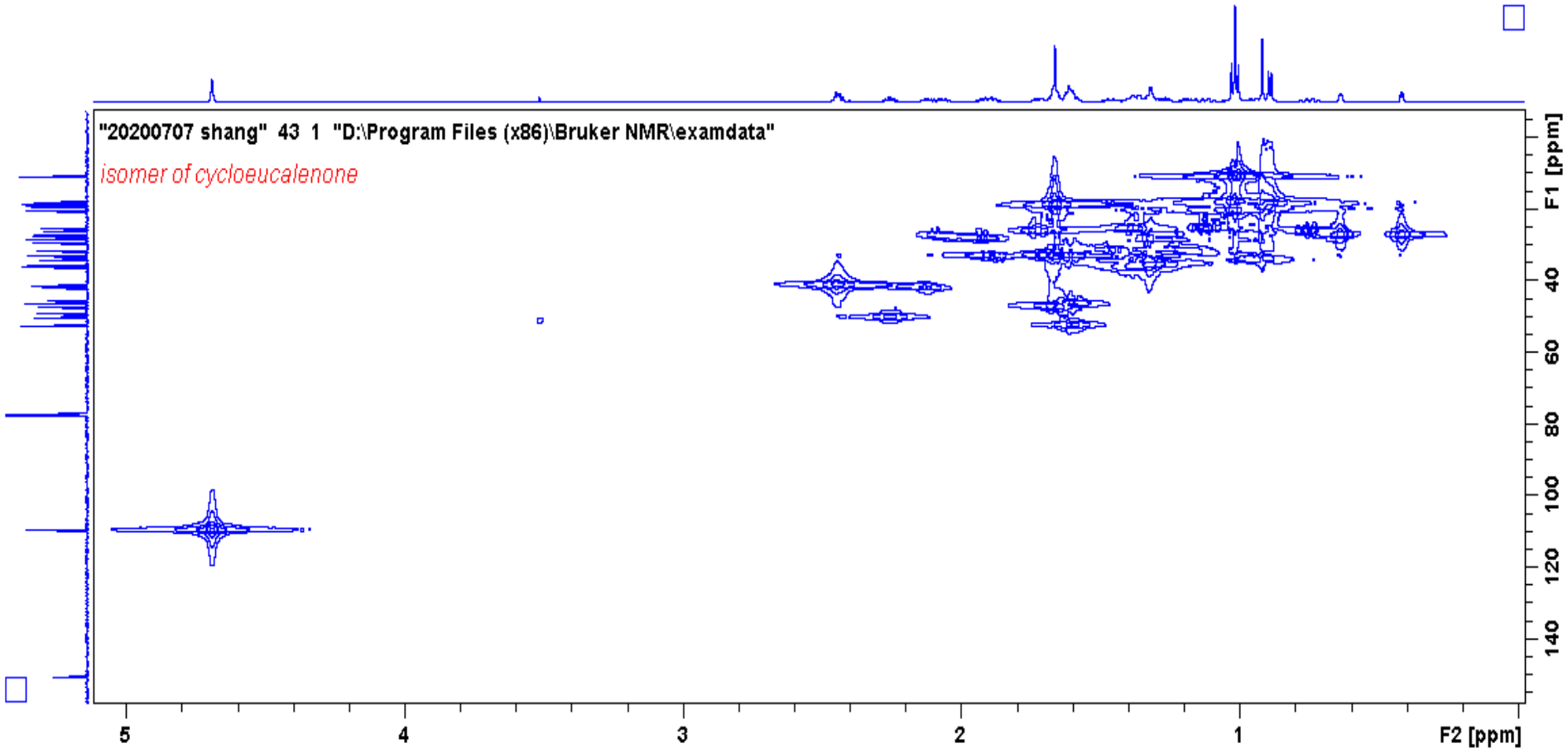

Fig. 10 HMQC (2D NMR spectrum) for isoCE-one (600 MHz NMR in $CDCl_3$).

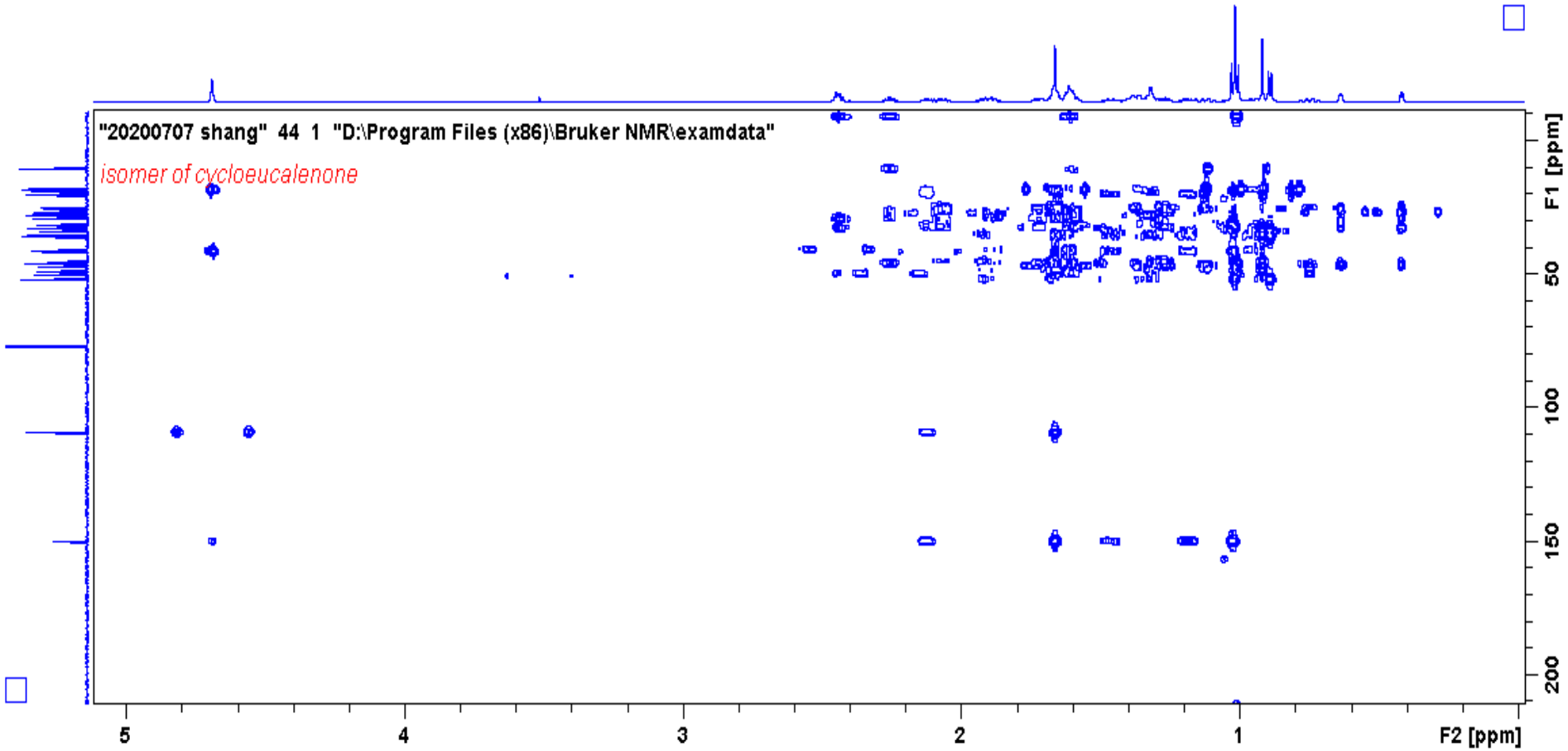

Fig. 11 HMBC (2D NMR spectrum) for isoCE-one (600 MHz NMR in $CDCl_3$).

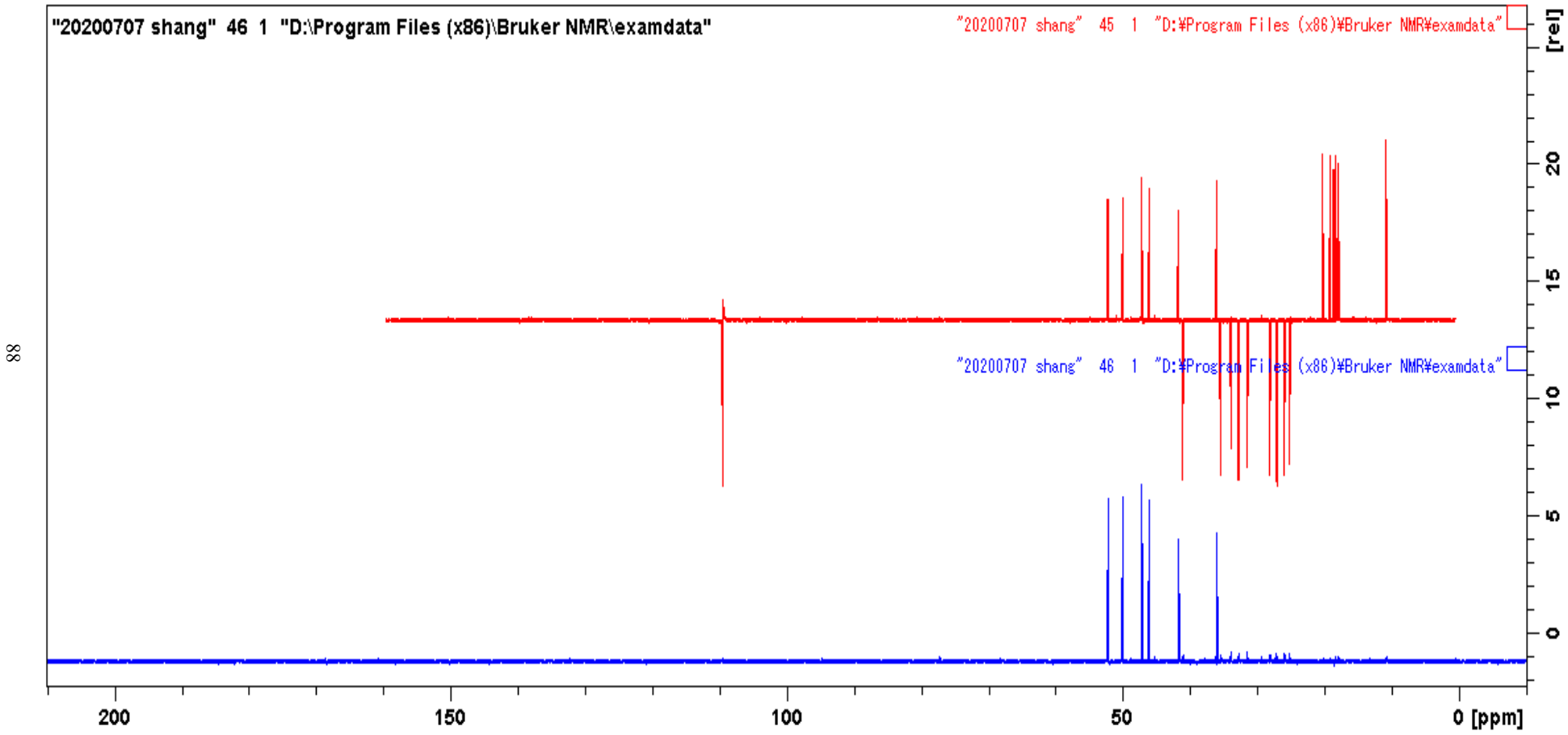

Fig. 12 DEPT 135 (top) and DEPT 90 (bottom) for isoCE-one (150 MHz NMR in $CDCl_3$).

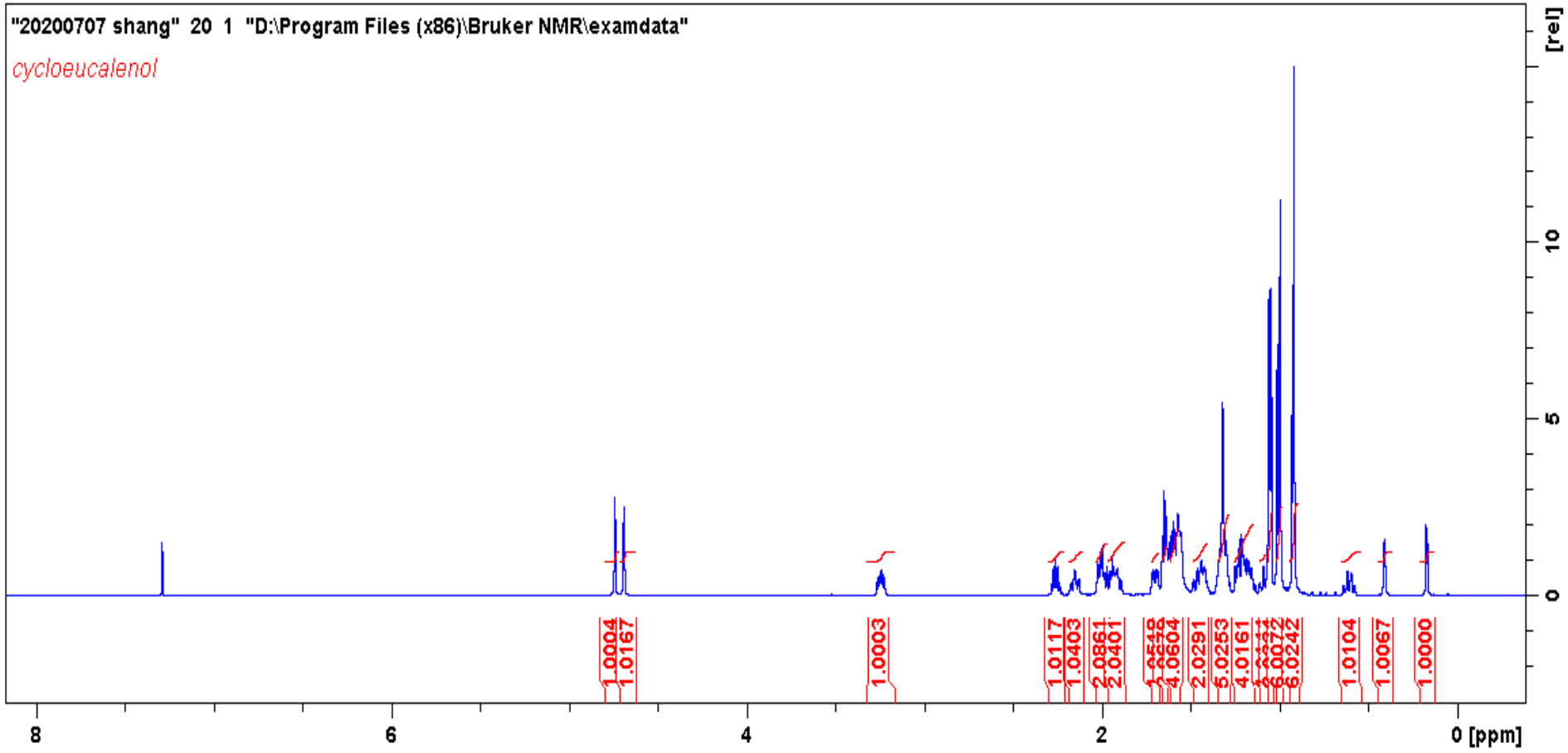

Fig. 13 ^{1}H NMR spectrum for CE-ol (600 MHz NMR in $CDCl_3$).

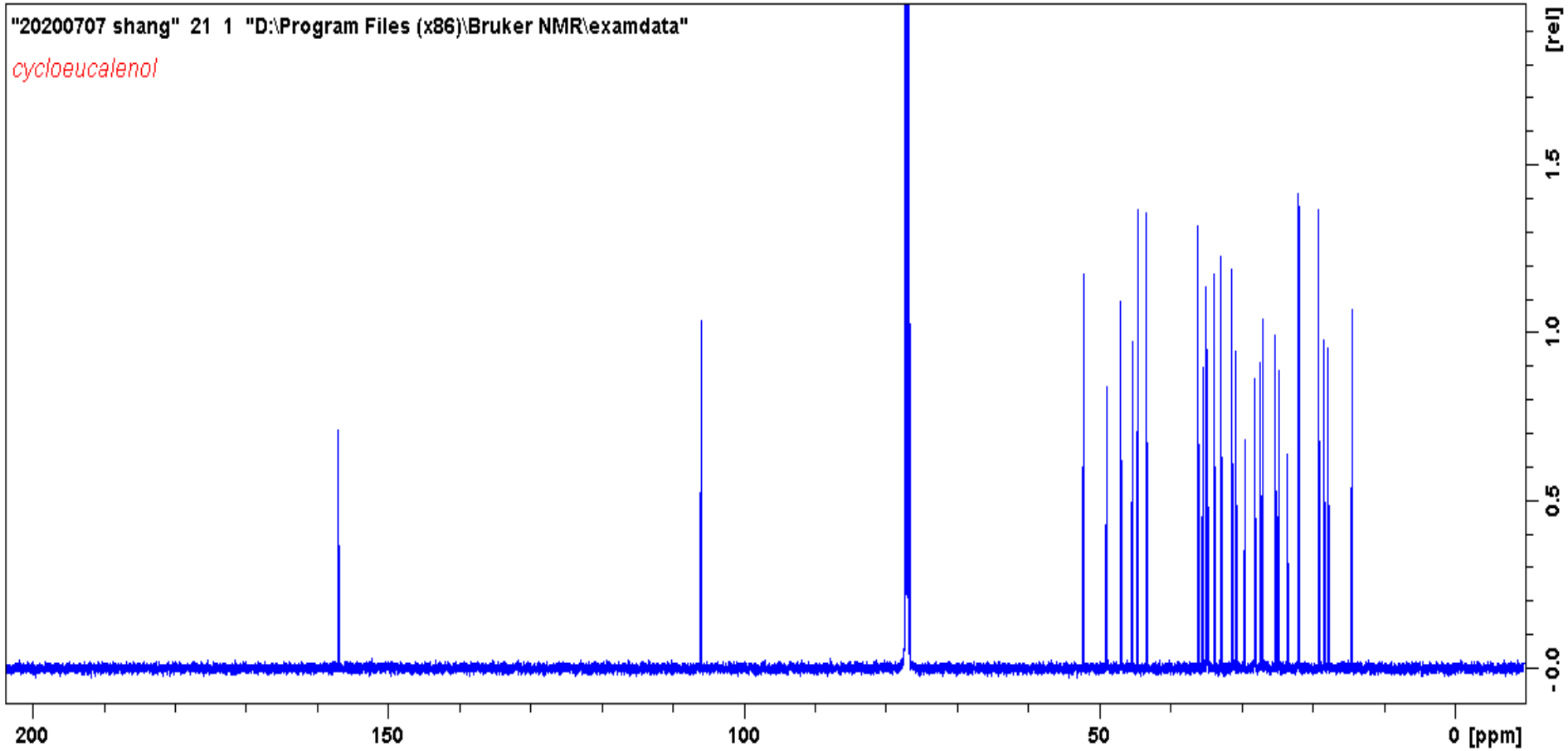

Fig. 14 ^{13}C NMR spectrum for CE-ol (150 MHz NMR in $CDCl_3$).

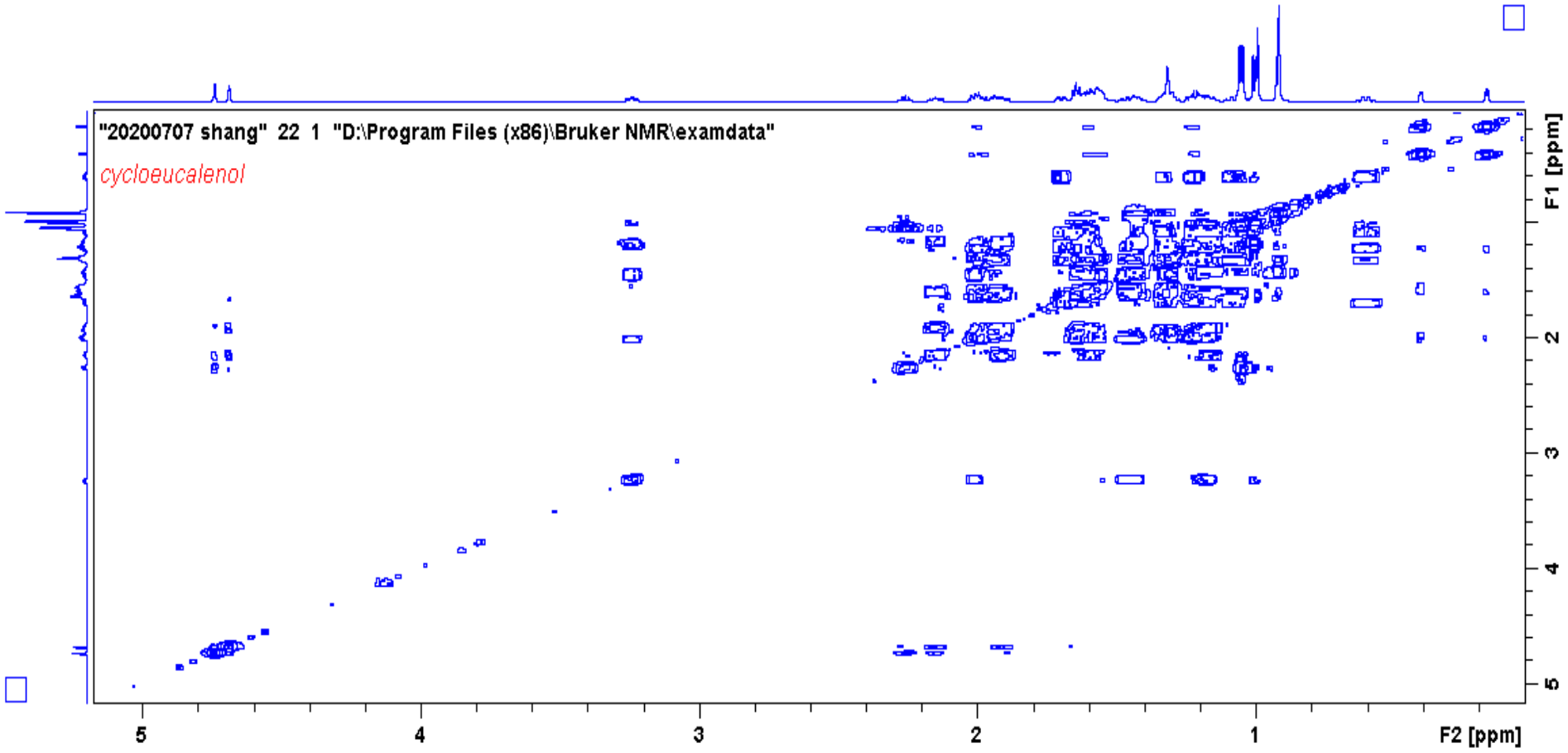

Fig. 15 COSY (2D NMR spectrum) for CE-ol (600 MHz NMR in $CDCl_3$).

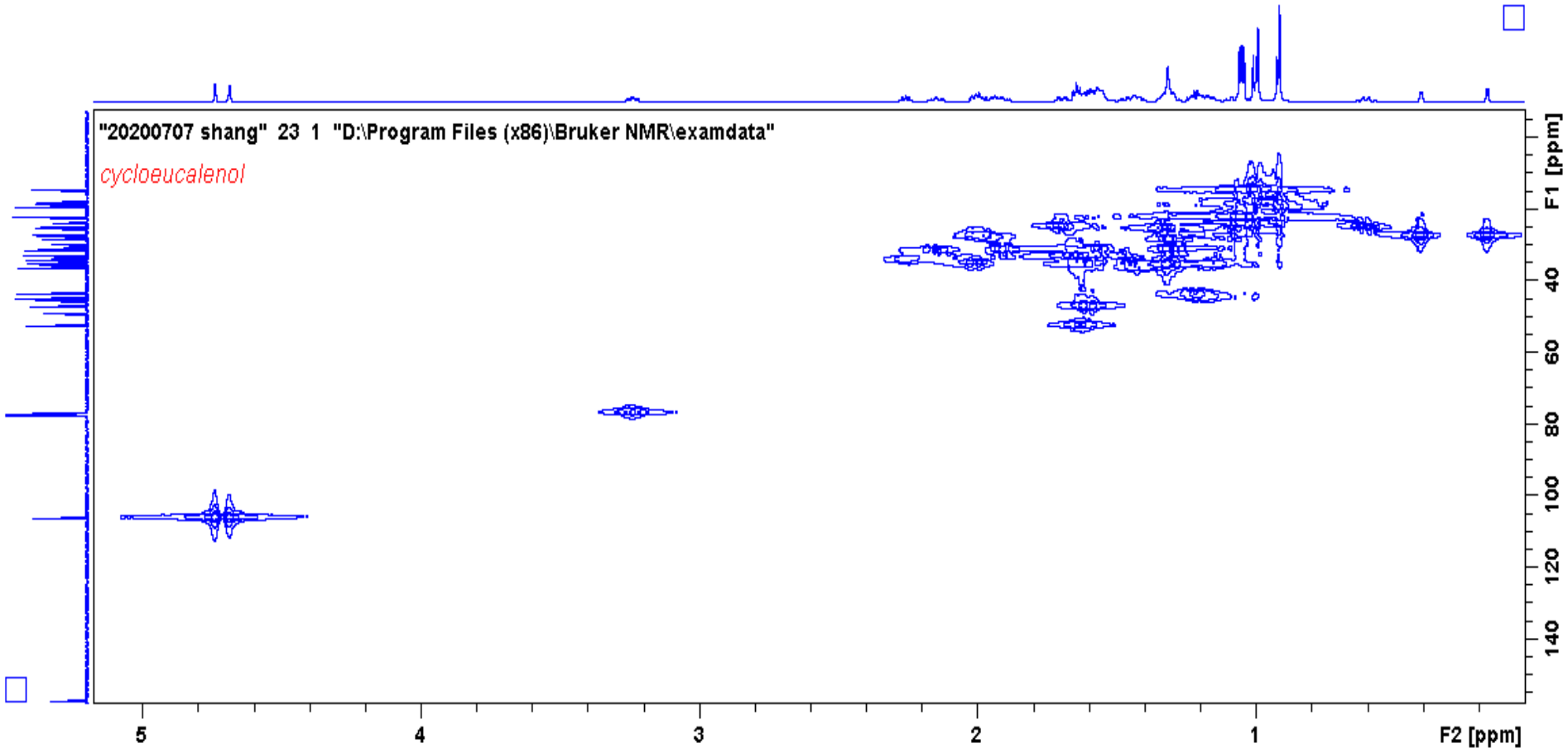

Fig. 16 HMQC (2D NMR spectrum) for CE-ol (600 MHz NMR in $CDCl_3$).

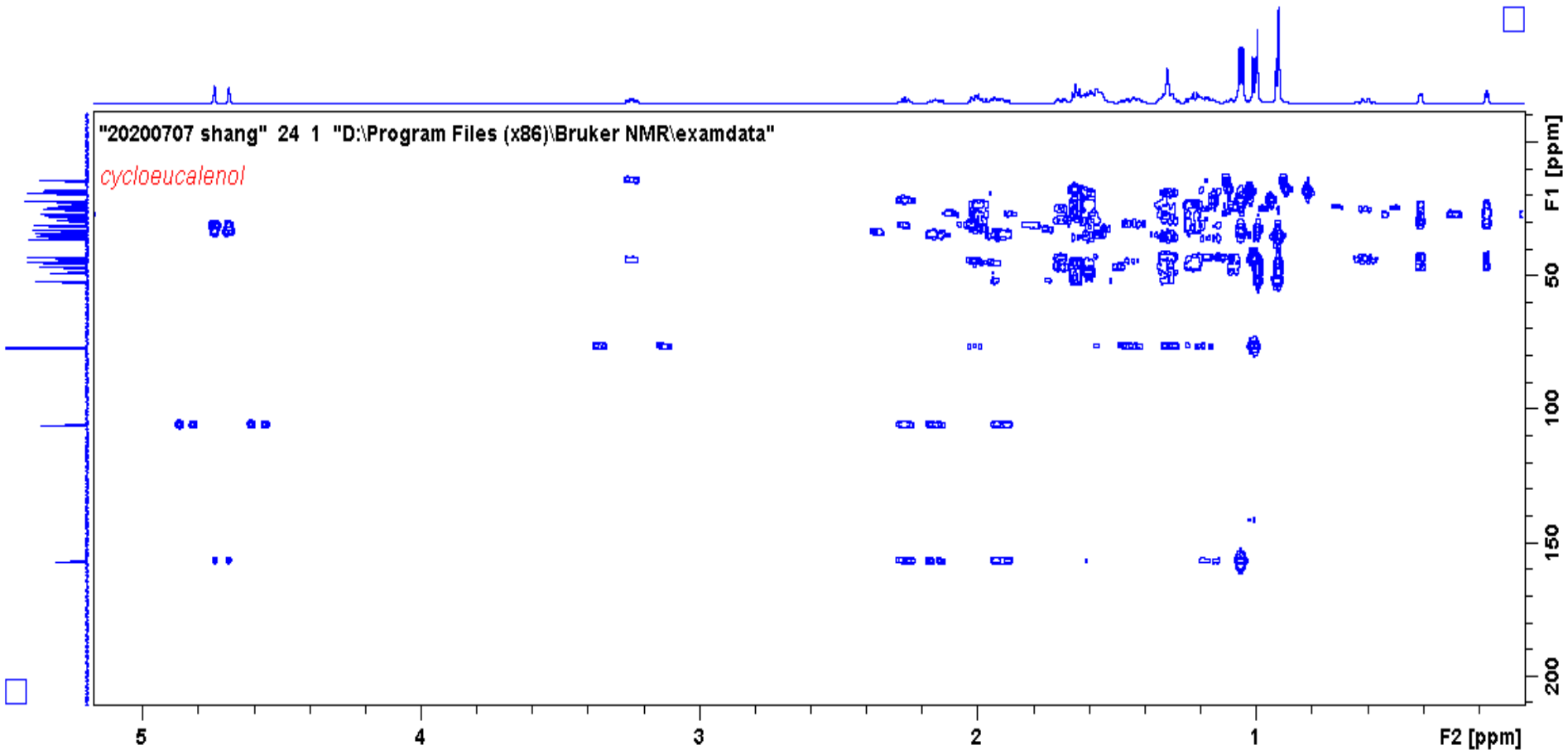

Fig. 17 HMBC (2D NMR spectrum) for CE-ol (600 MHz NMR in $CDCl_3$).

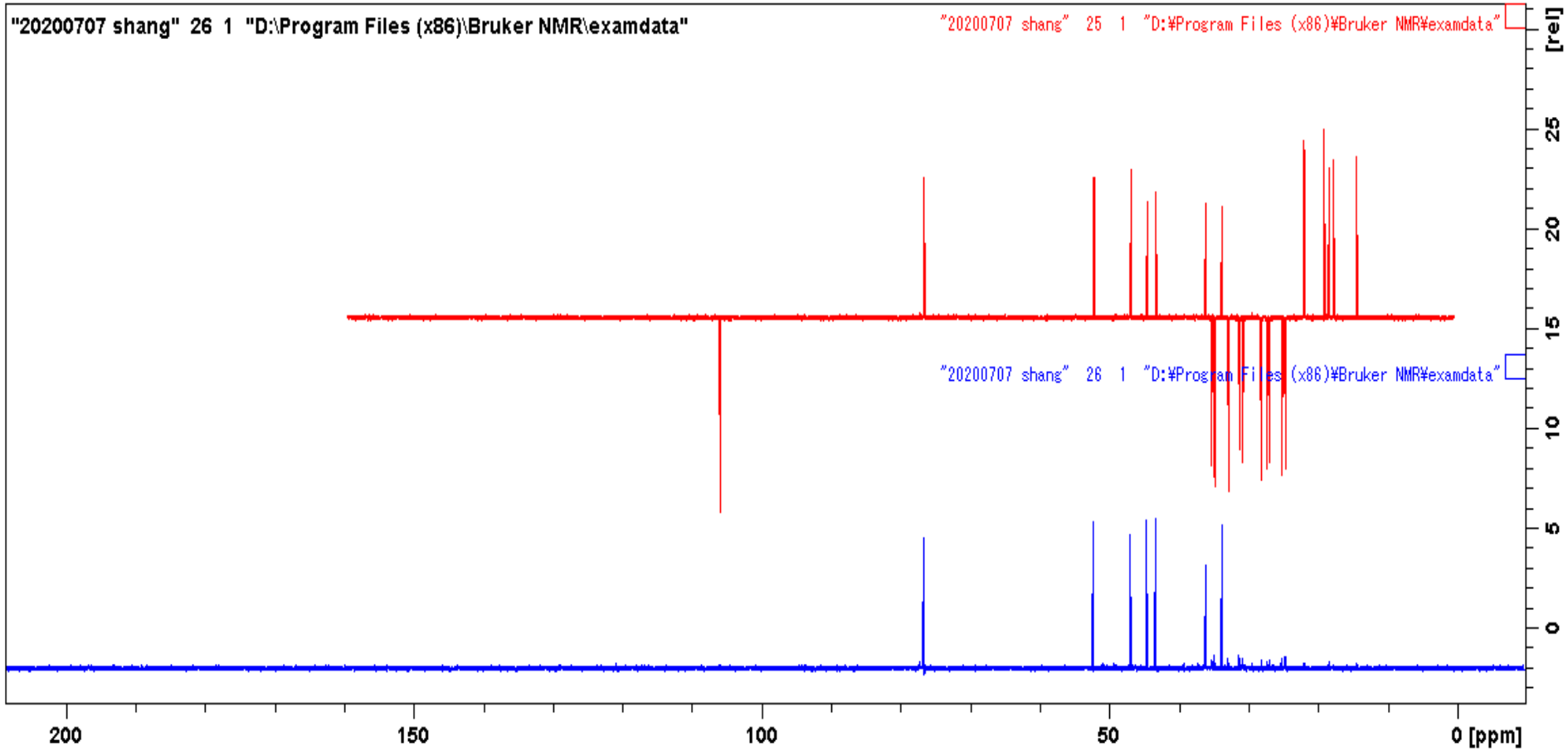

Fig. 18 DEPT 135 (top) and DEPT 90 (bottom) for CE-ol (150 MHz NMR in $CDCl_3$).

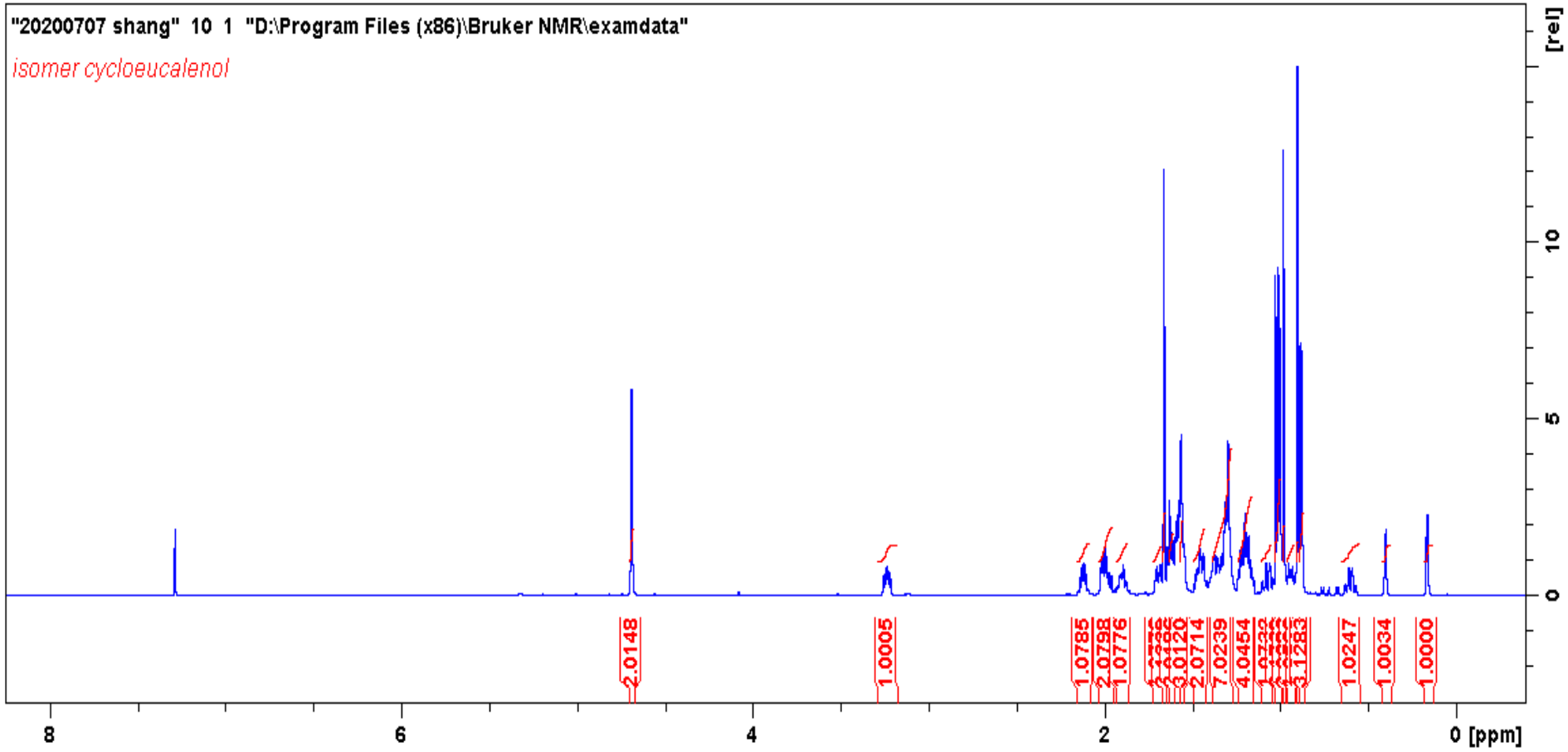

Fig. 19 ^{1}H NMR spectrum for isoCE-ol (600 MHz NMR in $CDCl_3$).

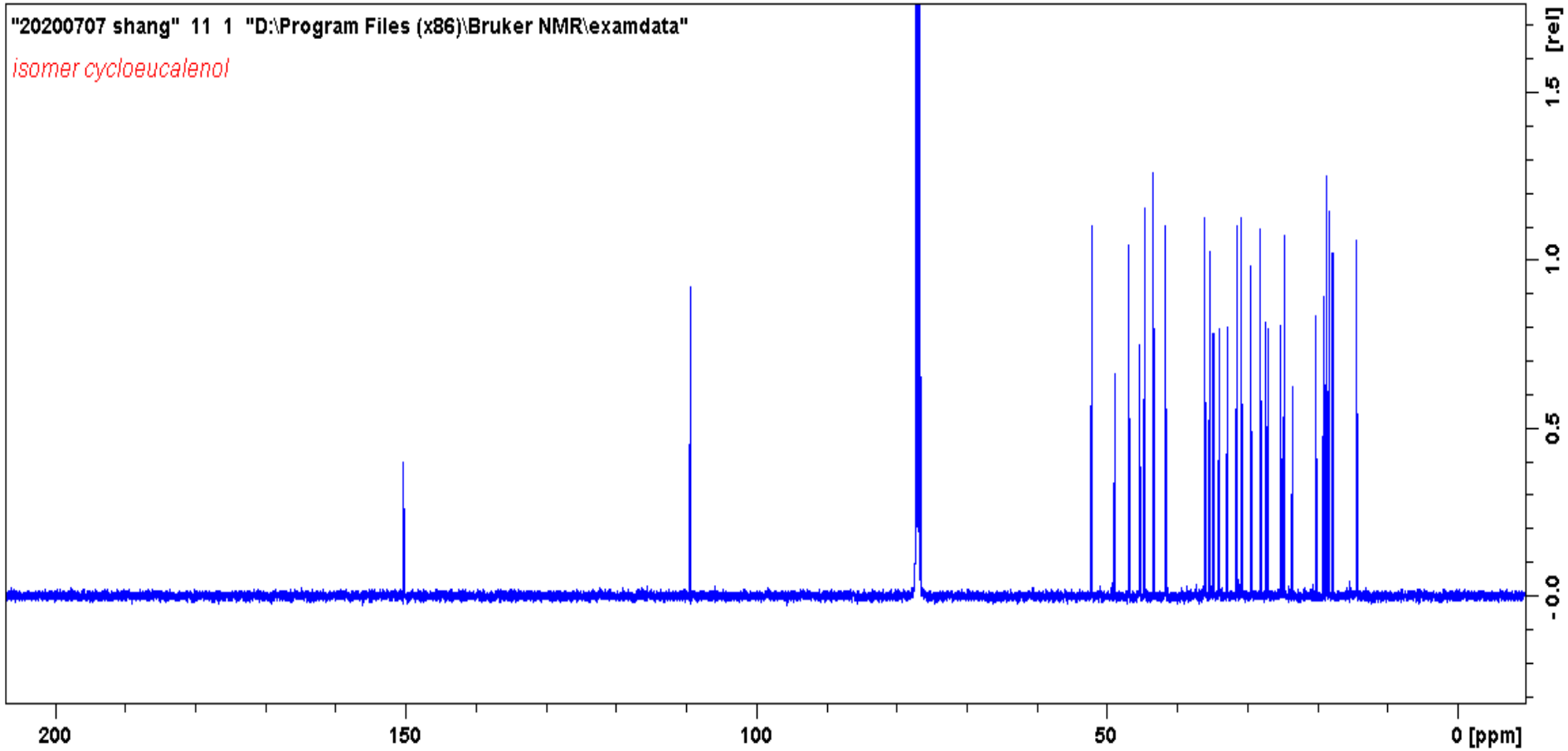

Fig. 20 ^{13}C NMR spectrum for isoCE-ol (150 MHz NMR in $CDCl_3$).

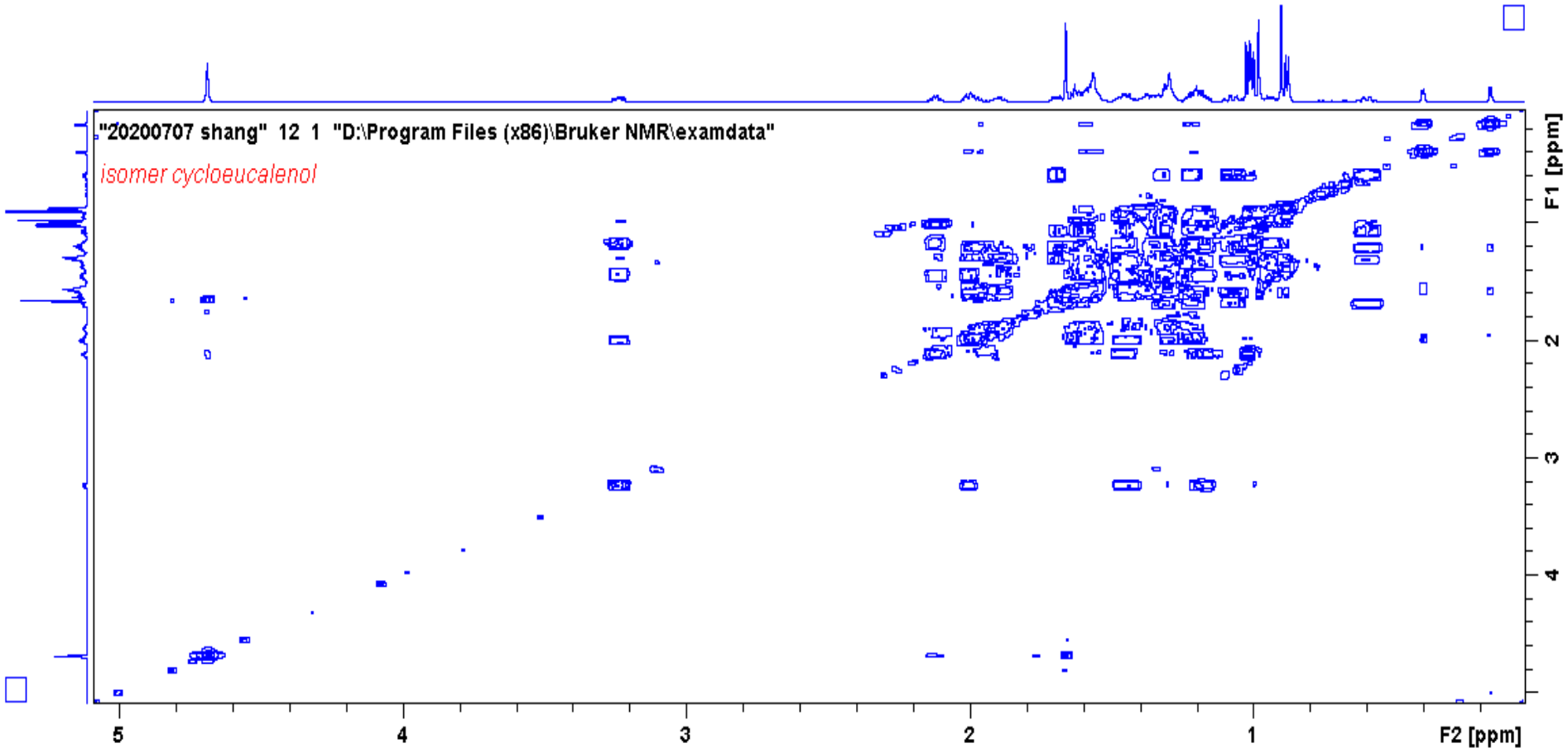

Fig. 21 COSY (2D NMR spectrum) for isoCE-ol (600 MHz NMR in $CDCl_3$).

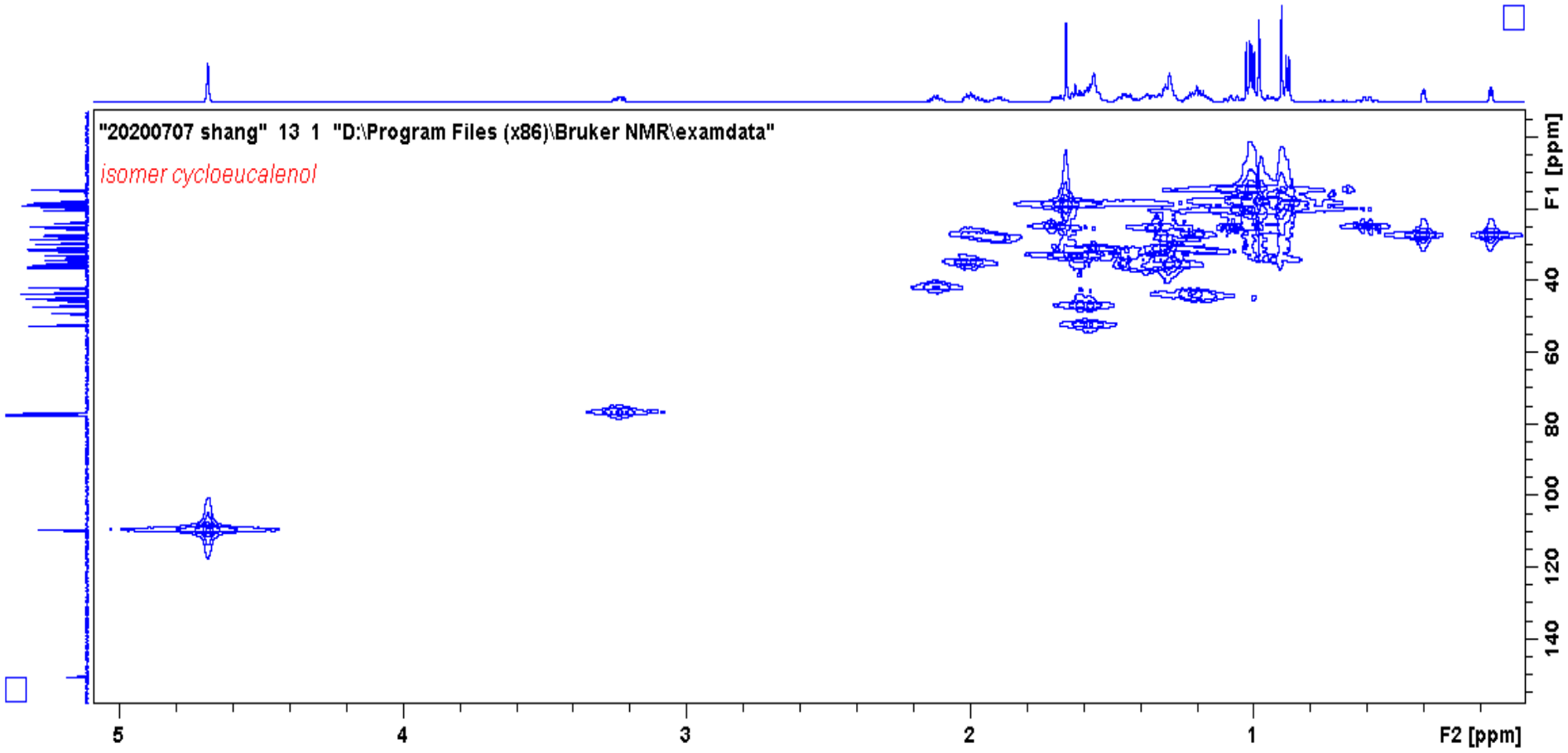

Fig. 22 HMQC (2D NMR spectrum) for isoCE-ol (600 MHz NMR in $CDCl_3$).

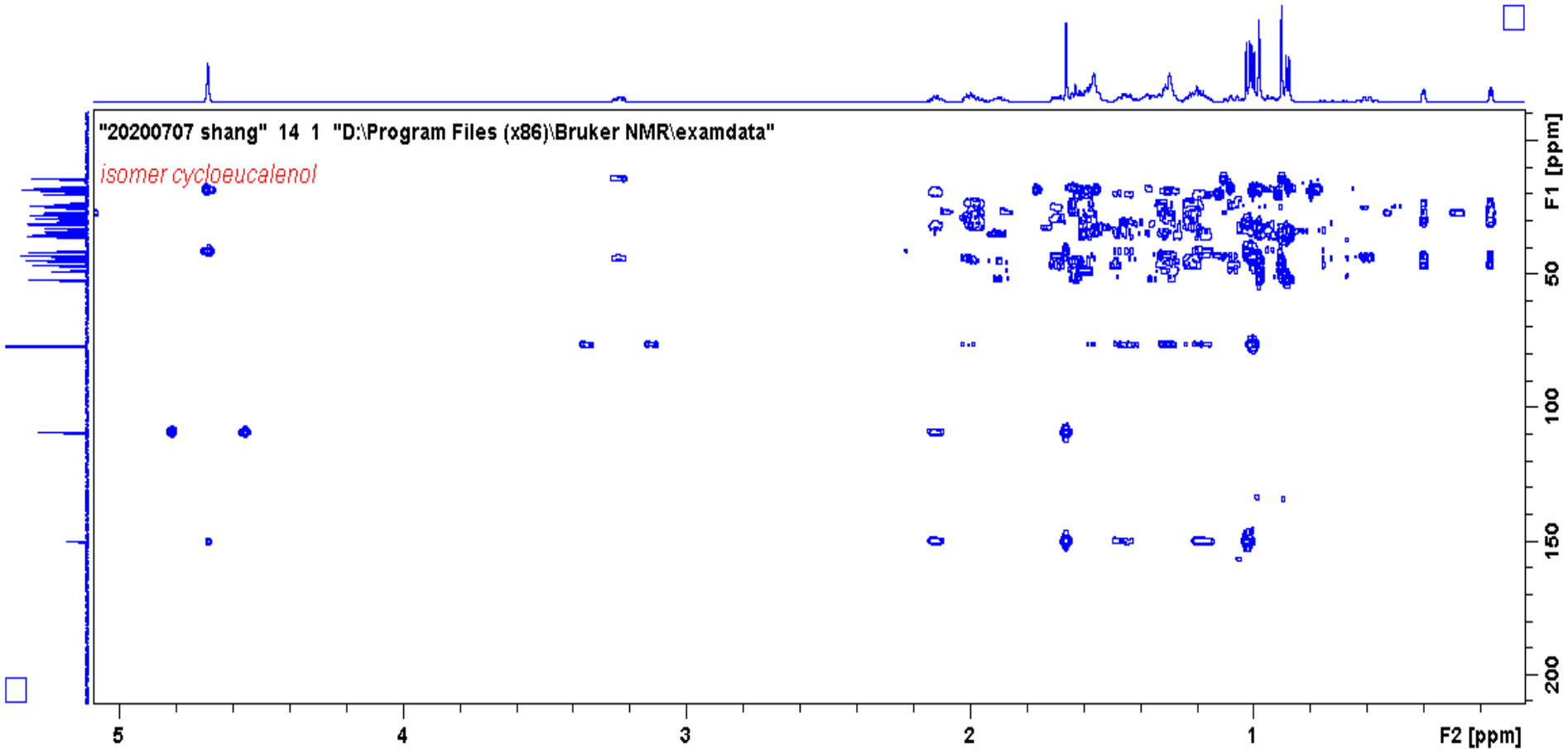

Fig. 23 HMBC (2D NMR spectrum) for isoCE-ol (600 MHz NMR in $CDCl_3$).

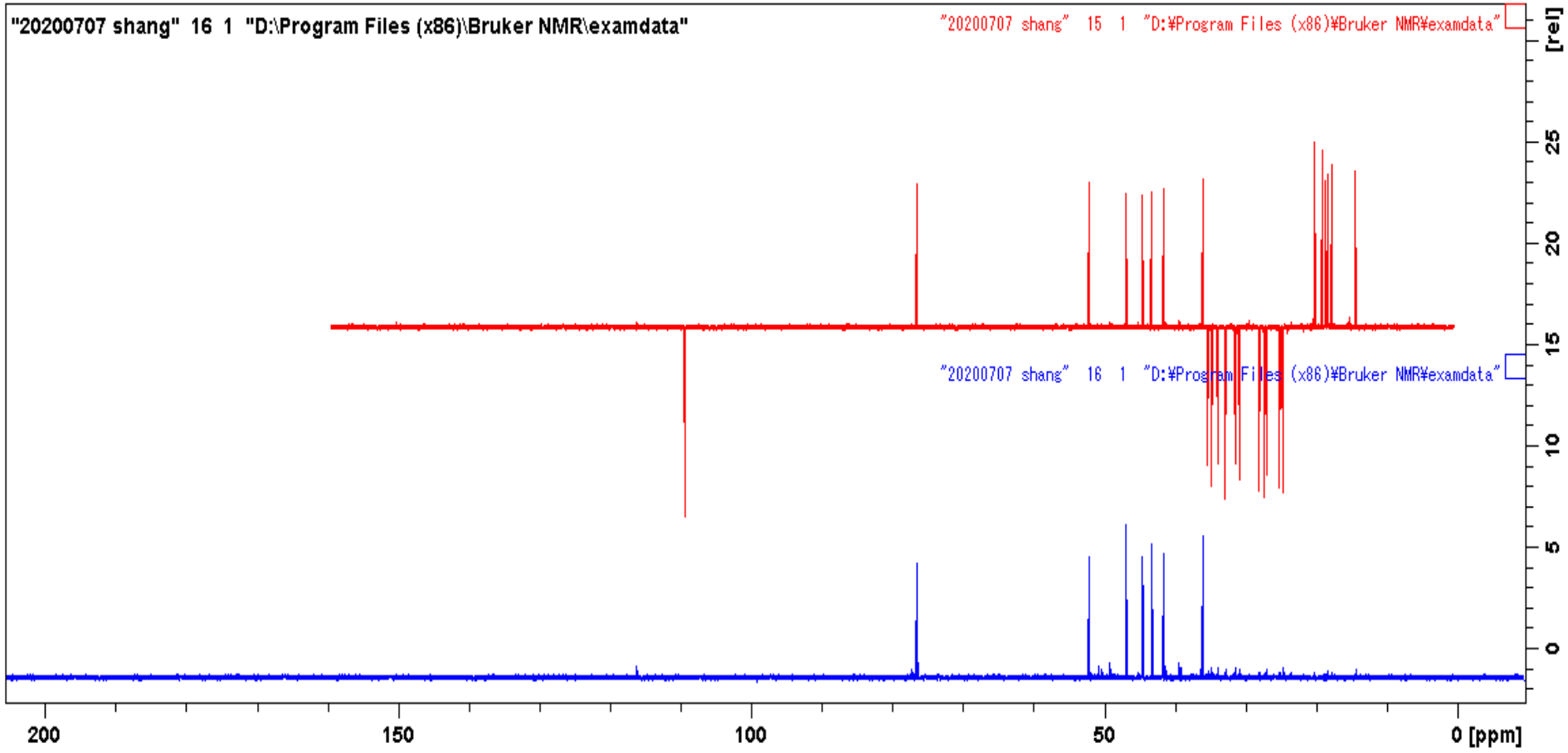

Fig. 24 DEPT 135 (top) and DEPT 90 (bottom) for isoCE-ol (150 MHz NMR in $CDCl_3$).