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นางมณธนา มะหะหมัด

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SYNTHESIS OF SESAMOLIN DERIVATIVES VIA NUCLEOPHILIC SUBSTITUTION REACTION AND THEIR BIOLOGICAL ACTIVITIES

Mrs. Monthana Mahamad

จุฬาลงกรณ์มหาวิทยาลัย
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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Chemistry
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ได้ศึกษาการสังเคราะห์ที่มีประสิทธิภาพของอนุพันธ์อัลคิลออกซีซามินชนิดใหม่ 10 สาร (2a-2j) ด้วยวิธีการที่ง่ายเพียงขั้นตอนเดียวจากเซซาโมลิน แนวทางการสังเคราะห์เกี่ยวข้องกับปฏิกิริยาการแทนที่ด้วยนิวคลีโอไฟล์โดยมีกรดเป็นตัวเร่งปฏิกิริยาที่บริเวณอะซิทัลโดยกลุ่มของแอลกอฮอล์ที่มีความหลากหลาย ซึ่งให้ผลผลิตที่ดีร้อยละ 55-82 ของสารผลิตภัณฑ์ตามที่ต้องการ คอนฟิกุเรชันสัมพัทธ์เป็นแบบ retention ซึ่งได้จากการวิเคราะห์ค่า coupling constant และ NOESY correlations มีการนำสารที่สังเคราะห์ได้ทั้งหมด 2a-2j มาทดสอบฤทธิ์การต้านอนุมูลอิสระวิธี DPPH และการยับยั้งเอนไซม์แอลฟากลูโคซิเดส ผลการต้านอนุมูลอิสระของสารในกลุ่มอนุพันธ์ที่ดัดแปลงโครงสร้างด้วยแอลกอฮอล์ 2a-2g แสดงให้เห็นว่าออกฤทธิ์ยับยั้งน้อยกว่าเล็กน้อยประมาณ 1.2-1.5 เท่า มีค่า SC_{50} อยู่ในช่วง 27.32-34.00 mM ในขณะที่อนุพันธ์กลุ่มที่ดัดแปลงโครงสร้างด้วยไดออล 2h-2j ออกฤทธิ์ยับยั้งน้อยกว่าประมาณ 1.6-2.3 เท่า อย่างมีนัยสำคัญเมื่อเปรียบเทียบกับสารตั้งต้นเซซาโมลิน (SC_{50} 22.59 mM) เมื่อสารมีความยาวของสายโซ่ที่เท่ากัน พบว่ากลุ่มที่มีหมู่ไฮดรอกซิลเพิ่มเข้าไป 1 หมู่ มีผลทำให้ฤทธิ์การต้านอนุมูลอิสระลดลงด้วย ในกรณีของการยับยั้งเอนไซม์แอลฟากลูโคซิเดส อนุพันธ์อัลคิลออกซีซามินทุกสารไม่ออกฤทธิ์การยับยั้งทั้งในยีสต์และในเอนไซม์ลำไส้เล็กของหนู เมื่อเปรียบเทียบฤทธิ์ทางชีวภาพของเซซาโมลินและอนุพันธ์มีฤทธิ์พอที่จะเทียบเคียงกันได้ ก็เป็นที่น่าสนใจที่จะประเมินความเสถียรของอนุพันธ์ต่อสภาวะกรด จึงทำการทดสอบใน CD_3OD โดยใช้ CF_3CO_2D เป็นตัวเร่งปฏิกิริยา พบว่า เซซาโมลินมีการสลายตัวอย่างสมบูรณ์ภายในเวลา 3 ชั่วโมง ขณะที่อัลคิลออกซีซามินยังคงไม่มีการเปลี่ยนแปลง

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สาขาวิชา เคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....
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MONTHANA MAHAMAD: SYNTHESIS OF SESAMOLIN DERIVATIVES VIA NUCLEOPHILIC SUBSTITUTION REACTION AND THEIR BIOLOGICAL ACTIVITIES. ADVISOR: ASST. PROF. PREECHA PHUWAPRAISIRISAN, Ph.D., CO-ADVISOR: ASST. PROF. SUMRIT WACHARASINDHU, Ph.D., 60 pp.

The synthesis of 10 new alkyloxy samins (2a-2j) was achieved by a simple one-step approach from sesamolin. The synthetic pathway involved acid-catalyzed nucleophilic substitution at acetal moiety with a variety of alcohol, affording fair to good yield (55-82%) of the desired products. The relative configuration established by analysis of coupling constant and NOESY correlations indicated that this nucleophilic substitution underwent with retention of configuration. All newly synthesized 2a-2j compounds were evaluated for DPPH radical scavenging and α-glucosidase inhibitory activity. Alcohol-derived analogues 2a-2g showed slightly weaker activity (~1.2-1.5 times) with SC_{50} values in range 27.32-34.00 mM whereas diol-derived analogues 2h-2j revealed significantly weaker scarvening activity (~1.6-2.3 times) compared with the parent starter sesamolin (SC_{50} 22.59 mM). Having identical chain length, the analogue possessing one additional hydroxyl group was likely to reduce scavenging activity in some extend. As for α-glucosidase inhibitory activity, all alkyloxy samins showed no inhibitory activity against both yeast and rat intestinal glucosidases. Because bioactivities of sesamolin and its analogues were comparable, it is of interest to evaluate their stability toward acid condition. On examination in CD_3OD catalyzed by CF_3CO_2D, sesamolin underwent degradation completely within 3 hours whereas alkyloxy samin remained unchanged.

Department:	Chemistry	Student's Signature	.....
Field of Study:	Chemistry	Advisor's Signature	.....
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List of Abbreviations

acetone-d_6	deuterated acetone
brs	broad singlet (NMR)
calcd	calculated
^{13}C NMR	carbon-13 nuclear magnetic resonance
$CDCl_3$	deuterated chloroform
CD_3OD	deuterated methanol
COSY	correlated spectroscopy
DMSO- d_6	deuterated dimethyl sulfoxide
DMSO	dimethylsulfoxide
d	doublet (NMR)
dd	doublet of doublet (NMR)
2D NMR	two dimensional nuclear magnetic resonance
1D NMR	one dimensional nuclear magnetic resonance
ESIMS	electrospray ionization mass spectrometry
equiv	equivalent (s)
FT-IR	fourier transform infrared spectroscopy
g	gram (s)
^{1}H NMR	proton nuclear magnetic resonance
HMBC	heteronuclear multiple bond correlation experiment
Hz	hertz
HRESIMS	high resolution electrospray ionization mass spectrum
h	hour (s)
IC_{50}	concentration that required for 50% inhibition in vitro

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IR	infrared
J	coupling constant
mg	milligram (s)
mL	milliliter (s)
mmol	millimole (s)
m/z	mass per charge
m	multiplet (NMR)
M.W.	molecular weight
M	molar
NOESY	nuclear overhauser enhancement spectroscopy
PDC	pyridinium dichromate
PNP-G	*p*-nitrophenyl-α-D-glucopyranoside
rt	room temperature
s	singlet (NMR)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TMS	tetramethylsilane
TLC	thin layer chromatography
U	unit
UV	ultraviolet
δ	chemical shift
δ_C	chemical shift of carbon
δ_H	chemical shift of proton
°C	degree celsius

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μL	microliter (s)
μM	micromolar (s)
% yield	percentage yield
$[\alpha]_D$	specific optical rotation

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CHAPTER I

INTRODUCTION

Sesamolin is a lignan that consisting of dimers of phenyl propane (C6-C3) units. The C6-C3 units are linked by the central carbon of their propyl side chains. Under IUPAC nomenclature, the lignans are 8,8″- coupled dimers of coniferyl or cinnamyl alcohol. Based on the way in which oxygen is incorporated into the skeleton and the cyclization pattern, the lignans are classified into eight subgroups, including furofuran, furan, dibenzylbutane, dibenzylbutylrolactone, arytetralin, arylnaphthalene, dibenzocyclooctadiene and dibenzyl butyrolactol (Umezawa, 2004). Sesamolin could be classified as furofuran lignan, one of the largest classes of naturally occurring lignans. Sesame (*Sesamum indicum*) oil is a rich source of lignans. There are sixteen types of lignans isolated from sesame (Figure 1.1) (Dar & Arumugam, 2013). The main lignans in sesame oil are sesamin and sesamolin.

Sesame lignans has been reported to exhibit many pharmacological properties, e.g. antioxidant activity (Hu *et al.*, 2004, Suja *et al.*, 2005) antihypertensive (Sankar *et al.*, 2005), anticancer (Miyahara *et al.*, 2001), hypocholesteremic (Tsai *et al.*, 2006), neuroprotective effects againt hypoxia or brain damage (Cheng *et al.*, 2006) including antidiabetic (Wikul *et al.*, 2012). Among the lignans from sesame, sesamol were shown to have the most potent antioxidative activity in vitro experimental system, having higher antioxidative activity than α-tocopherol and BHT (Ohtsuki *et al.*, 2003).

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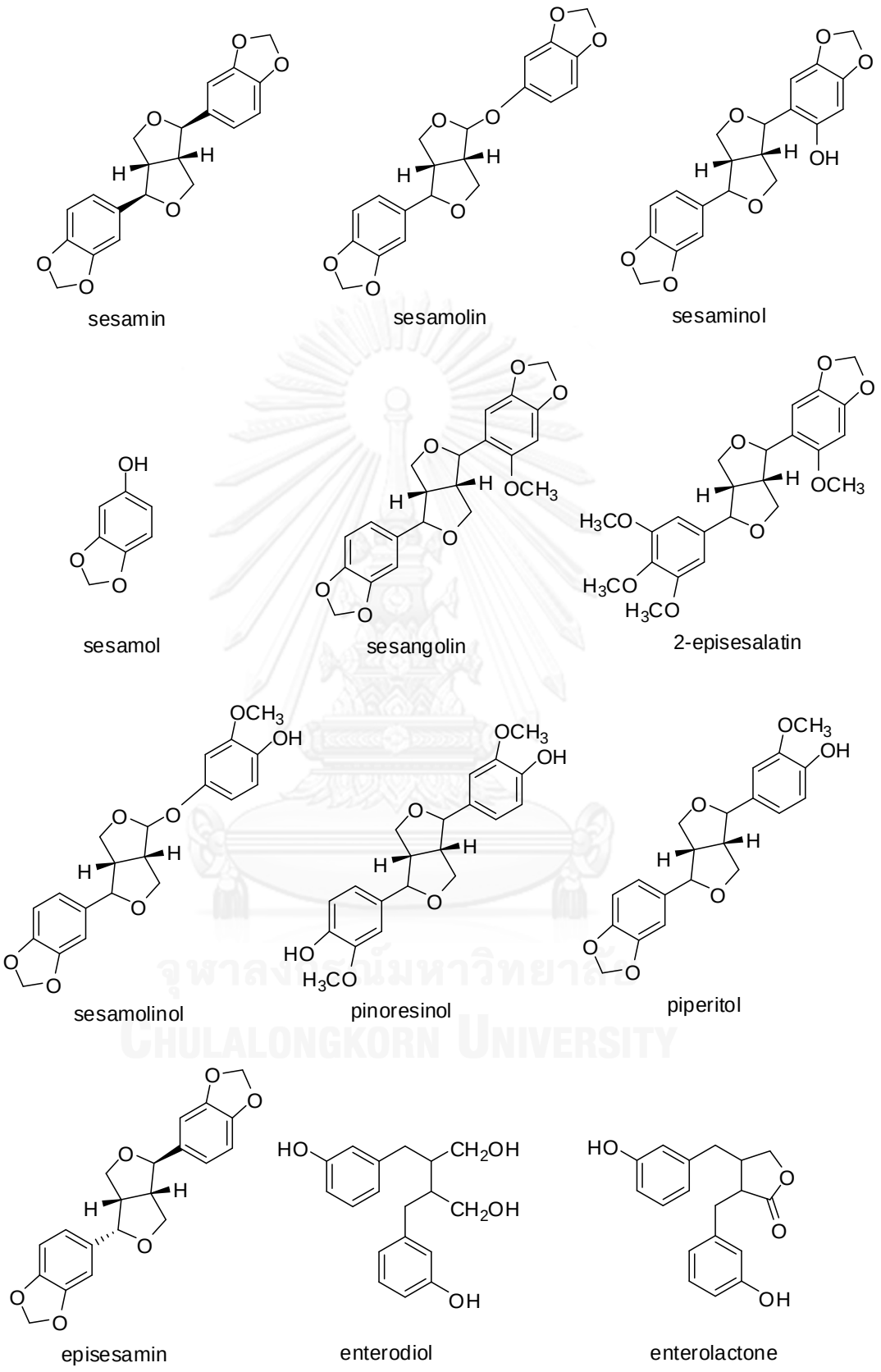

Figure 1.1 Structures of different lignans reported from *Sesamum indicum*

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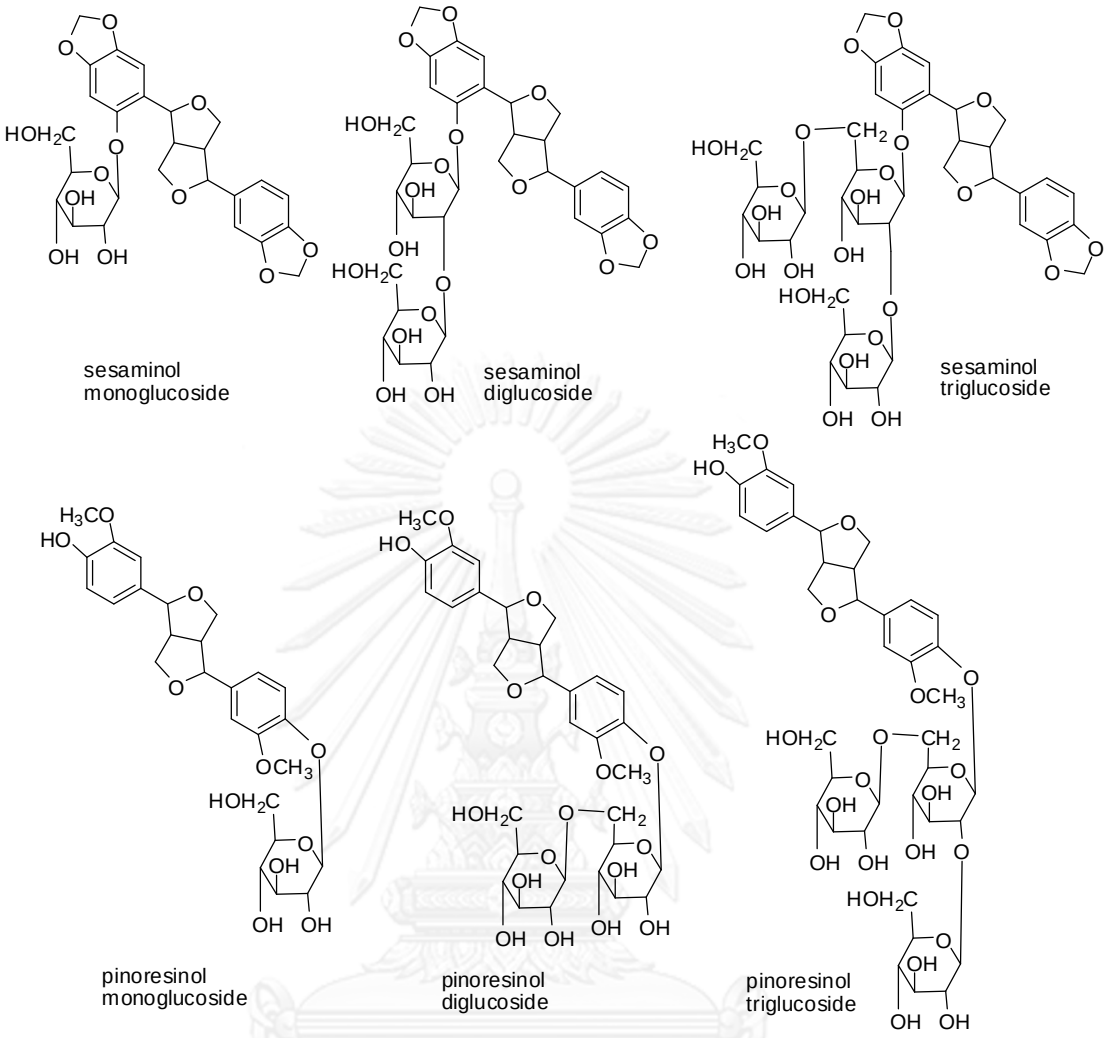
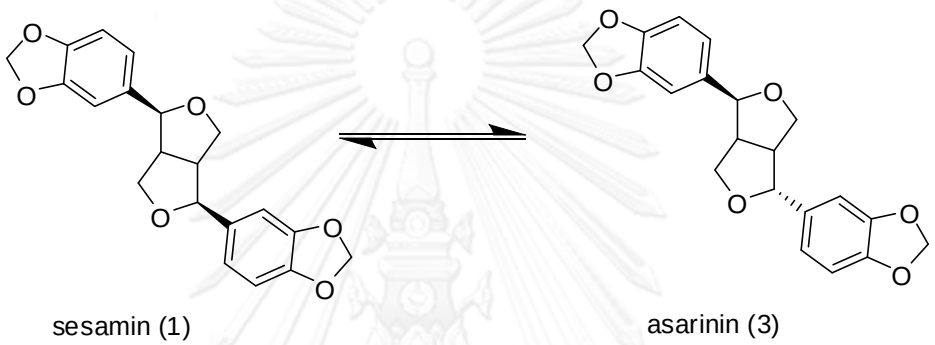


Figure 1.1 (continued)

Importantly, sesamin and sesamolin have many promising bioactivities, however there are a few reports on synthesis of these derivatives.

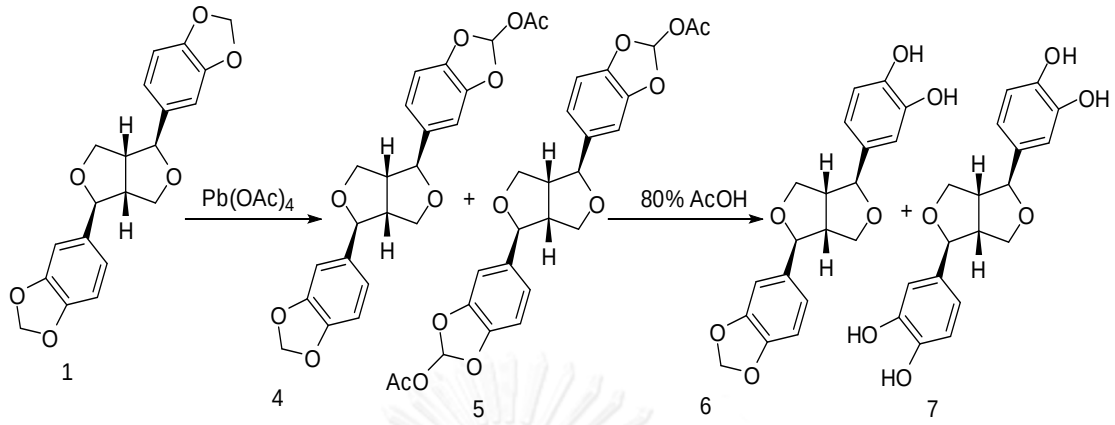
In 2005, (Li *et al.*, 2005) studied epimerization of sesamin (**1**) and azarinin (**3**). Both sesamin and azarinin (Scheme 1.1) have a furofuran backbone, which is similar to those of norbornane and bicycle[3.3.0]octane. It is well-known that the *exo* isomer is more stable than the *endo* isomer. Sesamin has two substitutuents in *exo* positions and the other in *endo* position. Therefore, the conformation of sesamin is

supposed to be more stable than that of azarinin. This results showed that the variations occurred due to acidic condition leading to a 44.8/55.2 equlibrium ratio of sesamin : azarinin. The ratio of these indicated that structural changes are dependent on the conditions of the extraction processes.



Scheme 1.1 Epimerization of sesamin (**1**) and azarinin (**3**)

Urata *et al*. (Urata *et al.*, 2008) reported the synthesis of oxidative products of sesamin. The structures of sesamin (**1)** have two methylenedioxyphenyl moieties, which are generally inactive to any chemical derivatizations. The synthesis was achieved by a simple two-step approach consisting of acetoxylation of the methylenedioxy moiety(ies) with lead(IV) tetraacetate followed by acid hydrolysis of the resulting hemiorthoester, thus yielding compounds **6** and **7** (Scheme 1.2).



Scheme 1.2 Synthesis of oxidative metabolites of sesamin

In 1997, Marchand *et al.* (Marchand *et al.*, 1997) reported hemisynthesis of (+)-episesaminone from sesamolin (**2**) (Scheme 1.3). The approach was based on the known chemistry of sesamolin (**2**), which can be hydrolyzed in acid condition to give samin (**8**) as the major product. Oxidation of **8** with pyridinium dichromate (PDC) yielded the corresponding lactone named (+)-acuminatelide (**9**) in 78% yield. Further condensation of **9** with Grignard reagent at room temperature, followed by hydrolysis of the intermediate lactol **10**, afforded (+)-episesaminone (**11**).

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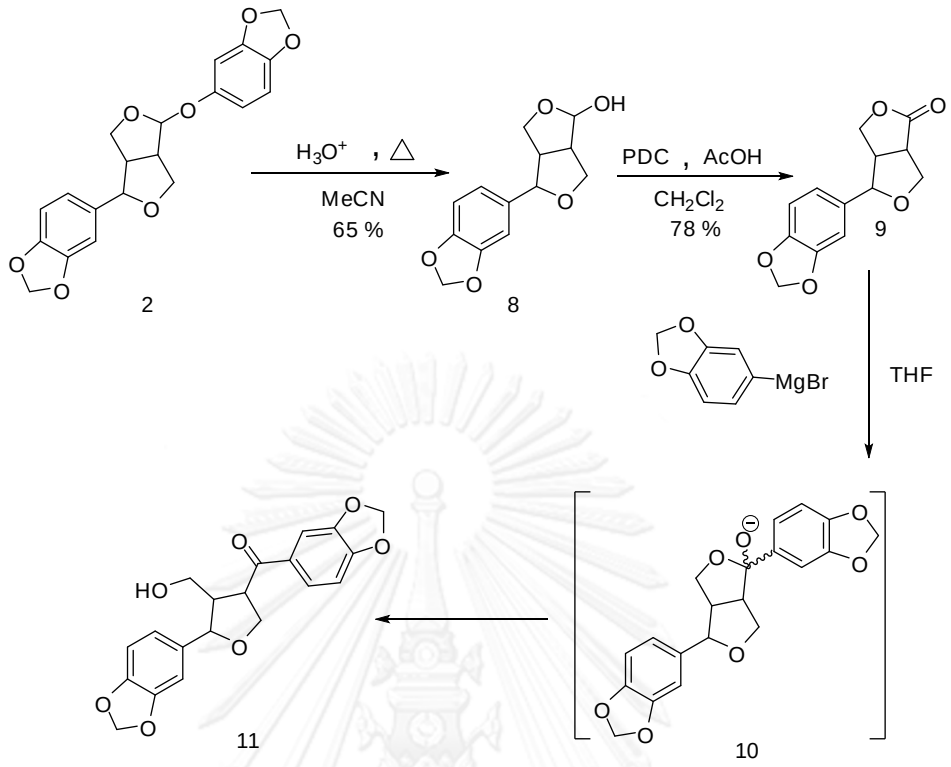
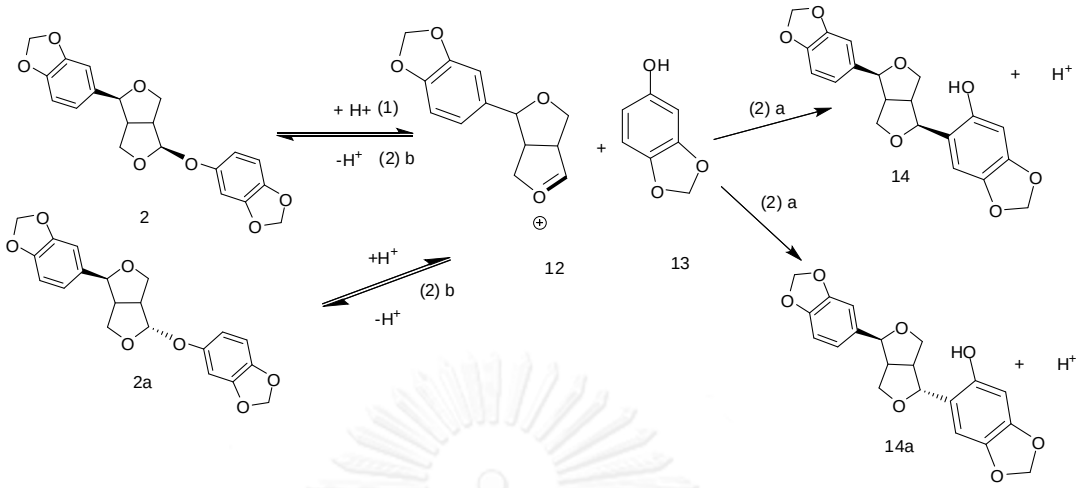


Scheme 1.3 Hemisynthesis of (+)-episesaminone (**11**) from sesamolin (**2**)

Recently, synthesis of sesaminol (**14**) starting from sesamolin (**2**) was described by Huang and co-workers (Huang *et al.*, 2012). Under optimal condition, utilizing cation exchange resin 16.66 g/mmol and reaction temperature of 90 °C, sesaminol (**14**) was produced in 75% yield as well as small amount of related products. They proposed (Scheme 1.4) that after protonation of sesamolin (**2**), an intermediate oxonium ion **12** was produduced together with the release of sesamol (**13**). Subsequent reactions of **12** can be proceeded by two different pathways, (2)a and (2)b. Friedel-Crafts or electrophilic substitution of **12** onto sesamol (**13**) generated sesaminol (**14**) and its epimer **14a**. On the other hand, the reverse of protonation (pathway 1) led to the concurrent production of sesamolin epimer (**2a**).



Scheme 1.4 Conversion of sesamolin (**2**) to sesaminol (**14**) and related products

During the last decade, the furofuran lignans particularly **1** and **2** have been most widely transformed in organic synthesis. The modified moiety, piperonyl moiety (3,4-methylenedioxyphynyl) and furofuran ring, have received much focus because it was found that the hydroxyl group on the furufuran ring and phenolic part affected the degree of antioxidant activity. To the best of our knowledge, lignans have scaffold for the synthesis and a potential source of biological activity. They are composed of ether and benzene moieties, which are relatively inactive. However, sesamolin has acetal group which was active to acid-catalyzed reaction. Therefore, chemical derivatization on acetal moiety would produce a series of diverse sesamolin analogues. In this study, the target of this research is to synthesize sesamolin derivatives by nucleophilic substitution (Scheme 1.5). This plan involved protonation of sesamolin to generate oxocarbenium ion. Further substitution by a series of alcohols as nucleophiles would provide alkyloxy samins as the desired products.

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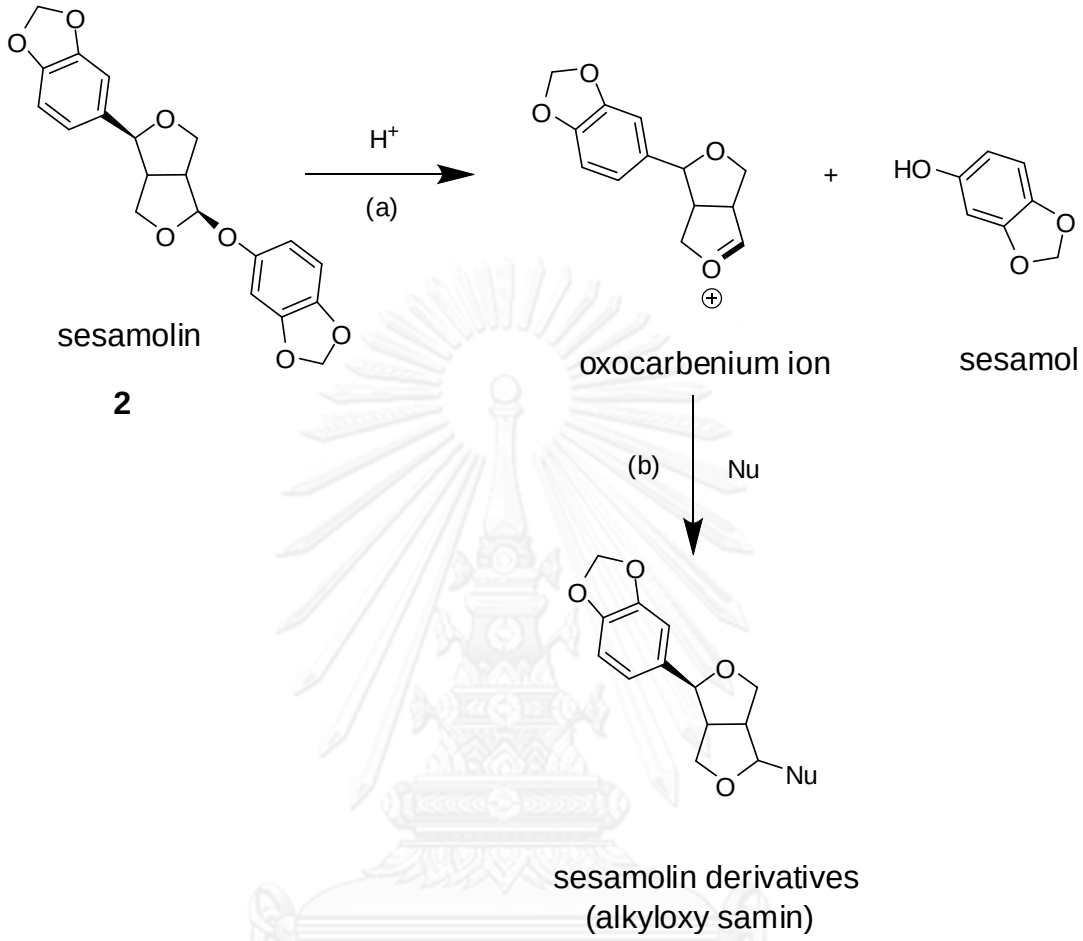

Scheme 1.5 Synthetic plan for the synthesis of alkyloxy samins starting from sesamolin

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CHAPTER II

SYNTHESIS OF ALKYLOXY SAMIN

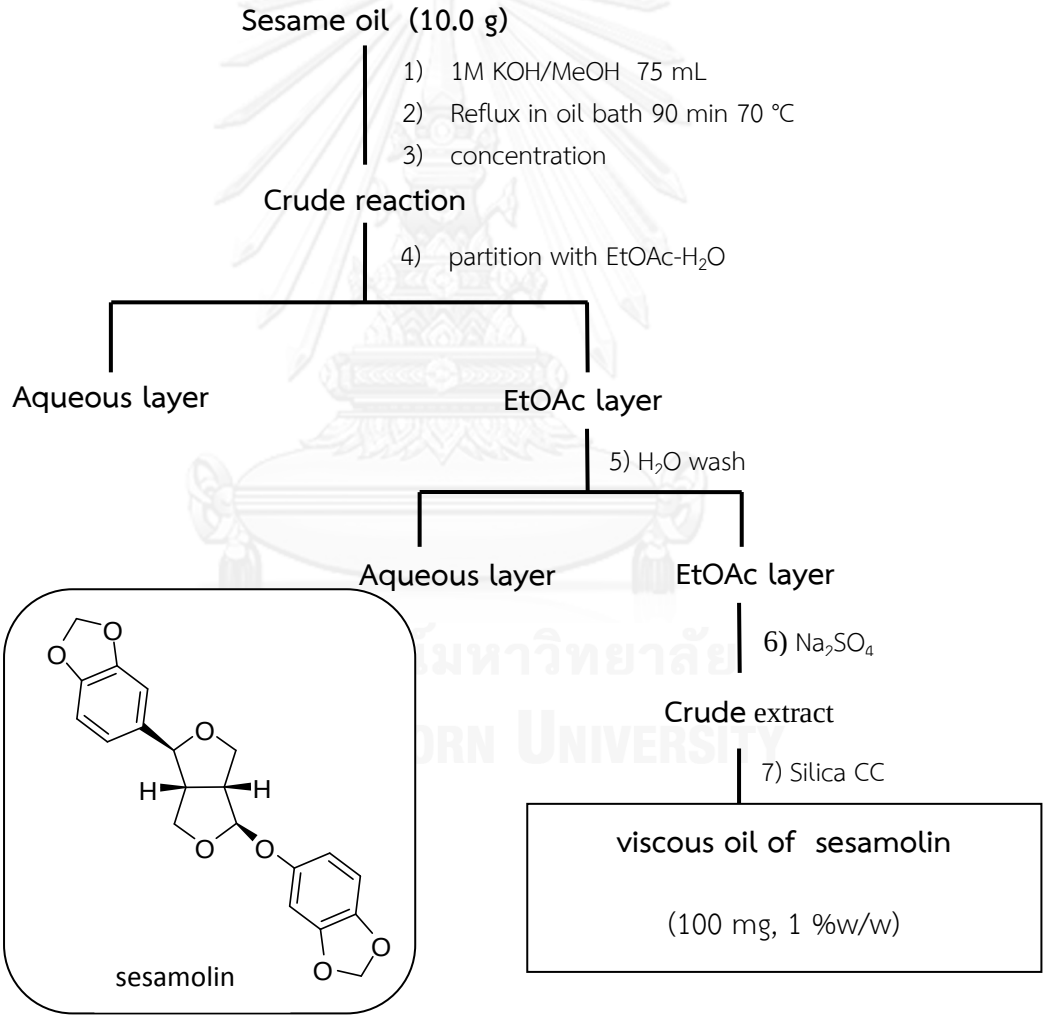
2.1. Isolation of sesamolin from sesame oil

Sesame oil is an important antioxidant oil with about 90% triglyceride, in which the major fatty acids are oleic and linoleic acids. The nonglyceride fraction of oil contains a variety of biologically active components including tocopherols, sterols, triterpene alcohols as well as lignans. The main lignans in sesame oil are furofuran lignans named sesamin (**1**) and sesamolin (**2**), which are reported to be in the concentration renge of 1-2%.

Isolation of lignans **1** and **2** from triglyceride matrix using chromatography technique is challenge as they are lipophilic and have similar polarity. To solve this problem, the oil was subject to saponification, followed by liquid extraction. This technique transformed triglycerides to water soluble potassium salt of a carboxylate (soap) whereas lignan remained unchanged. Saponification of sesame oil was carried out by adding of potassium hydroxide (KOH) in methanol in order to make soft soap which was readily soluble in water. Potassium hydroxide used in this experiment was based on saponification value. Saponification value (SV) of sesame oil is about 187-193 mg KOH/1g of oil. However, to ensure complete removal of triglyceride, slightly excess (~1.5-2 times of SV) KOH was applied. After completion of saponification, water was added to the mixture, which was then extracted with ethyl acetate two times, The ethyl acetate layer was washed with water to remove excess KOH. The combined ethyl acetate extract was evaporated under reduce pressure to

obtain lignan crude extract. The crude extract was furture purified by silica gel chromatography to obtain sesamolin (**2**). The isolation procedure is summarized in Scheme 2.1.

Sesamolin was obtained as a viscous oil. The structure was deduced by the results from ^{1}H, ^{13}C NMR data, which were consistent with previous report (Kang *et al.*, 1995).



Scheme 2.1 The isolation procedure of sesamolin from sesame oil

2.2 Synthesis of alkyloxy samins

Sesamolin is a furofuran lignan, which comprises a large group of natural products characterized by coupling of two phenylpropane units. Normally, sesamolin is relatively inactive because it is composed of ether and benzene moieties, which are inactive to be derivertised. However, it has acetal group, which is active to acid-catalyzed reaction.

After protonation, oxocarbenium ion generated at acetal group could undergo nucleophilic substitution. Therefore, a variety of sesamolin analogues would be abtained by applying diverse nucleophiles.

Initially, the reaction conditions for the synthesis of sesamolin derivatives were optimized (Table 2.1). As a model system, we selected methanol as nucleophilic reagent with variation of acids. Amberlyst-15, an acidic resin, not only provided the best yield (55%) of the target product **2a** under reflux (Entry 6). In contrast, Dowex resin showed no reaction at both room temperature and reflux (Entries 3-4). Although HCl catalyst showed a good product yield (Entry 2), the removal of this acid is more difficult than acidic resin.

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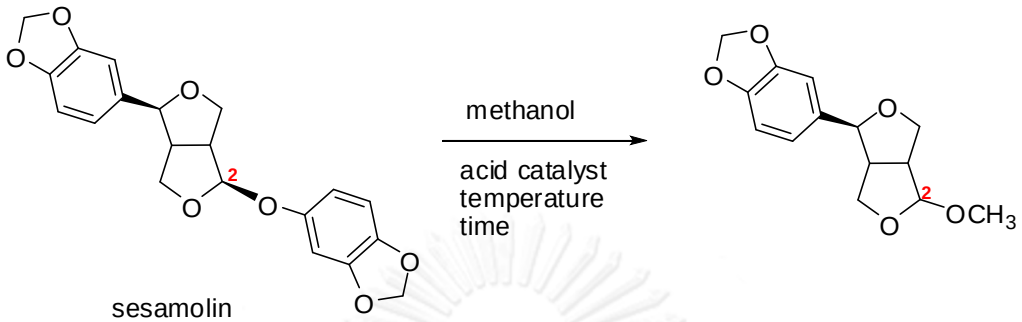
Table 2.1 Optimization of reaction parameters[a]



sesamolin **2** **2a**

Entry[a]	Acid catalyst	Temperature (°C)	Time (h)	Isolated yield of **2a** (%)
1	HCl	rt	24	10
2	HCl	reflux	5	40
3	Dowex 50W-X8 (H^+)	rt	24	No reaction
4	Dowex 50W-X8 (H^+)	reflux	12	No reaction
5	Amberlyst-15 (H^+)	rt	24	No reaction
6	Amberlyst-15 (H^+)	reflux	2	55

[a] Screening reaction conditions: 1 equiv of **2**, 20 equiv of methanol, catalytic amount of HCl, 50 mg of acidic resin (Dowex and Amberlyst) / mmol of sesamolin.

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With a set of optimal reaction conditions in hand, we next investigated the synthesis of sesamolin derivatives using various alcohols as both solvent and nucleophile (Table 2.2). Sesamolin derivatives generally named alkyloxy samins (**2a-2j**) were prepared by a one-step routine.

We started to use MeOH as a small nucleophile , affording the methoxy samin (**2a**). We next synthesized, alkyloxy samins having bigger alkyl groups such as ethyl, propyl and butyl group. Ethoxy (**2b**), propoxy (**2c-2d**) and butoxy (**2e-2g**) samins were successfully synthesized. However, this approach could not be applied to bigger alcohol such as octanol. Reaction between sesamolin and octanol generated epi-sesaminol (**14a**) instead of the expected octoxy samin. This observation was possibly contributed by weaker nucleophilicity of octanol, compound with the released sesamol. Therefore, synthesis of alkyloxy samins having bigger alkyl group than octyl moiety was terminated. We switched our target to alkyloxy samins having diol moiety. The diol-derived analogues (**2h-2j**) such as ethane-1-ol-2-oxy (**2h**), propane-1-ol-3-oxy (**2i**) and butane-1-ol-3-oxy (**2j**) samins were synthesized.

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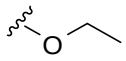
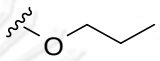
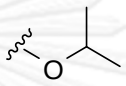
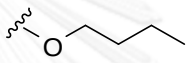
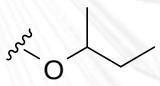
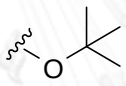
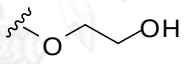
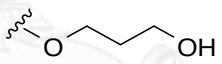
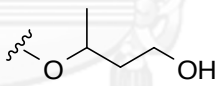
Table 2.2 Alkyloxy samins obtained from reactions of alcohols and sesamolin.

Sesamolin derivatives	Alcohol	R	Isolated yield (%)
2a	methanol		55
2b	ethanol		65
2c	propanol		74
2d	2-propanol		70
2e	butanol		75
2f	2-butanol		67
2g	2-methyl-2-propanol		63
2h	1,2-ethanediol		82
2i	1,3-propanediol		78
2j	1,3-butanediol		80

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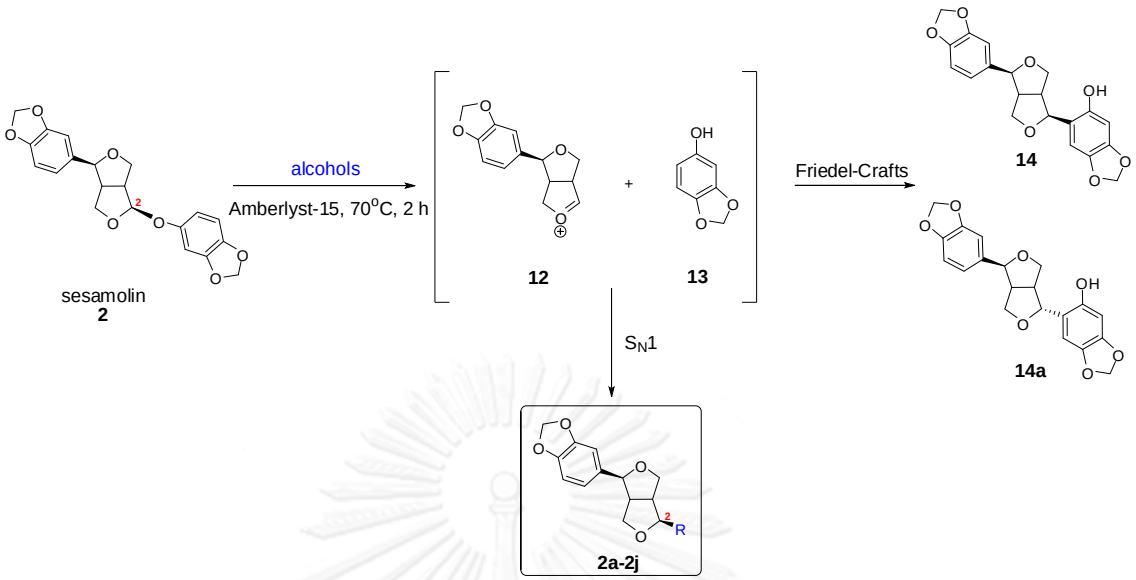
The reaction mechanism of nucleophilic substitution of sesamolin could be proposed as shown in Scheme 2.2. The acid hydrolysis of sesamolin under heating possibly generated the intermediate oxocarbenium ion (1**2**) and sesamol (1**3**). Nucleophilic substitution of **12** with various alcohol afforded alkyloxy samins (**2a-2j**) in good to excellent yields (55-82%). On the other hand, sesaminol (**14**) could be generated via the Friedel-Crafts reaction of **12** and 1**3** in a low yield. This observation was noticed when alcohol having longer alkyl chain such as octanol was applied.



Scheme 2.2 Proposed mechanism of nucleophilic substitution of sesamolin (**2**)

2.3 Structural characterization of alkyloxy samins

There were 10 synthesized alkyloxy samins obtained from the nucleophilic substitution reaction of sesamolin and the corresponding alcohol. In this research, we chose MeOH as the first nucleophile because of its smallest size and stronger nucleophile than sesamol. Treatment of sesamolin (**2**) with this reagent in the presence of Amberlyst-15 afforded the methoxy samin (**2a**) in 55% yield. Compound **2a** was obtained as a pale yellow oil. The molecular formula was determined as $C_{14}H_{16}O_5$ by the HREIMS *m/z* 287.0898 $[M+Na]^+$ (calcd for $C_{14}H_{16}NaO_5$, 287.0895). The ^1H

NMR spectrum of **2a** showed well-saparated signals compared with those of sesamolin (**2**), the starting material of this reaction.

The ^{1}H NMR spectrum of **2a** was close to that of sesamolin except for the presence of additional substituent. Particularly, the remaining signals of one 1,3,4-trisubstituted benzene ring indicated that aryloxy substituent at C-2 of sesamolin (**2**) was removed after reaction completed. In addition, the presence of methylenedioxy proton of piperonyl moiety at δ_H 5.88, which was assigned as H-7″ of **2a,** supported the above observation. Moreover, it showed additional subtituent as one methoxy group by a sharp singlet at δ_H 3.27 (H-1′). In ^{13}C NMR spectrum, the aromatic resonances of C-1′ in **2a** and **2** were obviously different, in which the carbon chemical shifts of C-1′ in **2** was δ_C 151.8 whereas that of C-1′ in **2a** was δ_C 54.7. respectively.

In addition, the signal of one remaining dioxymethylene group (OCH_2O) at δ_H 5.88 (s, 2H) could also be recognized for methoxy samin (**2a**), after the release of aryloxy (OAr). Other ^{1}H and ^{13}C NMR signals of **2a** could be tentatively assigned by comparison with those of sesamolin. All assignments were summarized in experimental section. It was of interest to deduce configuration of C-2 because the nucleophilic substitution took place at this chiral center.

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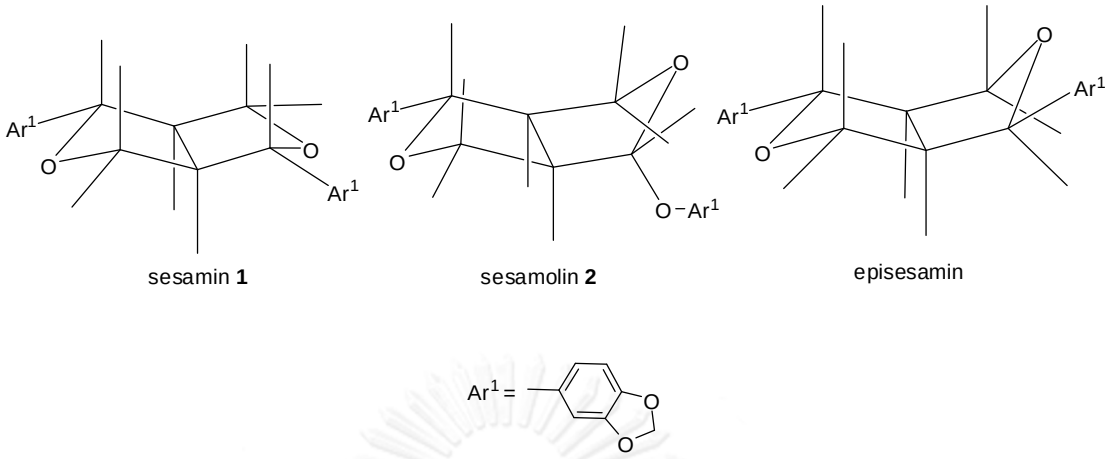


Figure 2.1 Boat-chair conformation of selected furofuran lignans

Nuclear Overhauser effects, lanthanide induced shifts, force-field calculations, and X-ray analysis have been used to study the conformation of sesamolin (**2**) (Figure 2.1) (Lutz *et al.*, 1997). The conformation was found to differ from that of sesamin (**1**) due to the anomeric effect of the cyclic acetal favouring a pseudo-axial position for the aryloxy substituent. The 'chair-boat' of sesamolin (**2**) has more in common with that of episesamin.

The relative stereochemistry of alkyloxy substituents was deduced using *J* value, which is related to dihedral angle as described by Karplus. The Karplus relationship is based on the observation, that vicinal H-H couplings will be maximal with protons with 180° and 0° dihedral angles (anti or eclipsed rerationship results in optimal orbital overlap) and that coupling will be minimal (near 0) for protons that are 90° for each other. The equation gives as approximate values for $^{3}J_{HH}$ as a function of dihedral angle between the protons. The Bothner-By equation provides an empirical "Karplus" curve that does not require different *J* values for the 0-90° vs 90-180° section

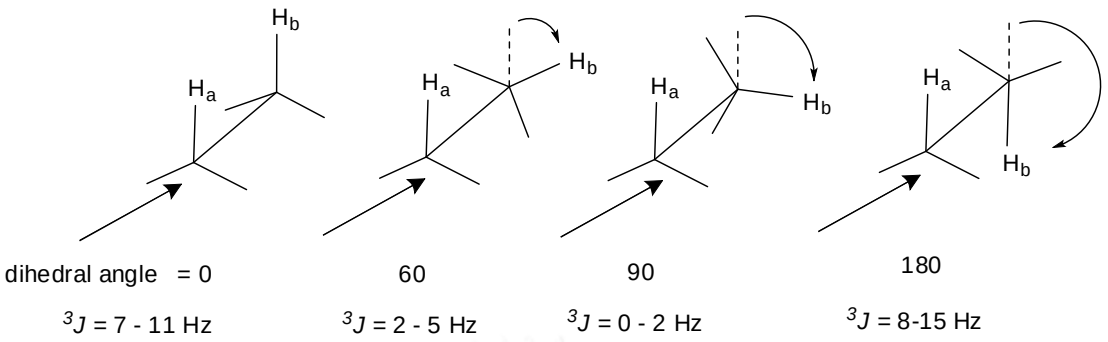
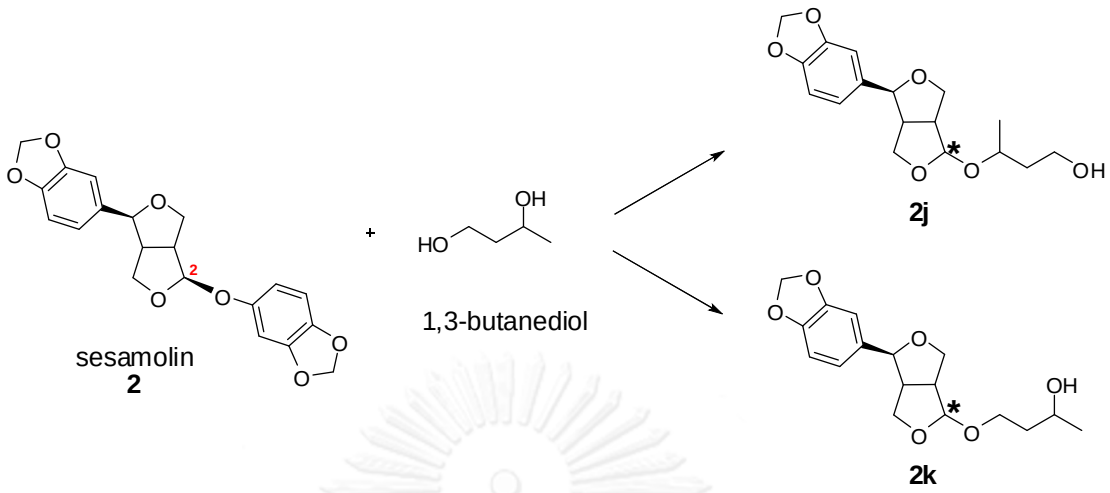


Figure 2.2 Relationship between dihedral angle and coupling constant.

To gain insight into the relative configuration at the chiral center C-2 of **2a**, we analyzed coupling constant of H-2. The H-2 of compound **2a** signal appeared as a board singlet at δ_H 4.77. The observation indicated that the dihedral angle of H-1/H-2 was nearly 90°, which was consistent with pseudo-axial orientation of the methoxy group at C-2. In addition, the unchanged relative configuration at C-2 of sesamolin and 2a suggested that this reaction occurred with retention of configuration.

In addition, chemoselectivity of two different alcohols toward nucleophilic substitution was also examined. Substitution of sesamolin **(2)** by 1,3-butanediol, an alcohol having both primary and secondary hydroxyl groups in the same molecule, was carried out. If these two different hydroxyl groups could undergo nucleophilic substitution equally, a pair of alkyloxy samins (**2j** and **2k**) should be obtained (Scheme 2.3). After reaction completed, only single product was obtained. On the basis of MS and NMR data, especially COSY, HSQC and HMBC, the product was identified as butane-1-ol-3-oxy samin or **2j**.



Scheme 2.3 Possible structures of alkyloxy samins (**2j** and **2k**) from reaction of **2** with 1,3-butanediol.

Butane-1-ol-3-oxy samin (**2j**) was obtained as a colorless oil. The molecular formular $C_{17}H_{22}O_6$ was determined by HRESIMS of pseudo molecular ion at *m/z* 345.1310 $[M+Na]^+$ (calcd for $C_{17}H_{22}NaO_6$, 345.1314).

The ^{1}H NMR spectrum of **2j** displayed signals in four notable regions: benzene signals (δ_H 6.87-6.84), methine and methylene dioxy protons (δ_H 5.94, 4.94), methine and methylene oxygenated protons (δ_H 4.35-2.81) as well as methylene and methyl signals (δ_H 1.64-1.19). Three downfield signals at δ_H 6.84 – 6.77 were assigned as H-2″, H-5″and H-6″. Two signals at δ_H 5.94 and δ_H 4.94 were also assigned as methylene dioxy proton (H-7″) and acetal proton (H-2), respectively. Two quartet methine protons at δ_H 3.02 and 2.81 were assigned H-1 and H-5, respectively. All signals was definitely assigned and to be summarized in experimental section.

In HMBC spectrum (Figure 2.3), the diagnostic correlations of δ_H 4.19 (H-4′) and δ_H 4.94 (H-2) with δ_C 67.3 (C-1′) confirmed that the methyl group was connected to C-1′ (Figure 2.4). Therefore, the isolated product was formed by substitution of sesamolin by secondary alcohol as described in structure 2j.

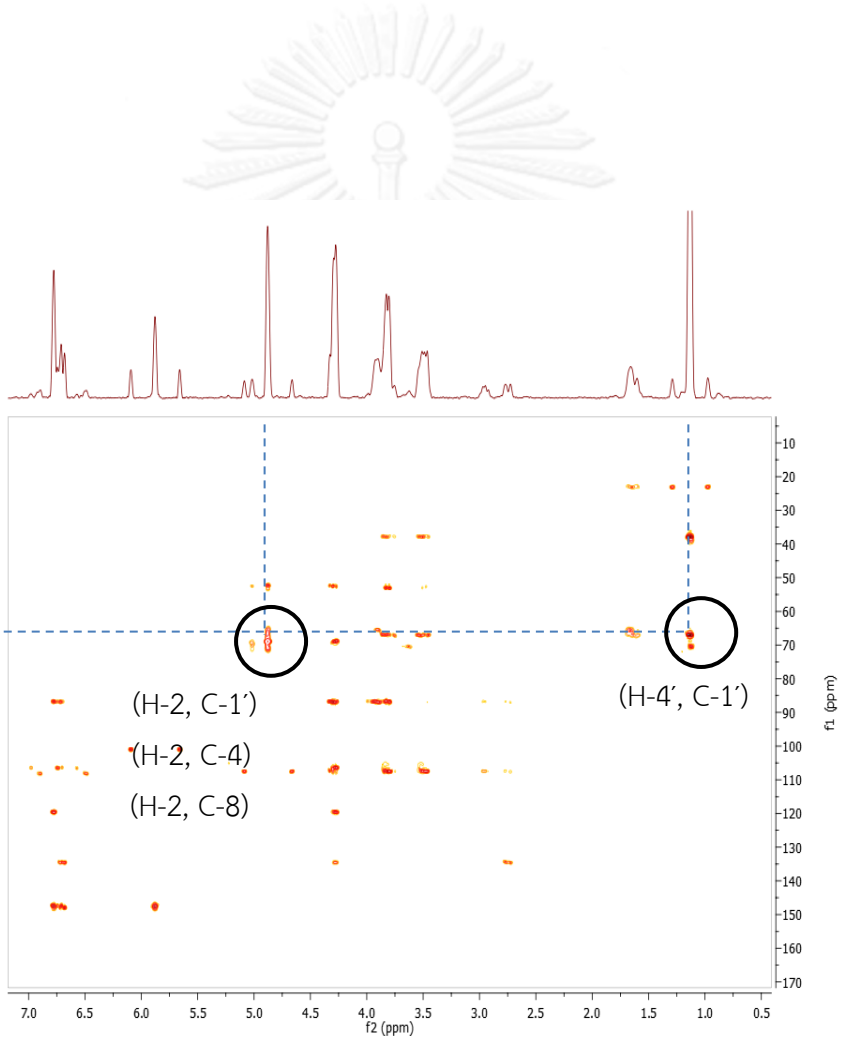


Figure 2.3 Diagnostic HMBC correlations observed in **2j.**

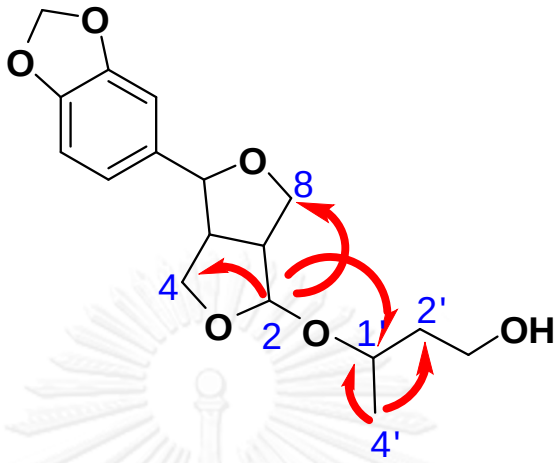


Figure 2.4 Selected HMBC correlations of **2j**

In addition, the relative configuration assigned by NOESY correlations (H-2/H-8_{ax}, H-1/H-5, H-1/H-8_{eq}, and H-6/H-8_{ax}) shown in Figure 2.6 indicated pseudoaxial orientation of the substituent group at C-2, the same as assigned by coupling constant.

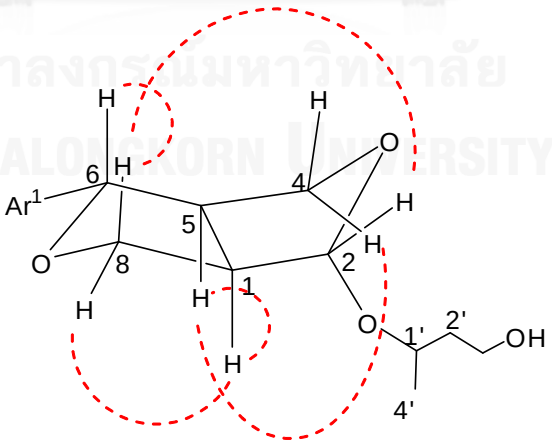


Figure 2.5 Selected NOESY correlations of **2j**.

2.4 Experimental section

2.4.1 General experiment procedures

^{1}H and ^{13}C NMR spectra were recorded ($CDCl_3$ as solvent) at 400 and 100 MHz, respectively, on a Varian Mercury^{+}400 NMR spectrometer. The chemical shifts were reported in ppm downfield from TMS. HRESIMS spectra were obtained from a micrOTOF Bruker mass spectrometer. Thin layer chromatography (TLC) was performed on pre-coated Merck silica gel 60 F_{254} plates (0.25 mm thick layer) and visualized under 254 nm UV. Sephadex LH-20 and silica gel 60 Merck cat. No. 7734 and 7729 were used for open column chromatography.

2.4.2 Chemical

Methyl alcohol, ethyl alcohol, *n*-propyl alcohol, *iso*-propyl alcohol, *n*-butyl alcohol, *2*-butyl alcohol, *t*-butyl alcohol, ethyleneglycol, 1,3-propane diol, 1,3-butane diol, potassium hydroxide and Amberlyst-15 were purchased from sigma. Sesame oil was purchased from Suan-pana (Bangkok, Thailand).

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2.4.3 Isolation of sesamolin (2)

General procedures for isolation of sesamolin from sesame oil are as follows. Sesame oil (10 g) was saponified by reflux with 1M methanolic potassium hydroxide for 90 min. After removal of methanol, the reaction mixture was suspended in water and extracted twice with EtOAc. The organic layer was further purified by silica gel chromatography (20 % EtOAc-Hexane) to obtain sesamolin (60 mg, 0.6 %w/w).

sesamolin **2**: as viscous oil ; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.87 (s, 1H, H-2″), 6.80 (m, 2H, , H-5″ and H-6″), 6.70 (d, *J* = 8.4 Hz, 1H, H-5′), 6.62 (d, *J* = 2.4 Hz, 1H, H-2′), 6.50 (dd, *J* = 2.4, 8.4 Hz, 1H, H-6′), 5.96 (s, 2H, H-7′), 5.92 (s, 2H, H-7″), 5.50 (s, 1H, H-2), 4.44 (t, *J* = 9.0 Hz, 1H, H-8), 4.39 (d, *J* = 7.2 Hz, 1H, H-6′), 4.12 (m, 1H, H-4), 3.96 (d, *J* = 9.2 Hz, 1H, H-4), 3.63 (m, 1H, H-8), 3.30 (q, *J* = 8.8 Hz, 1H, H-1), 2.94 (q, *J* = 8.8 Hz, 1H, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 151.8 (C-1′), 148.1 (C-3′), 148.0 (C-3″), 147.3 (C-4″), 142.6 (C-4′), 134.3 (C-1″), 119.6 (C-6″), 108.9 (C-6′), 108.1 (C-5″), 108.0 (C-5′), 106.8 (C-2), 106.5 (C-2″), 101.2 (C-7″), 101.0 (C-7′), 100.1 (C-2″), 87.0 (C-6), 71.2 (C-8), 69.7 (C-4), 53.2 (C-1), 52.7 (C-5).

2.4.4 General procedure for synthesis of alkyloxy samins

To a solution of sesamolin (**2**, 1 equiv) in alcohol (20 equiv) was treated with Amberlyst-15 (H^+) (50 mg/mmol of sesamolin). After stirring at 70 °C for 2 h, the reaction mixture was evaporated to dryness and further purified by Sephadex LH-20 or silica gel flash column chromatography.

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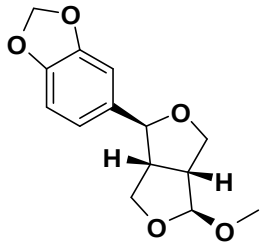
Methoxy samin (2a)



Following the general procedure above, reaction of sesamolin **2** (200 mg, 0.54 mmol), methyl alcohol (0.5 mL) and Amberlyst-15 (20 mg), after 2 h, yielded methoxy samin (**2a**, 78.4 mg, 55%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (s, 1H, H-2″), 6.72 (m, 2H, H-5″ and H-6″), 5.88 (s, 2H, H-7″), 4.77 (s, 1H, H-2), 4.29 (m, 2H, H-6 and H-8), 3.89 (m, 1H, H-4), 3.80 (d, *J* = 8.8 Hz, 1H, H-4), 3.48 (m, 1H, H-8), 3.27 (s, 3H, H-1′), 2.97 (m,1H, H-1), 2.72 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.1, 147.4, 134.8, 119.8, 108.7, 108.3, 106.7, 101.2, 87.2, 71.4, 69.1, 54.7, 53.2, 52.9; HRESIMS *m/z* 287.0898 $[M+Na]^+$ (calcd for $C_{14}H_{16}NaO_5$, 287.0895).

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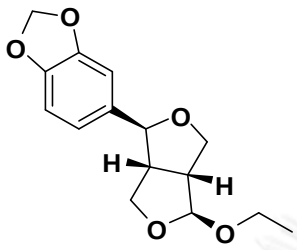
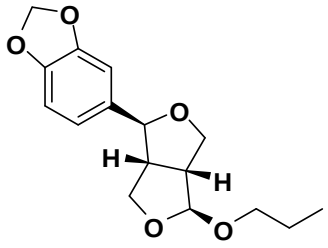
Ethoxy samin (2b)



Following the general procedure above, reaction of sesamolin **2** (70 mg, 0.19 mmol), ethyl alcohol (0.2 mL) and Amberlyst-15 (10 mg), after 2 h, yielded ethoxy samin (2b, 34.2 mg, 65%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 (s, 1H, H-2″), 6.71 (m, 2H, H-5″ and H-6″), 5.78 (s, 2H, H-7″), 4.89 (s, 1H, H-2), 4.29 (m, 2H, H-6 and H-8), 3.91 (m, 1H, H-4), 3.79 (d, *J* = 8.4 Hz, 1H, H-4), 3.65 (m, 1H, H-1′), 3.48 (m, 1H, H-8), 3.38 (m, 1H, H-1′), 2.97 (m,1H, H-1), 2.75 (m, 1H, H-5), 1.13 (t, J = 7.2 Hz, 3H, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.1, 147.3, 134.9, 119.7, 108.3, 107.4, 106.7, 101.2, 87.2, 71.6, 69.1, 62.9, 53.3, 53.0, 15.3; HRESIMS m/z 301.1054 $[M+Na]^+$ (calcd for $C_{15}H_{18}NaO_5$, 301.1052).

***n*-Propoxy samin (2c)**



Following the general procedure above, reaction of sesamolin **2** (50 mg, 0.14 mmol), *n*-propyl alcohol (0.2 mL) and Amberlyst-15 (10 mg), after 2 h, yielded *n*-propoxy samin (**2c,** 29.2 mg, 74%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (s, 1H, H-2″), 6.71 (m, 2H, H-5″ and H-6″), 5.88 (s, 2H, H-7″), 4.87 (s, 1H, H-2), 4.29 (m, 2H, H-6 and H-8), 3.90 (m, 1H, H-4), 3.78 (d, *J* = 8.8 Hz, 1H, H-4), 3.55 (m, 1H, H-1′), 3.48 (m, 1H, H-8), 3.28 (m, 1H, H-1′), 2.97 (m,1H, H-1), 2.75 (m, 1H, H-5), 0.85 (t, *J* = 7.2 Hz, 3H, H-3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.1, 147.3, 134.9, 119.7, 108.3, 107.6, 106.7, 101.2, 87.2, 71.6, 69.1, 53.3, 53.0, 23.0, 10.7; HRESIMS *m/z* 315.1204 $[M+Na]^+$ (calcd for $C_{16}H_{20}NaO_5$, 315.1208).

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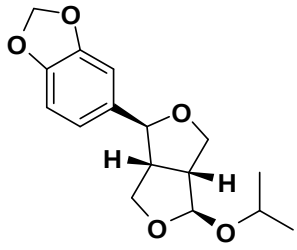
***iso*-Propoxy samin (2d)**



Following the general procedure above, reaction of sesamolin **2** (40 mg, 0.11 mmol), *iso*-propyl alcohol (0.2 mL) and Amberlyst-15 (10 mg), after 2 h, yielded *iso*-propoxy samin (**2d,** 22.1 mg, 70%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 (s, 1H, H-2″), 6.72 (m, 2H, H-5″ and H-6″), 5.88 (s, 2H, H-7″), 4.99 (s, 1H, H-2), 4.29 (m, 2H, H-6 and H-8), 3.92 (m, 1H, H-4), 3.83 (m, 1H, H-1′), 3.77 (d, *J* = 8.8 Hz, 1H, H-4), 3.48 (m, 1H, H-8), 2.93 (m,1H, H-1), 2.75 (m, 1H, H-5), 1.12 (d, *J* = 6.4 Hz, 3H, H-2′), 1.08 (d, *J* = 6.0 Hz, 3H, H-3′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.0, 147.1, 134.8, 119.8, 108.3, 106.7, 105.7, 101.2, 87.3, 71.6, 69.0, 68.9, 53.5, 53.0, 23.7, 21.7; HRESIMS *m/z* 315.1206 $[M+Na]^+$ (calcd for $C_{16}H_{20}NaO_5$, 315.1208).

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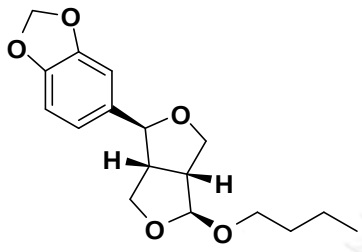
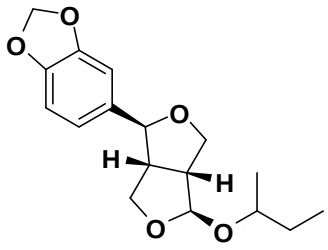
***n*-Butoxy samin (2e)**



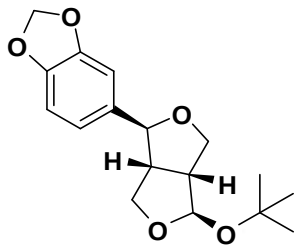
Following the general procedure above, reaction of sesamolin **2** (33 mg, 0.09 mmol), *n*-butyl alcohol (0.2 mL) and Amberlyst-15 (5 mg), after 2 h, yielded *n*-butoxy samin (**2e,** 20.5 mg, 75%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.85 (s, 1H, H-2″), 6.78 (m, 2H, H-5″ and H-6″), 5.95 (s, 2H, H-7″), 4.93 (s, 1H, H-2), 4.35 (m, 2H, H-6 and H-8), 3.96 (m, 1H, H-4), 3.85 (d, *J* = 8.8 Hz, 1H, H-4), 3.66 (m, 1H, H-1′), 3.54 (m, 1H, H-8), 3.37 (m, 1H, H-1′), 3.02 (m,1H, H-1), 2.85 (m, 1H, H-5), 1.36 (m, 1H, H-2′), 1.25 (m, 1H, H-3′), 0.91 (t, *J* = 7.4 Hz, 3H, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.0, 147.5, 134.9, 119.8, 108.3, 107.5, 106.7, 101.2, 87.2, 71.6, 69.0, 67.2, 53.3, 53.0, 31.9, 19.6, 14.0; HRESIMS *m/z* 329.1361 $[M+Na]^+$ (calcd for $C_{17}H_{22}NaO_5$, 329.1365).

***sec*-Butoxy samin (2f)**



Following the general procedure above, reaction of sesamolin **2** (40 mg, 0.11 mmol), *2*-butyl alcohol (0.2 mL) and Amberlyst-15 (5 mg), after 2 h, yielded *sec*-butoxy samin (**2f,** 22.1 mg, 67%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ6.85 (s, 1H, H-2″), 6.78 (m, 2H, H-5″ and H-6″), 5.94 (s, 2H, H-7″), 5.06 (s, 1H, H-2), 5.04 (s, 1H, H-2), 4.36 (m, 2H, H-6 and H-8), 3.99 (m, 1H, H-4), 3.83 (d, *J* = 9.2 Hz, 1H, H-4), 3.65 (m, 1H, H-1′), 3.54 (m, 1H, H-8), 3.02 (m,1H, H-1), 2.83 (m, 1H, H-5), 1.46 (m, 2H, H-2′), 1.17 (d, *J* = 8.0 Hz, 3H, H-4′), 1.12 (d, *J* = 8.0 Hz, 3H, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.0, 147.4, 134.9, 119.8, 108.2, 107.2, 106.7, 101.2, 87.3, 75.3 (73.2), 71.6, 69.1 (69.0), 53.3, 53.0, 30.4 (29.2), 21.1 (18.9), 10.2 (9.9). Chemical shifts in parenthesis indicated minor isomer; HRESIMS *m/z* 329.1363 $[M+Na]^+$ (calcd for $C_{17}H_{22}NaO_5$, 329.1365).

***t*-Butoxy samin (2g)**



Following the general procedure above, reaction of sesamolin **2** (38 mg, 0.10 mmol), *t*-butyl alcohol (0.2 mL) and Amberlyst-15 (5 mg), after 2 h, yielded *t*-butoxy samin (**2g,** 19.8 mg, 63%) as a pale yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 (s, 1H, H-2″), 6.71 (m, 2H, H-5″ and H-6″), 5.87 (s, 2H, H-7″), 5.17 (s, 1H, H-2), 4.27 (m, 2H, H-6 and H-8), 3.99 (m, 1H, H-4), 3.73 (d, *J* = 9.2 Hz, 1H, H-4), 3.50 (m, 1H, H-8), 2.88 (m,1H, H-1), 2.75 (m, 1H, H-5), 1.19 (s, 9H, H-2′ and H-3′ and H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.1, 147.3, 135.1, 119.7, 108.3, 106.7, 102.9, 101.2, 87.2, 74.6, 71.8, 68.8, 54.2, 53.2, 29; HRESIMS *m/z* 329.1366 $[M+Na]^+$ (calcd for $C_{17}H_{22}NaO_5$, 329.1365).

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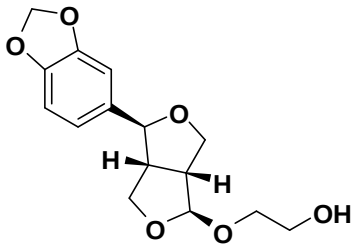
Ethane-1-ol-2-oxy samin (2h)



Following the general procedure above, reaction of sesamolin **2** (80 mg, 0.22 mmol), ethylene glycol (0.25 mL) and Amberlyst-15 (10 mg), after 2 h, yielded ethane-1-ol-2-oxy samin (**2h,** 52.1 mg, 82%) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.88 (s, 1H, H-2″), 6.82 (m, 2H, H-5″ and H-6″), 5.97 (s, 2H, H-7″), 5.32 (s, 1H, OH), 5.02 (s, 1H, H-2) 4.40 (m, 2H, H-6 and H-8), 4.04 (m, 1H, H-4), 3.93 (d, *J* = 9.2 Hz, 1H, H-4), 3.76 (m, 1H, H-2′), 3.67 (m, 1H, H-1′), 3.60 (m,1H, H-8), 3.12 (m, 1H, H-1), 2.87 (m, 1H, H-5); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.2, 147.5, 134.8, 119.8, 108.3, 106.7, 101.1, 87.1, 71.4, 70.0, 69.3, 62.4, 53.2, 52.8; HRESIMS *m/z* 317.0924 $[M+Na]^+$ (calcd for $C_{15}H_{18}NaO_6$, 317.1001).

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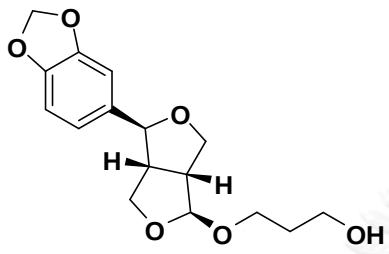
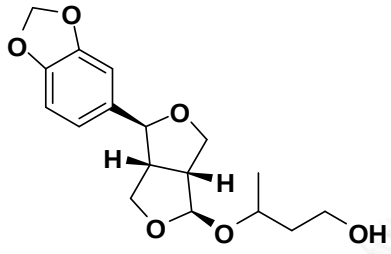
Propane-1-ol-3-oxy samin (2i)



Following the general procedure above, reaction of sesamolin **2** (70 mg, 0.19 mmol), 1,3-propane diol (0.2 mL) and Amberlyst-15 (10 mg), after 2 h, yielded propane-1-ol-3-oxy samin (**2i,** 45.4 mg, 78%) as a colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.78 (s, 1H, H-2″), 6.72 (m, 2H, H-5″ and H-6″), 5.88 (s, 2H, H-7″), 4.88 (s, 1H, H-2), 4.29 (m, 2H, H-6 and H-8), 3.91 (m, 1H, H-4), 3.80 (m, 2H, H-4 and H-3′), 3.69 (m, 2H, H-1′), 3.50 (m, 2H, H-8 and H-3′), 2.96 (m, 1H, H-1), 2.78 (m, 1H, H-5), 1.77 (m, 2H, H-2′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.2, 147.5, 134.8, 119.8, 108.3, 108.3, 107.8, 106.7, 101.2, 87.1, 71.5, 69.3, 65.9, 61.5, 53.3, 52.9, 32.1; HRESIMS *m/z* 331.1154 $[M+Na]^+$ (calcd for $C_{16}H_{20}NaO_6$, 331.1158).

Butane-1-ol-3-oxy samin (2j)



Following the general procedure above, reaction of sesamolin **2** (50 mg, 0.14 mmol), 1,3-butane diol (0.2 mL) and Amberlyst-15 (10 mg), After 2 h, yielded butane-1-ol-3-oxy samin (**2j,** 22.1 mg, 67%) as a colorless oil; (80%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.84 (s, 1H, H-2″), 6.77 (m, 2H, H-5″ and H-6″), 5.94 (s, 2H, H-7″), 4.94 (d, *J* = 4.4 Hz, 1H, H-2), 4.35 (m, 2H, H-6 and H-8), 3.90 (m, 2H, H-4 and H-1′), 3.82 (m, 2H, H-4 and H-3′), 3.48 (m, 2H, H-8 and H-3′), 3.02 (m, 1H, H-1), 2.81 (m, 1H, H-5), 1.64 (m, 2H, H-2′), 1.19 (d, *J* = 6.4 Hz, 3H, H-4′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 148.1, 147.5, 134.8, 119.7, 108.3, 107.8(107.7), 106.7, 101.2, 87.1, 71.4, 69.4(69.3), 67.3(67.1), 65.8(65.7), 53.3, 52.8, 38.3, 23.5. Chemical shifts in parenthesis indicated minor isomer; HRESIMS *m/z* 345.1310 $[M+Na]^+$ (calcd for $C_{17}H_{22}NaO_6$, 345.1314).

CHAPTER III

Bioactivities and Stability of Alkyloxy samins

3.1 Investigation of antioxidant activity (DPPH radical scavenging) and α-glucosidase inhibitory effect

For antioxidation of synthesized sesamolin derivatives, all new alcohol-modified sesamolins **2a-2j** showed radical scavenging activity toward DPPH with SC_{50} values in range of 27.32 – 51.46 mM (Table 3.1). Compared to the potent starter **2**, the synthesized derivatives **2a-2g** showed slightly weaker activity (~1.2-1.5 times) whereas diol-derived analogues **2h-2j** revealed significantly weaker scavenging (~1.6-2.3 times). The increase in alkyl chain length from C_1 in **2a** to C_4 in **2e-2f** did not significantly alter scavenging activity (Figure 3.1). Having identical chain length, the presence of one additional OH group was likely to reduce scavenging activity in some extend; these observations were exemplified by **2b** vs **2h** (SC_{50} 29.35 vs 36.66 mM) as well as **2c** vs **2i** (SC_{50} 30.27 vs 48.60 mM).

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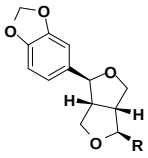
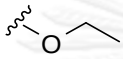
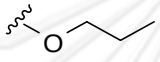
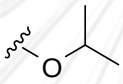
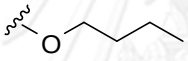
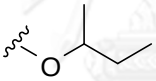
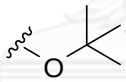
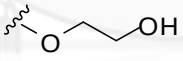
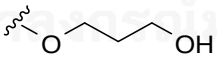
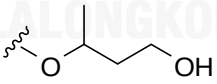
Table 3.1 Radical scavenging activity of alkyloxy samins

Compounds 	Radical scavenging (SC_{50}, mM)
2a R =	34.00±0.65
2b R = 	29.35±0.54
2c R = 	30.27±0.78
2d R = 	32.19±0.93
2e R = 	27.32±0.21
2f R = 	29.34±0.89
2g R = 	31.53±0.78
2h R = 	36.66±1.48
2i R = 	48.60±5.40
2j R = 	51.46±1.13
Sesamolin	22.59±0.10
BHT	19.81±0.02

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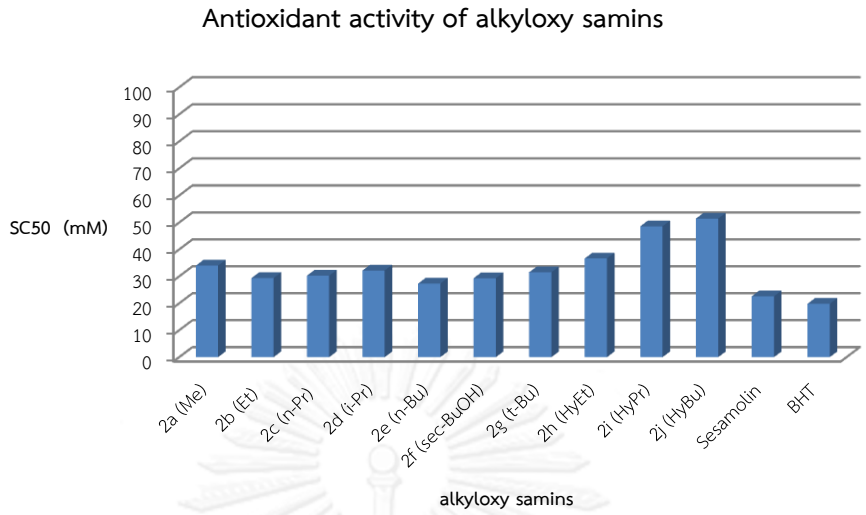

Figure 3.1 Inhibition trends of alkyloxy samins **2a**-**2j** compared to sesamolin and BHT.

All newly synthesized **2a-2j** were also evaluated for their α-glucosidase inhibitory effect. The commercial antidiabetic drug acarbose was used as the reference and the alkyloxy samins were also validated for comparative purpose. For α-glucosidase inhibitory activity, all synthesized compounds showed no inhibition (<30%), even at highest concentration examined (10 mg/mL), against both yeast and rat intestine α-glucosidases. The results are shown in Figures 3.2-3.4

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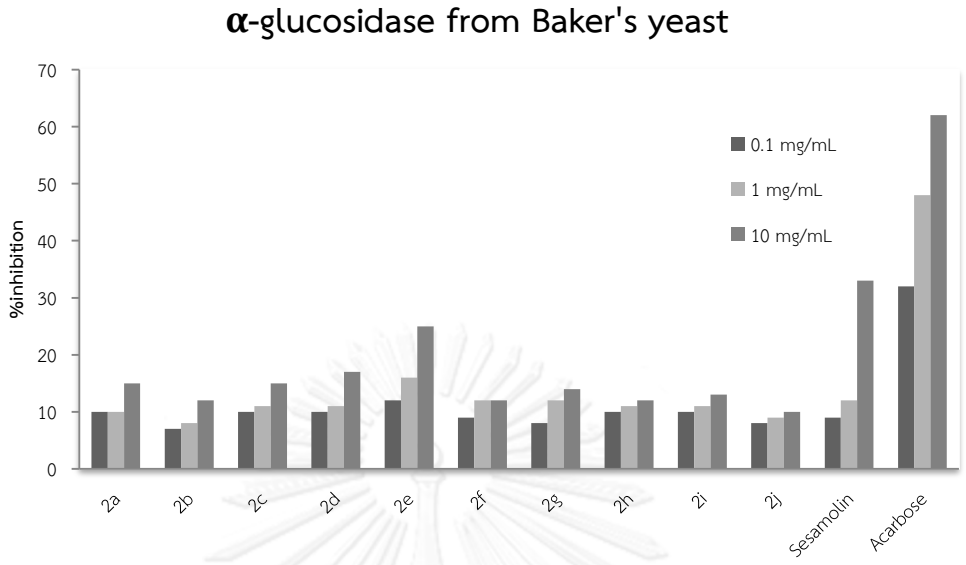

Figure 3.2 Yeast α-glucosidase inhibition trends of alkyloxy samins **2a**-**2j**

Figure 3.3 α-Glucosidase inhibition trends (from sucrase) of alkyloxy samins **2a**-**2j**

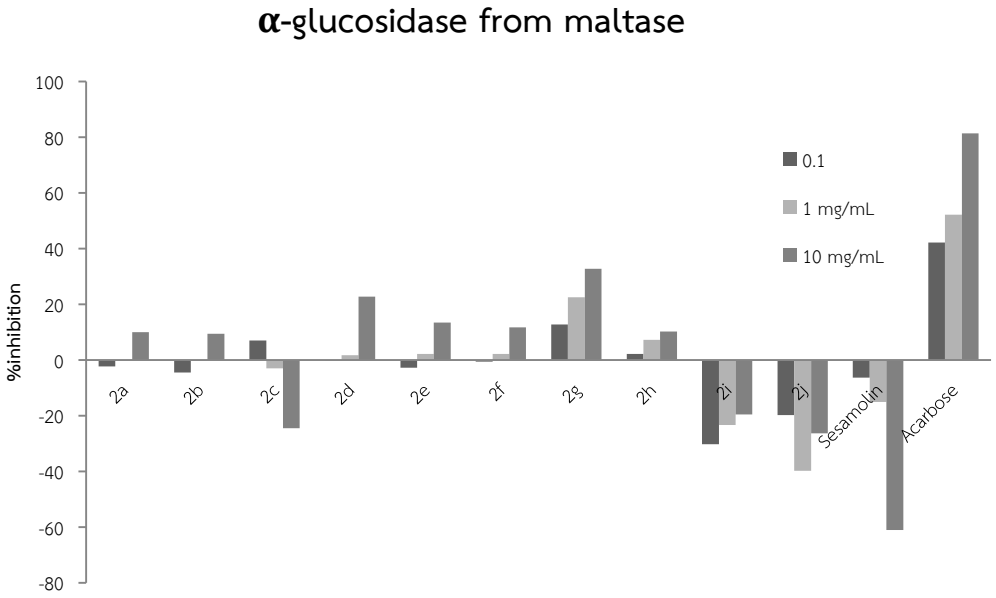

Figure 3.4 α-Glucosidase inhibition trends (from maltase) of alkyloxy samins **2a-2j**

3.2 Stability of alkyloxy samins in acid condition

Because alkyloxy samin has got the bioactivity which equals or isn't different from sesamolin especially the antioxidant, but sesamolin is unstable, especially under the acidic conditions. Thus, if alkyloxy samin is stable more than sesamolin, it can be used widely in applications. The testing the stability under acidic conditions is needed. Having the experiment for studying the stability in NMR by using CD_3OD for solvent and TFA-d for acid which was checked spectrum. Observing the reduction of compound in various times at every hours. Using **2j**, it is easy to follow signal which is clear from characterization by using 2D NMR technique.

Throughout the 3 hours, **2j** shows that the NMR has no signal from changing. No new signal occurs and the intensity of the signal is not changed. So alkyloxy samin is stable.

In contrast, sesamolin might have nucleophilic substitution reaction within 1 hour (t_1) by the appearance of H-a and H-6 which are deuterated sesamolin (**13a**). In addition to the H-2 and H-6 of the compound **2** are transformed to **13a** within 3 hours.

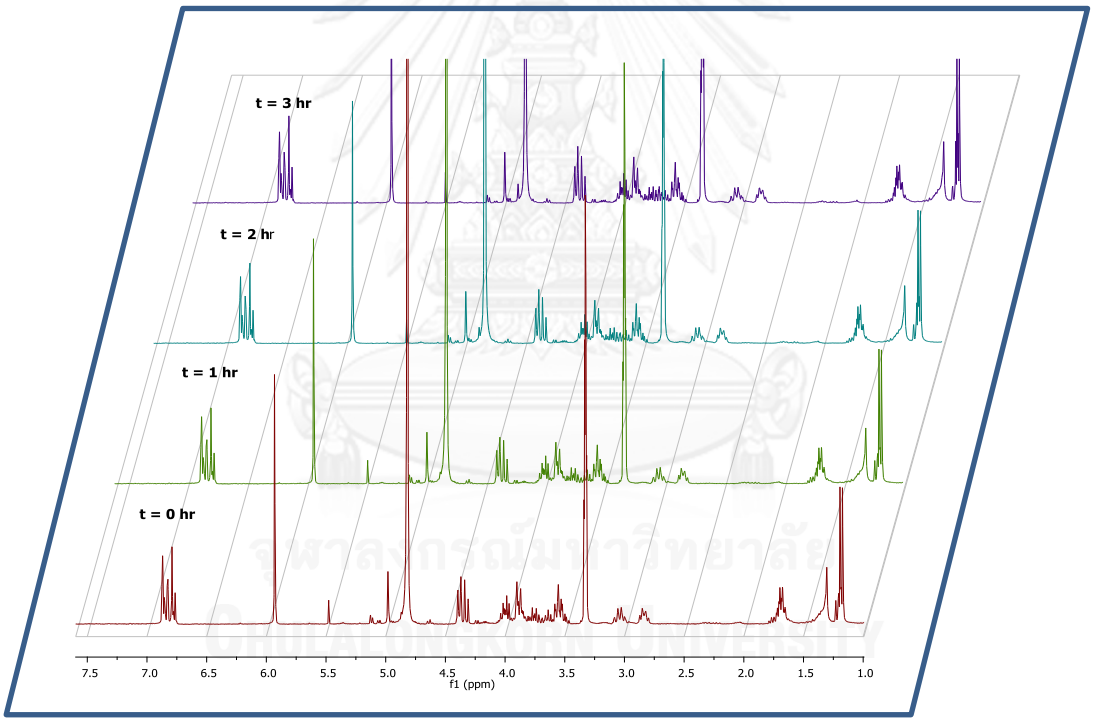

Figure 3.5 ^{1}H NMR sprectra of **2j** recorded in CD_3OD with one drop of CF_3CO_2D at t_0 – t_3

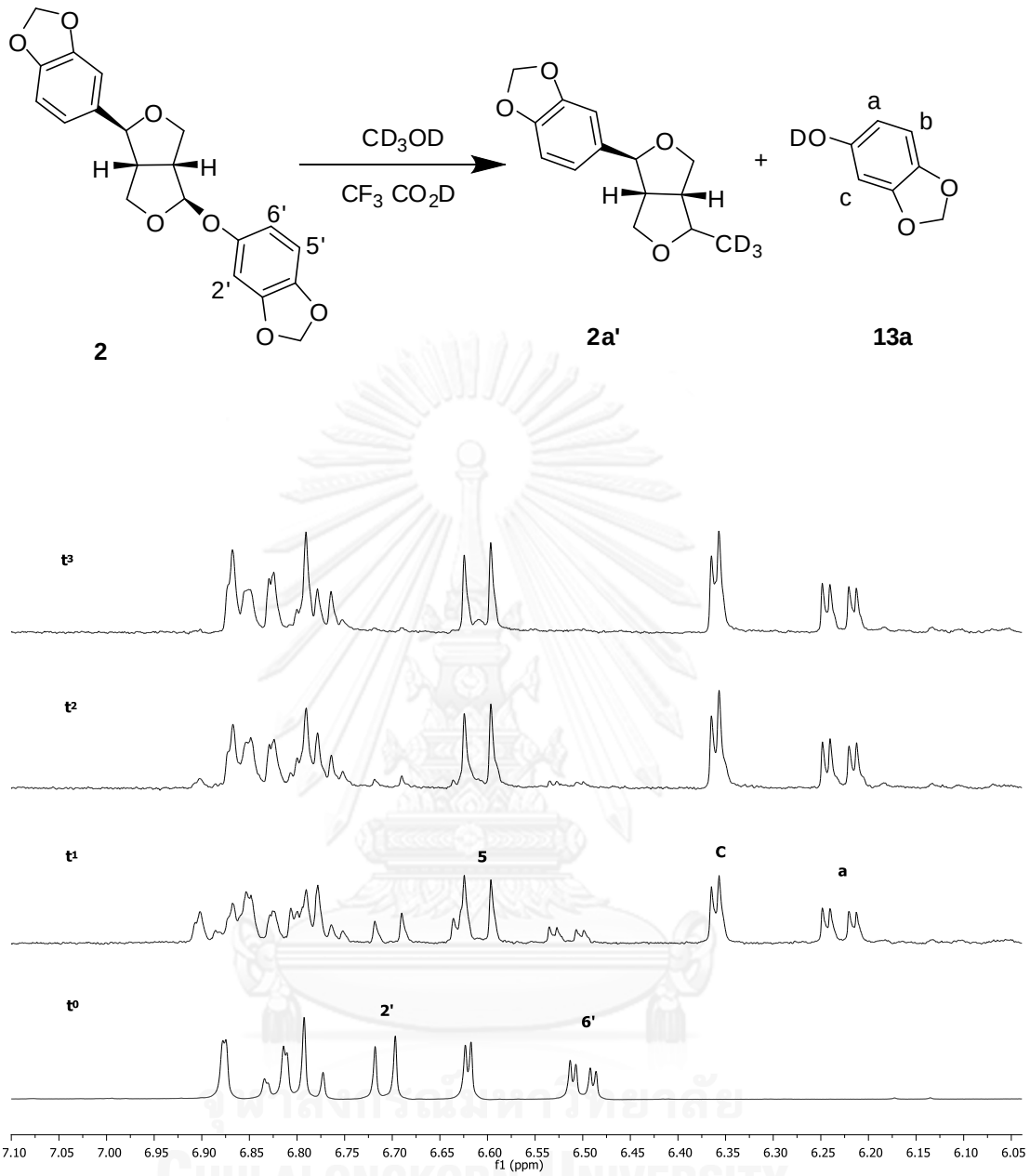

Figure 3.6 ^{1}H NMR spectra of **2** recorded in CD_3OD with one drop of CF_3CO_2D at t_0 – t_3

Over 3 hours of experiment, 2j remained unchanged while sesamolin is less than 50% within 1 hour, and the rest in disappeared within 3 hours (Figure 3.7).

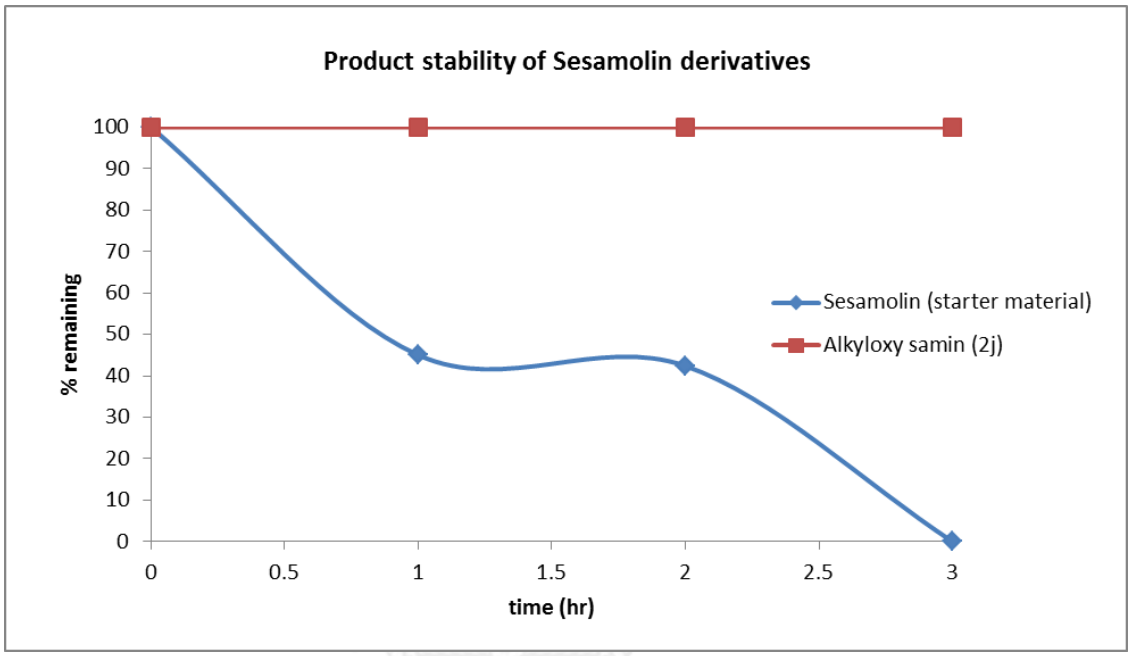

Figure 3.7 % remaining of **2** and **2j** under acid condition

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3.3 Experimental section

3.3.1 Chemical and equipment

Sucrose, maltose, baker's yeast α-glucosidase, rat intestinal acetone powder, and *p*-nitrophenyl-α-D-glucopyranoside, 2.2-diphenyl-1-picrylhydrazyl (DPPH) and BHT were purchased from Sigma-Aldrich (St. Louis, MO, USA). Glucose assay kit was purchased from Human Gesellschaft für Biochemica und Diagnostica mbH (Germany). Acarbose was obtained from Bayer (Germany).

3.3.2 DPPH radical scavenging

Radical scavenging activity was validated using DPPH colorimetric method (Dawidowicz *et al.*, 2012). Briefly, a sample solution (10 µL at concentrations of 0.1, 1.0 and 10.0 mg/mL) was added to 0.1 mM methanolic solution of DPPH (150 µL). The mixture was kept dark at room temperature in incubator shaker for 15 min. The absorbance of the resulting solution was measured at 517 nm with 96-well microplate reader. The percentage scavenging was calculated by $[(A_0 - A_1)/A_0] \times 100$, where A0 is the absorbance without the sample whereas A1 is the absorbance with the sample. The SC_{50} value was deduced from a plot of percentage scavenging versus sample concentration. BHT was used a standard antioxidant.

3.3.3 Assay for determining inhibitory effect against baker's yeast α-glucosidase

The α-glucosidase inhibition assay was performed according to the method discribed by Hwang 2012. The α-glucosidase (0.1 U/mL) and substrate (1 mM *p*-nitrophenyl-α-D-glucopyranoside) were dissolved in 0.1 M phosphate buffer, pH 6.9. Ten microlieds of isolated compounds (0.1, 1, 10 mg/mL in DMSO) was incubated with 40 µL of α- glucosidase at 37°C for 10 min. A 50 µL substrate solution was then added to the reaction mixture and incubated for additional 20 min, After the reaction was terminated by adding 100 µL of 1 M $Na_2CO_{3.,}$ the enzymatic hydrolysis of the *p*NPG was monitored at UV 405 based on the amount of *p*-nitrophenol released into the reaction mixture (Figure 3.7). The percentage inhibition was calculated by $[(A_0-A_1)/A_0]\times100$, where A_0 is the absorbance without the sample, while A_1 is the

absorbance with the sample, the IC_{50} value was determined from a plot of percentage inhibition versus sample concentration. Acarbose® was used as standard control and the experiment was performed in duplicate.

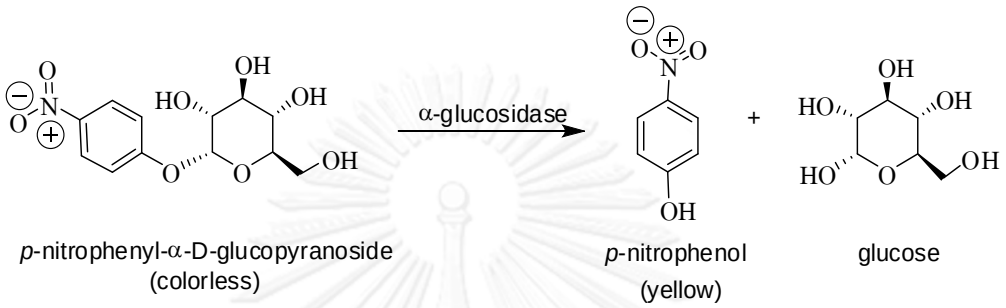

Figure 3.8 Hydrolysis of baker's yeast α-glucosidase

3.3.4 Assay for determining inhibitory effect against rat intestinal α-glucosidase

Rat intestinal α-glucosidase inhibitory activity is crude enzyme solution was prepared from rat intestinal acetone powder and used as a source of maltase and sucrase. Rat intestinal acetone powder (1 g) was homogenized in 30 mL of 0.9%NaCl solution. After centrifugation (12,000*g* × 30 min). Briefly, 10 μL of the test sample and substrate solution (maltase: 10 mM, 20 μL; sucrose: 100 mM, 20 μL, respectively) in 0.1 M phosphate, buffer (pH 6.9); therefore, the reaction mixture was then incubated at 37 ^{o}C for 20 min (for maltose) or 60 min (for sucrose). The mixtures were discontinued in boiling water for 10 min to stop reaction were determined by the glucose assay kit and measured at 500 nm.The percentage inhibition was calculated by $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance without the sample, and A_1 is the

absorbance with the sample. The IC_{50} value was determined from a plot of percentage inhibition versus sample concentration. Acarbose® was used as standard control and the experiment was performed in duplicate.

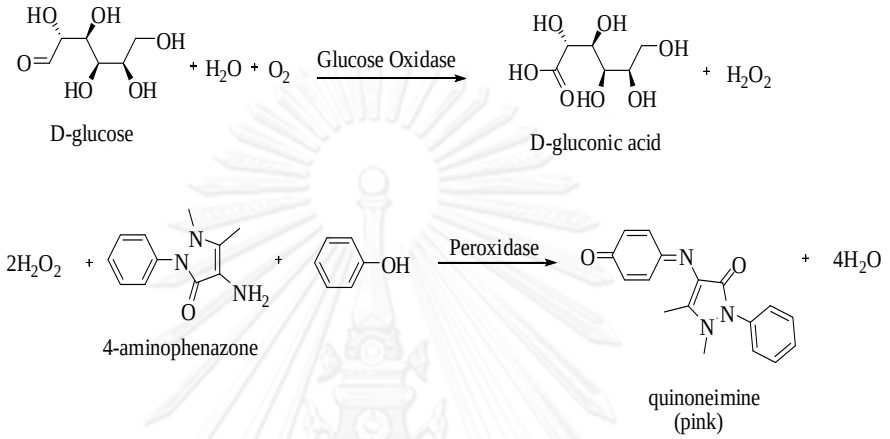

Figure 3.9 The reaction principle of α-glucosidase from rat small intestine

3.3.5 NMR-based monitoring stability of alkyloxy samins.

Determination of stability of alkyloxy samin (**2j**) and sesamolin (**2**), alkyloxy samin (**2j**, 5 mg) or sesamolin (**2**, 5 mg) was added with TFA (source of acid) 20 μL. The reaction mixture checked by ^{1}H NMR (300 MHz) in various times at every hours (t=0 h, 1 h, 2 h, 3 h).

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CHAPTER IV

CONCLUSION

The synthetic design was implemented in the hope high structure diversity by modification at C-2 acetal. Ten new sesamolin derivatives have been synthesized. The synthesis pathway was proceeded via oxocarbenium ion of sesamolin in acidic condition followed by nucleophilic substitution of alcohols and diols which resulted in retention of configuration. For structure-activity relationship, all new synthesized derivatives demonstrated slightly weaker antioxidant activity (~1.2-1.5 times) compared with sesamolin. The antioxidant activity of these alcohol-derived analogues (**2a-2g**) showed higher potent activity over diol-derived analogues (**2h-2j**). However, all synthesized compounds showed no α-glucosidase activity. Having identical chain length, the presence of one additional OH group was likely to reduce scavenging activity in some extend. Moreover, we also investigated the stability of alkyloxy samin and sesamolin. Alkyloxy samin is more stable than sesamolin.

The low cost and easy conversion of sesamolins to alkyloxy samins suggested prospects for synthesis alternative source of sesamolin. The research demonstrated the feasibility of chemical transformation of sesamolin to various derivatives. In addition, the replacement of aryloxy samin under acid condition was improved.

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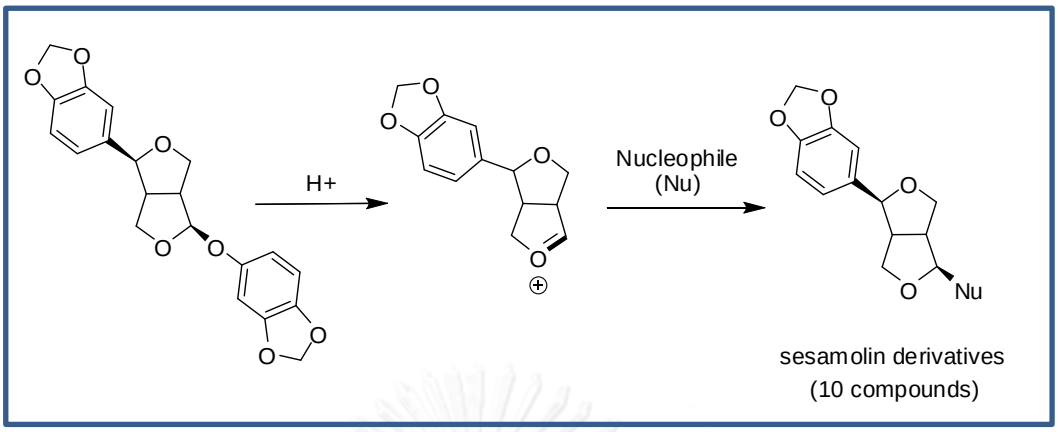
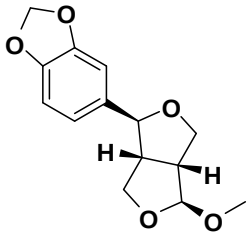
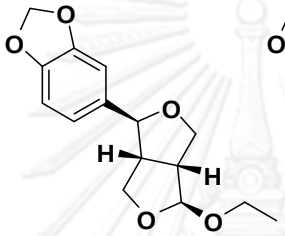




2a



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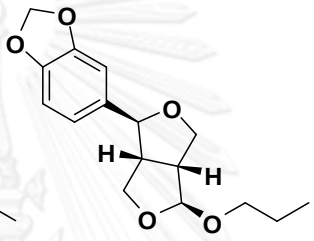
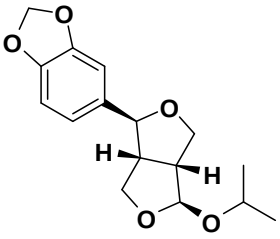


2c



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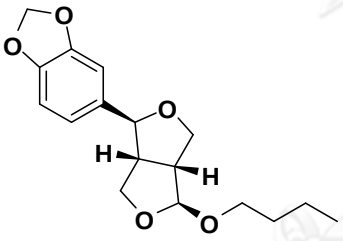
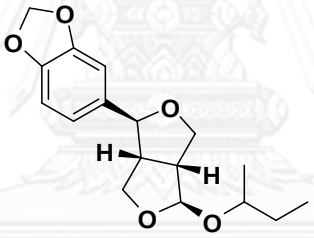
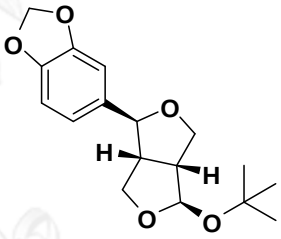


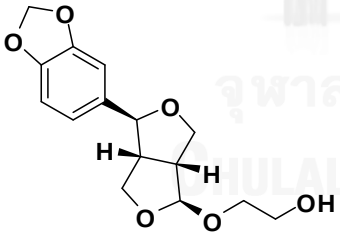
2e



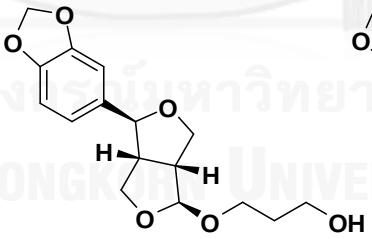
2f



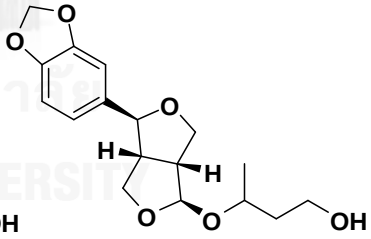
2g



2h



2i



2j

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APPENDIX

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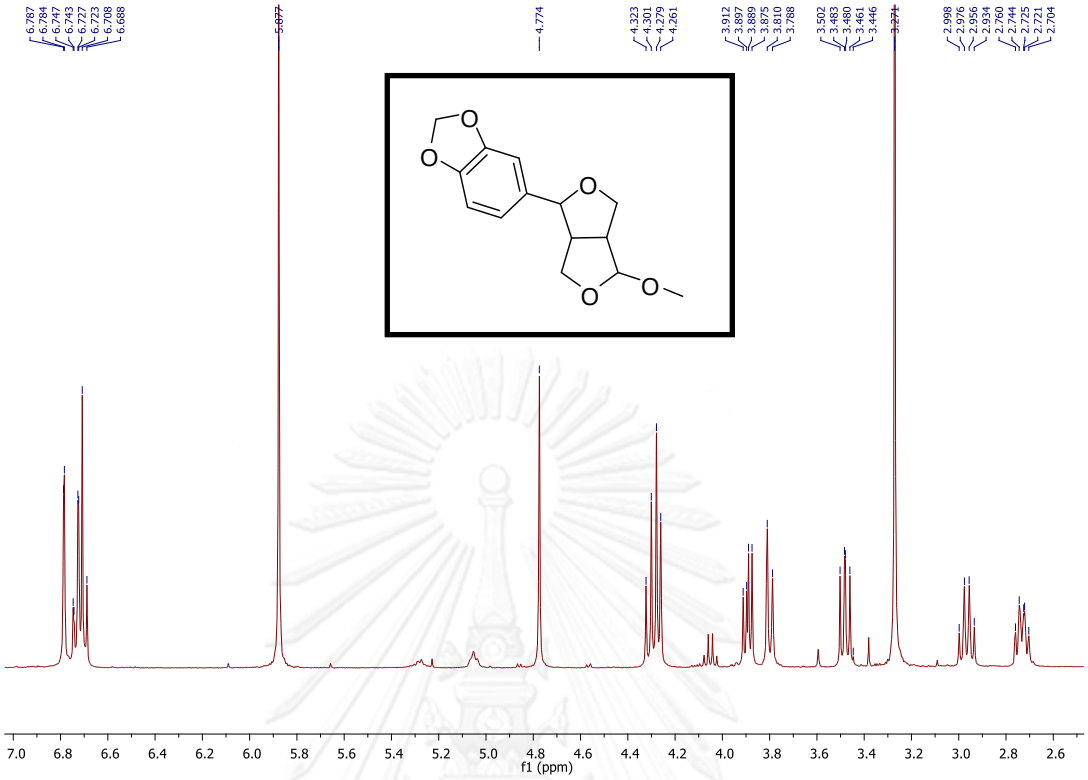

Figure 1 The ^{1}H NMR ($CDCl_3$) spectrum of methoxy samin **(2a)**

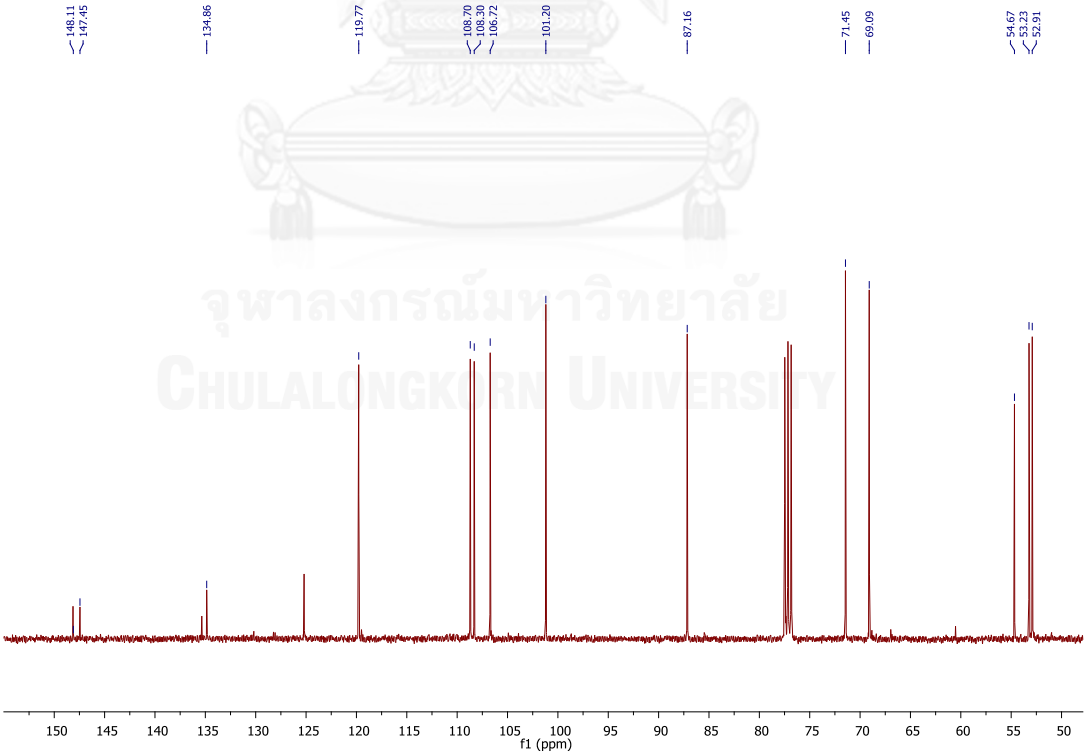

Figure 2 The ^{13}C NMR ($CDCl_3$) spectrum of methoxy samin **(2a)**

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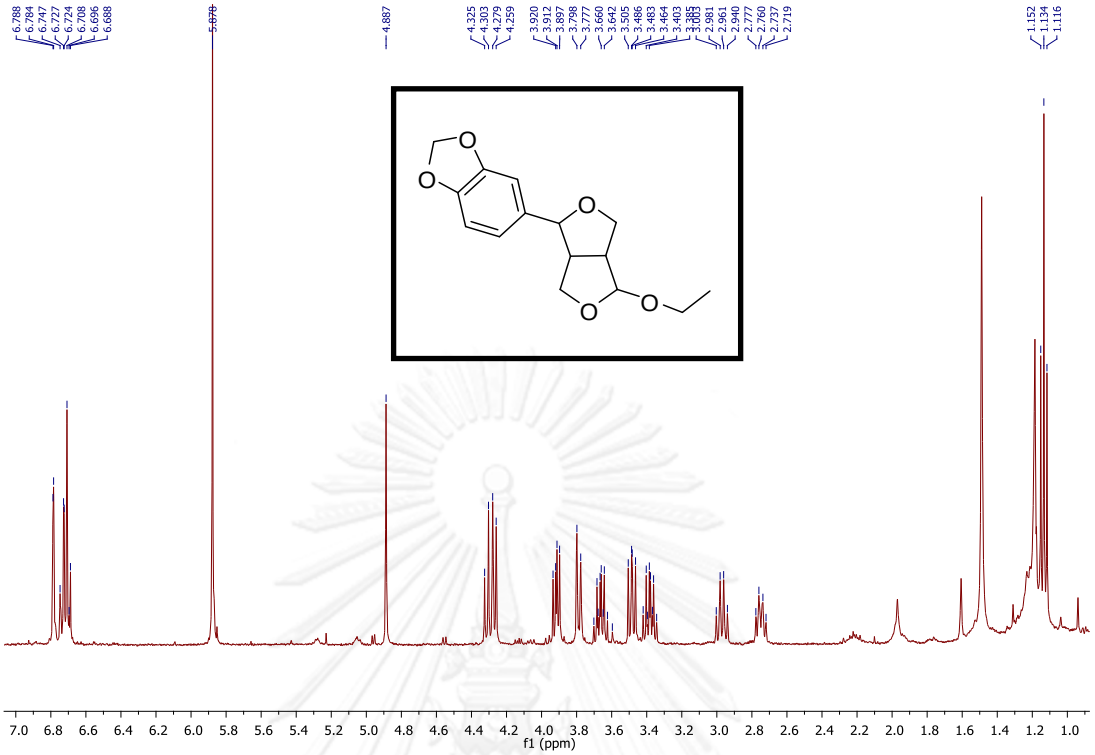

Figure 3 The ^{1}H NMR ($CDCl_3$) spectrum of ethoxy samin (**2b**)

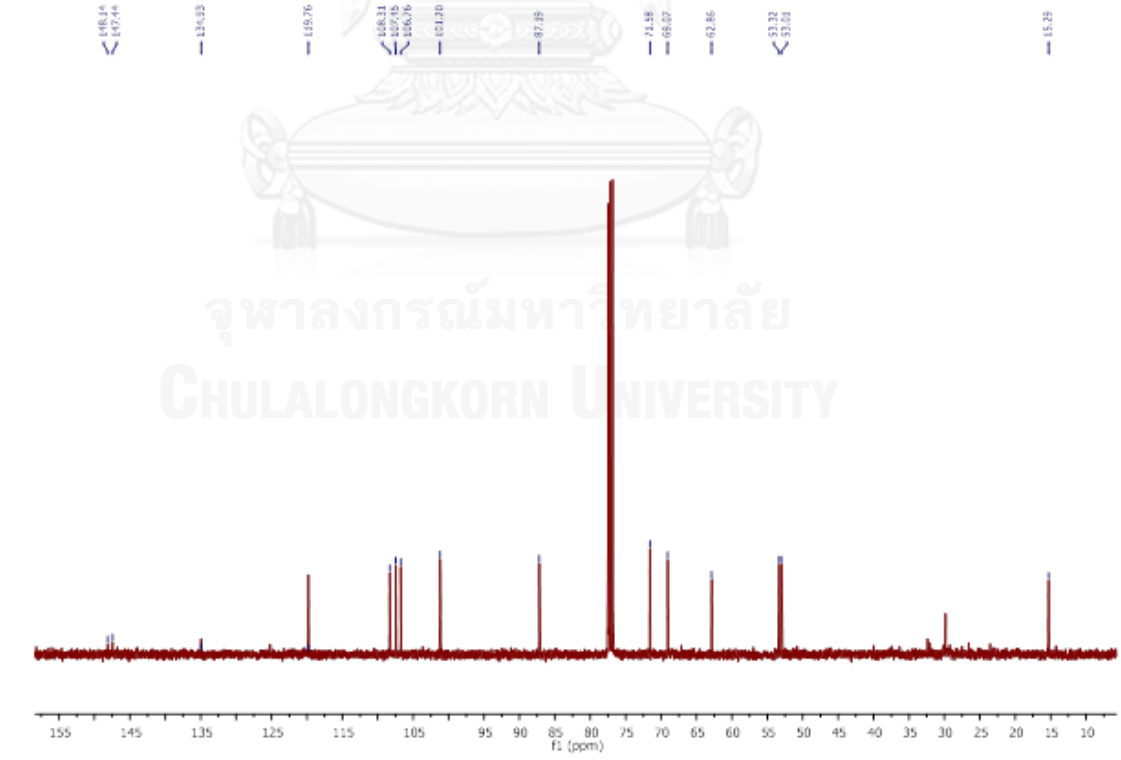

Figure 4 The ^{13}C NMR ($CDCl_3$) spectrum of ethoxy samin **(2b)**

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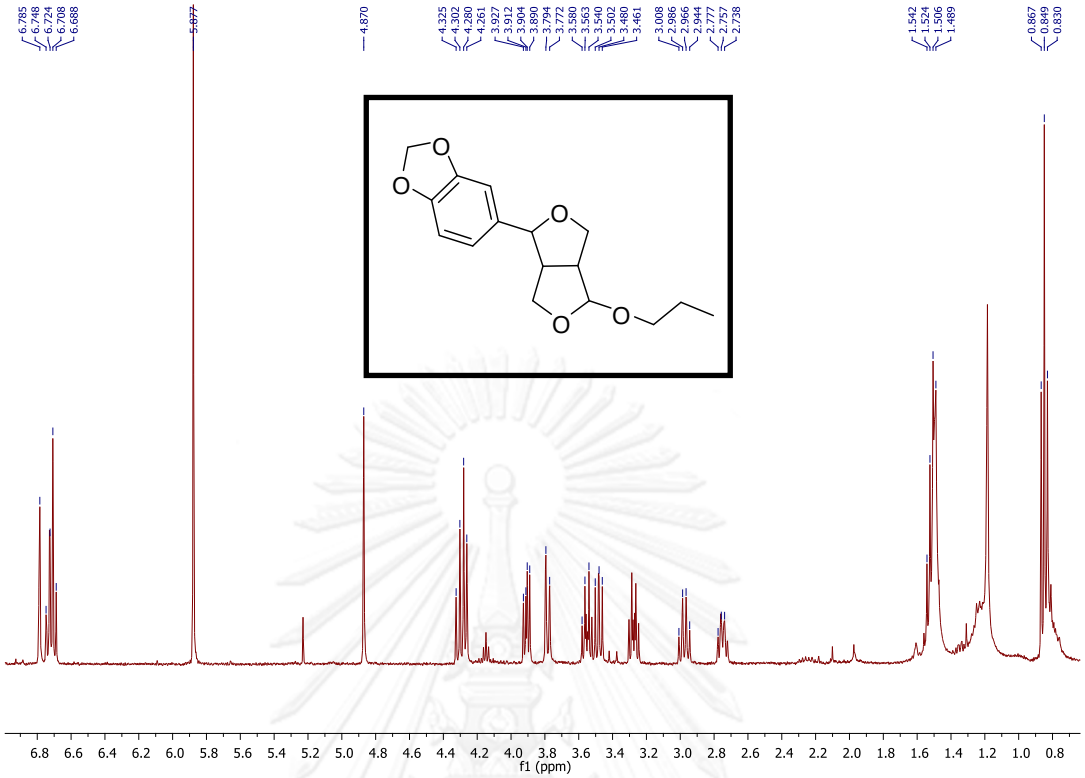

Figure 5 The ^{1}H NMR ($CDCl_3$) spectrum of ***n*-propoxy samin (2c)**

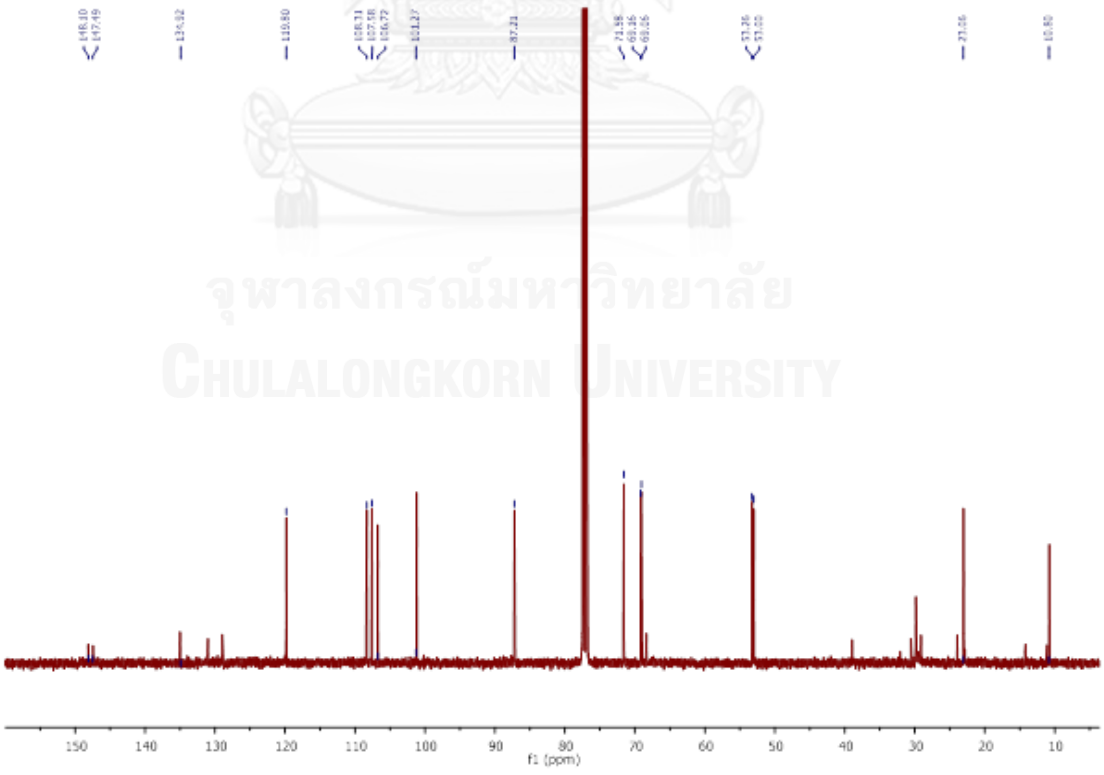

Figure 6 The ^{13}C NMR ($CDCl_3$) spectrum of ***n*-propoxy samin (2c)**

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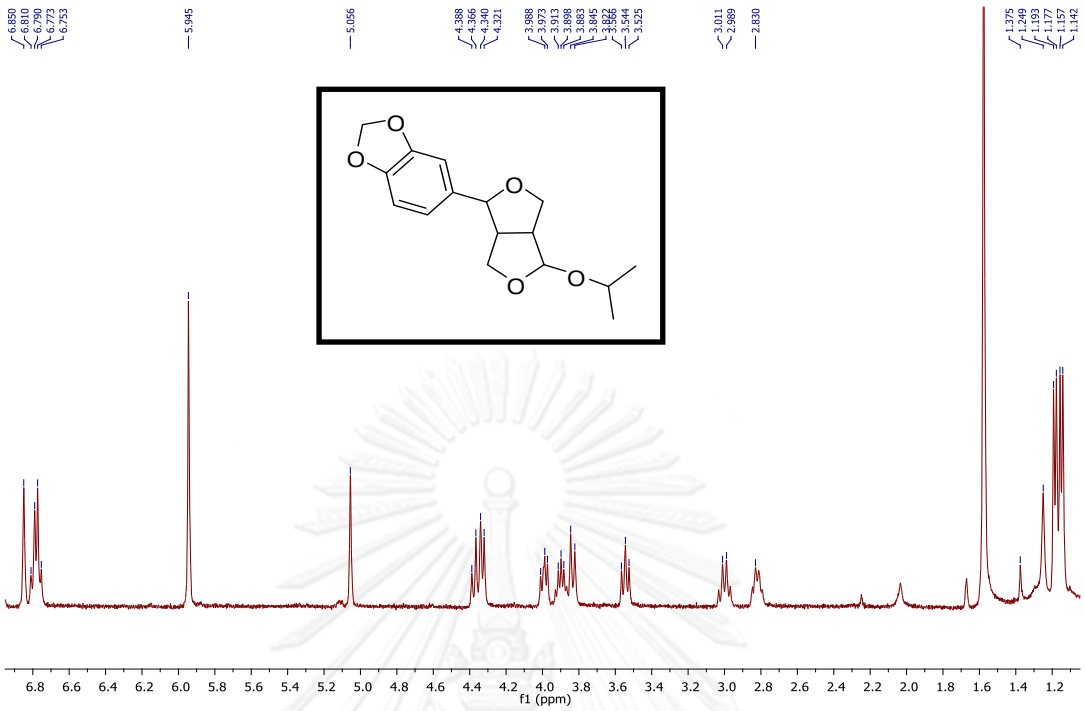

Figure 7 The ^{1}H NMR ($CDCl_3$) spectrum of ***iso*-propoxy samin (2d)**

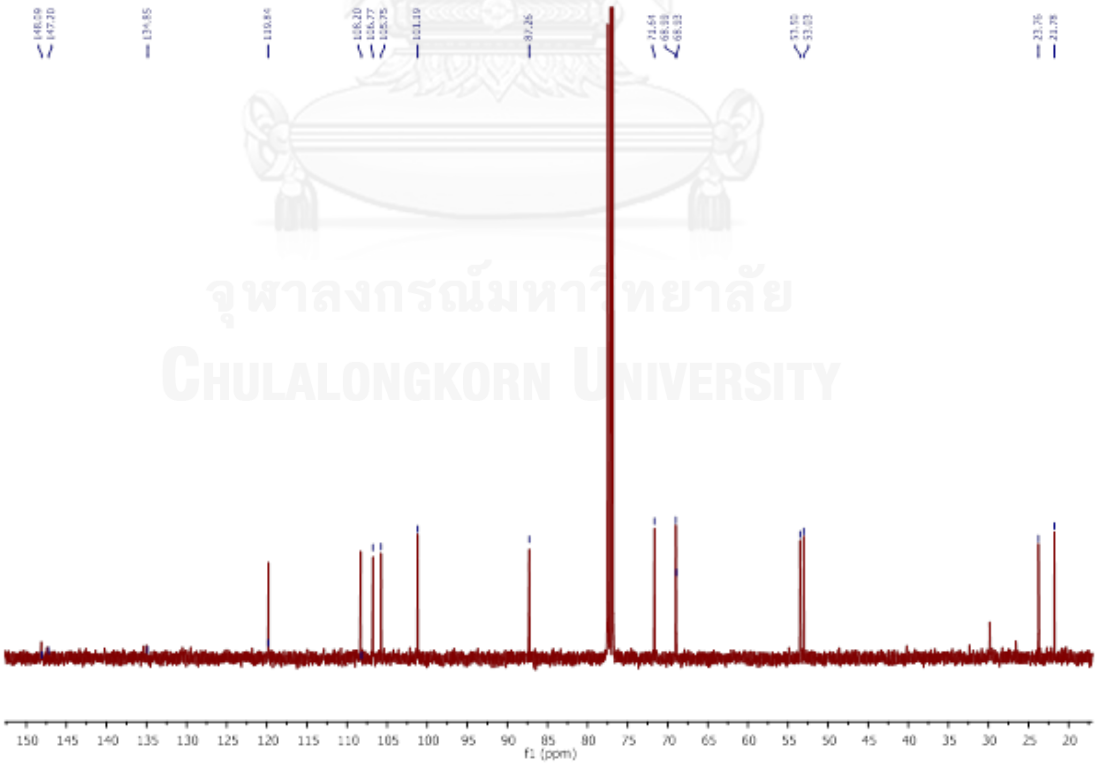

Figure 8 The ^{13}C NMR ($CDCl_3$) spectrum of ***iso*-propoxy samin (2d)**

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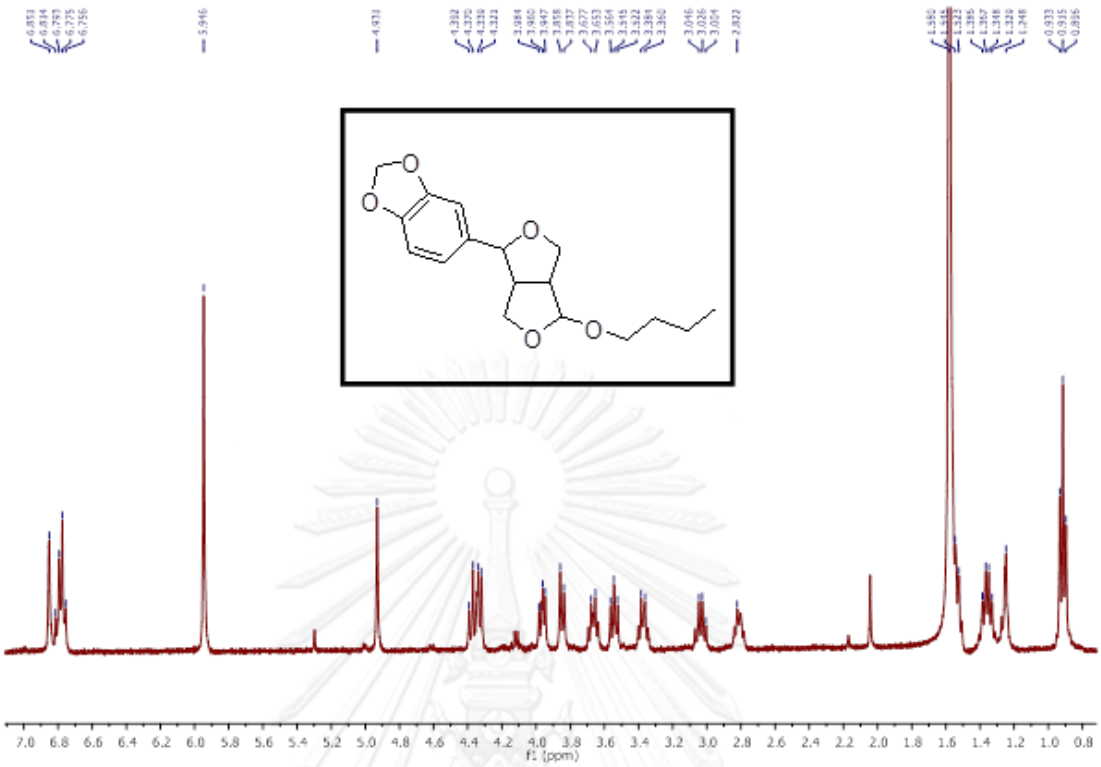

Figure 9 The ^{1}H NMR ($CDCl_3$) spectrum of ***n*-butoxy samin (2e)**

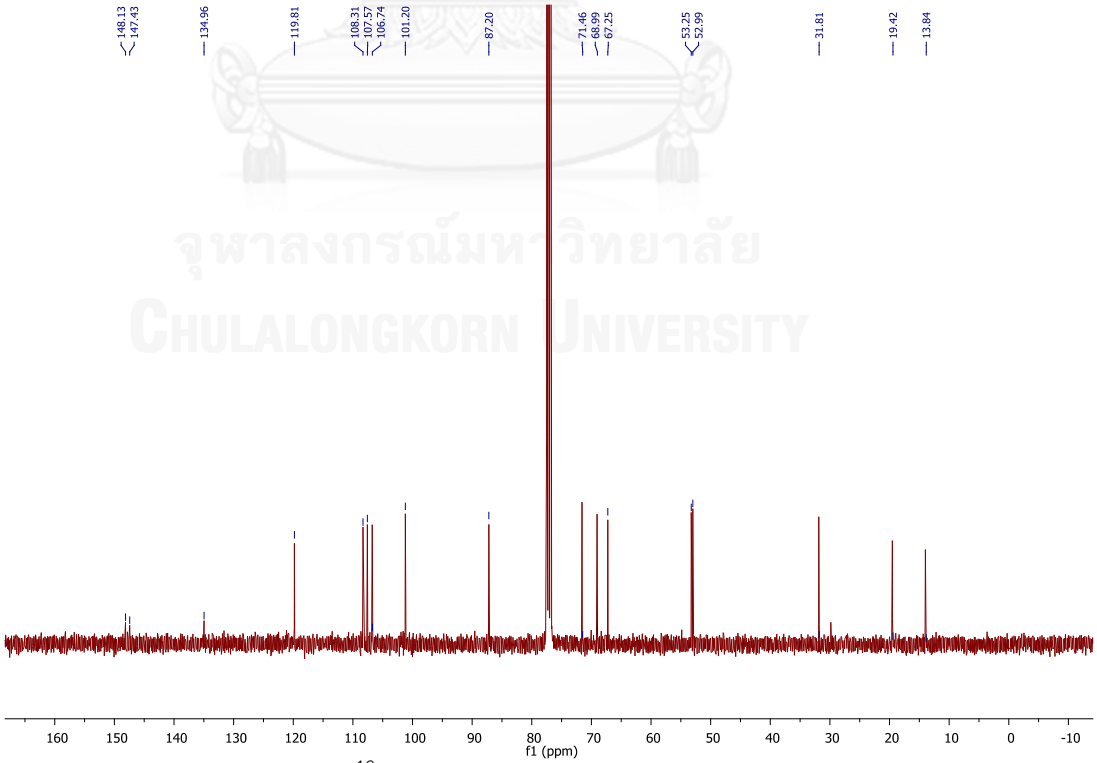

Figure 10 The ^{13}C NMR ($CDCl_3$) spectrum of ***n*-butoxy samin (2e)**

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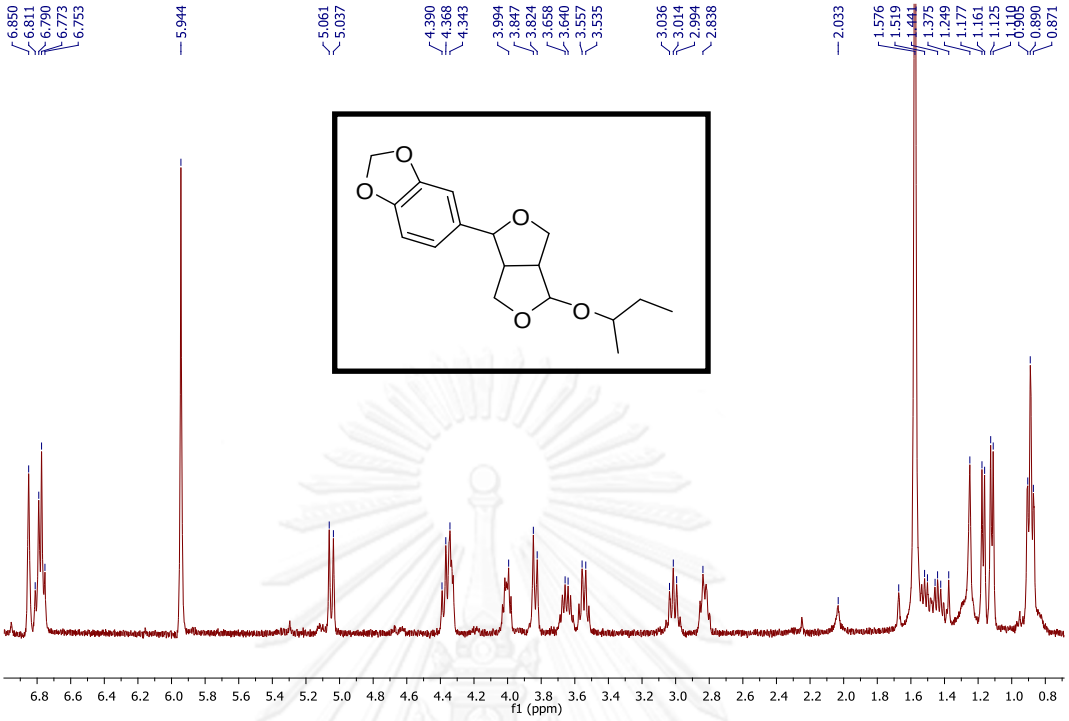

Figure 11 The ^{1}H NMR ($CDCl_3$) spectrum of ***sec*-butoxy samin (2f)**

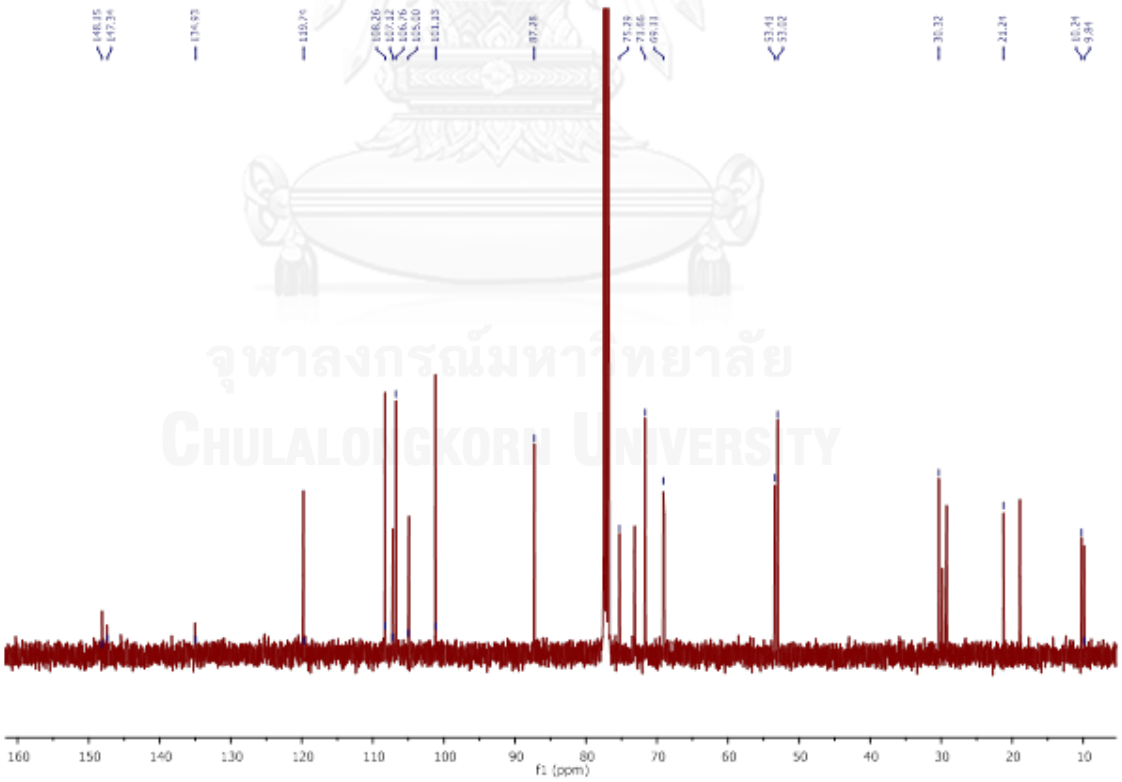

Figure 12 The ^{13}C NMR ($CDCl_3$) spectrum of ***sec*-butoxy samin (2f)**

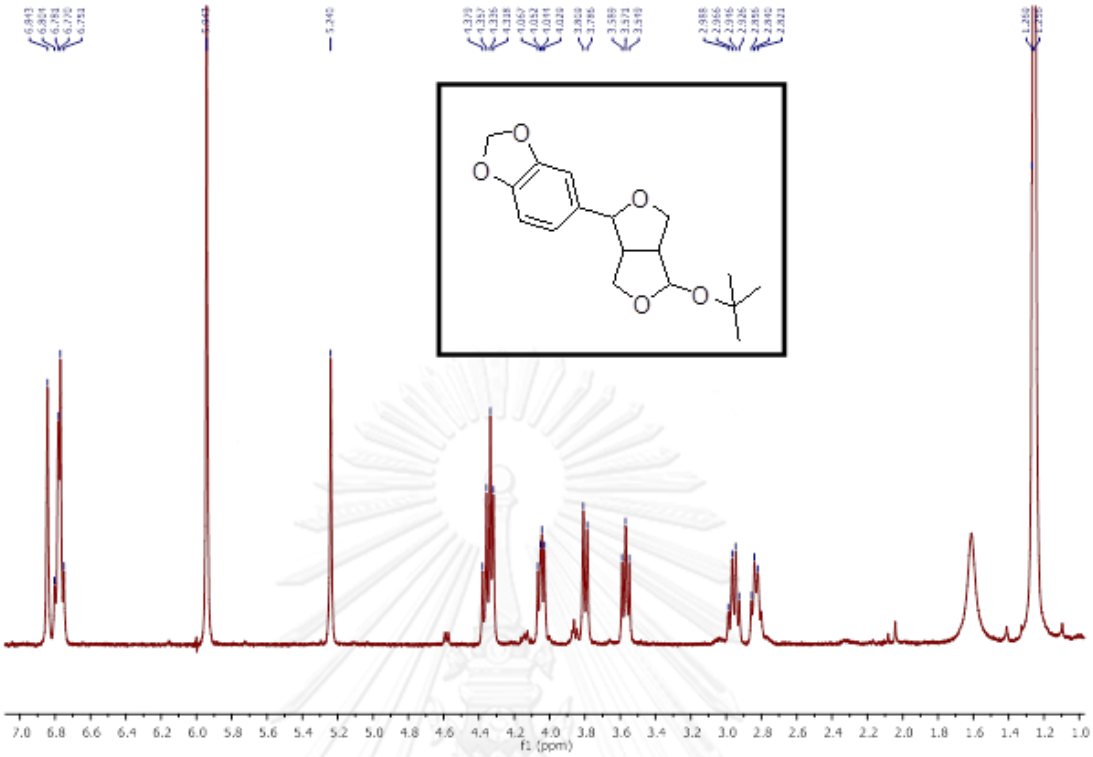

Figure 13 The ^{1}H NMR ($CDCl_3$) spectrum of ***t*-butoxy samin (2g)**

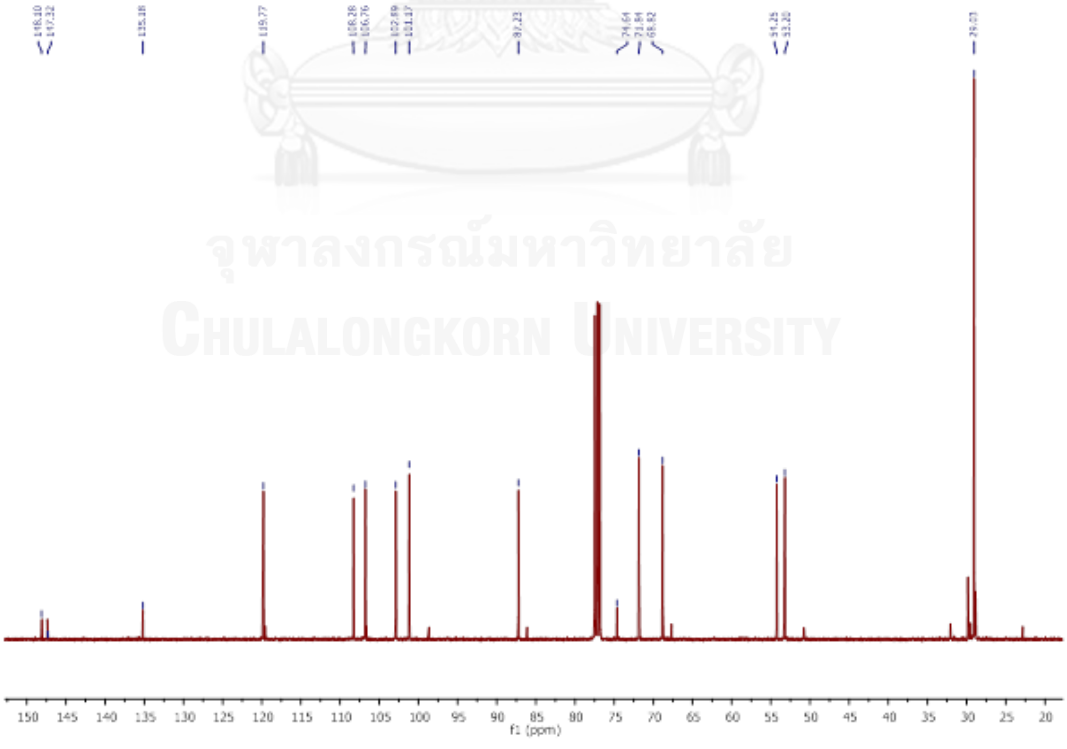

Figure 14 The ^{13}C NMR ($CDCl_3$) spectrum of ***t*-butoxy samin (2g)**

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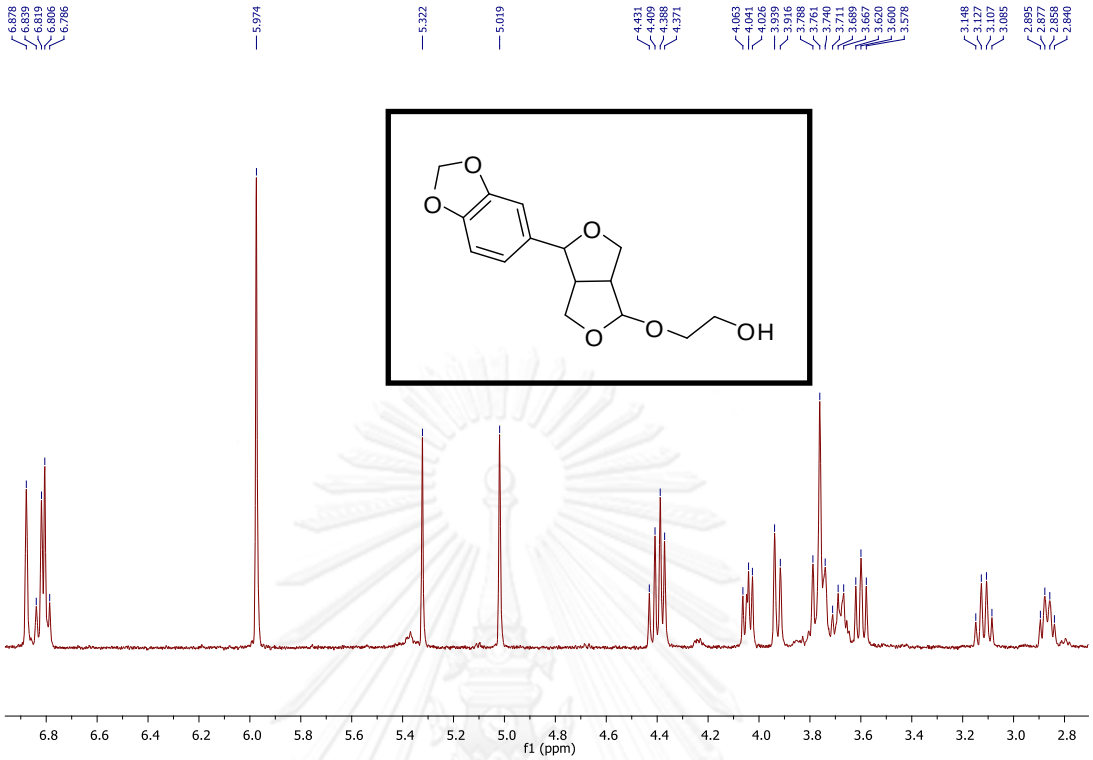

Figure 15 The ^{1}H NMR ($CDCl_3$) spectrum of **ethane-1-ol-2-oxy samin (2h)**

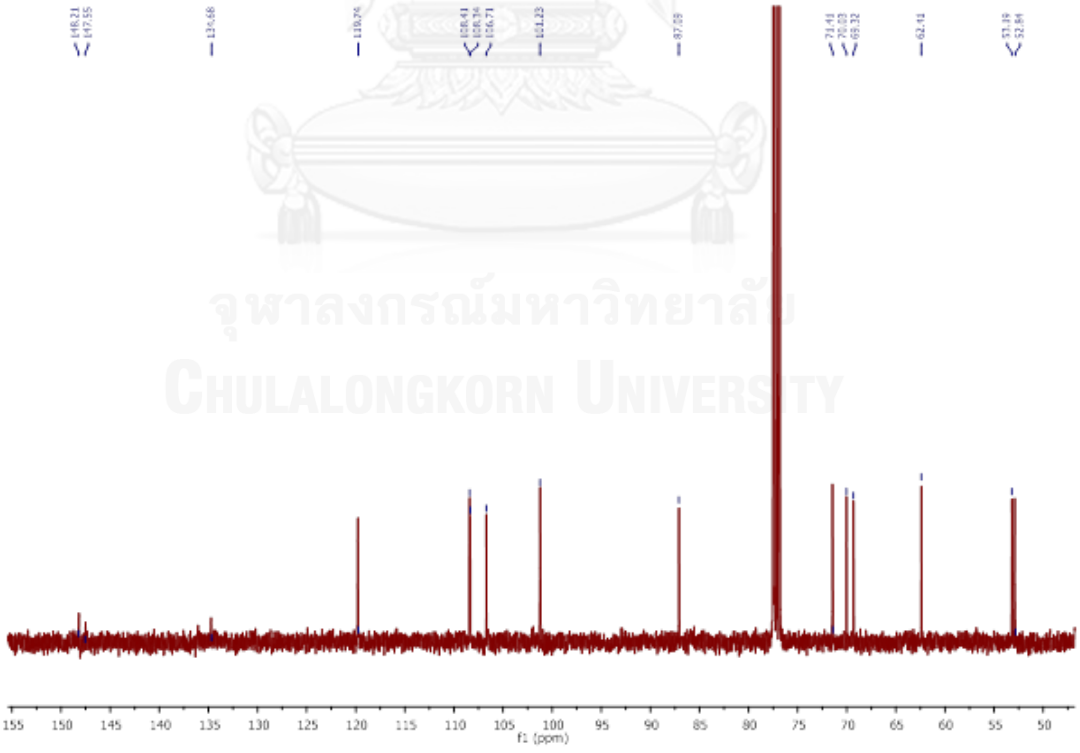

Figure 16 The ^{13}C NMR ($CDCl_3$) spectrum of **ethane-1-ol-2-oxy samin (2h)**

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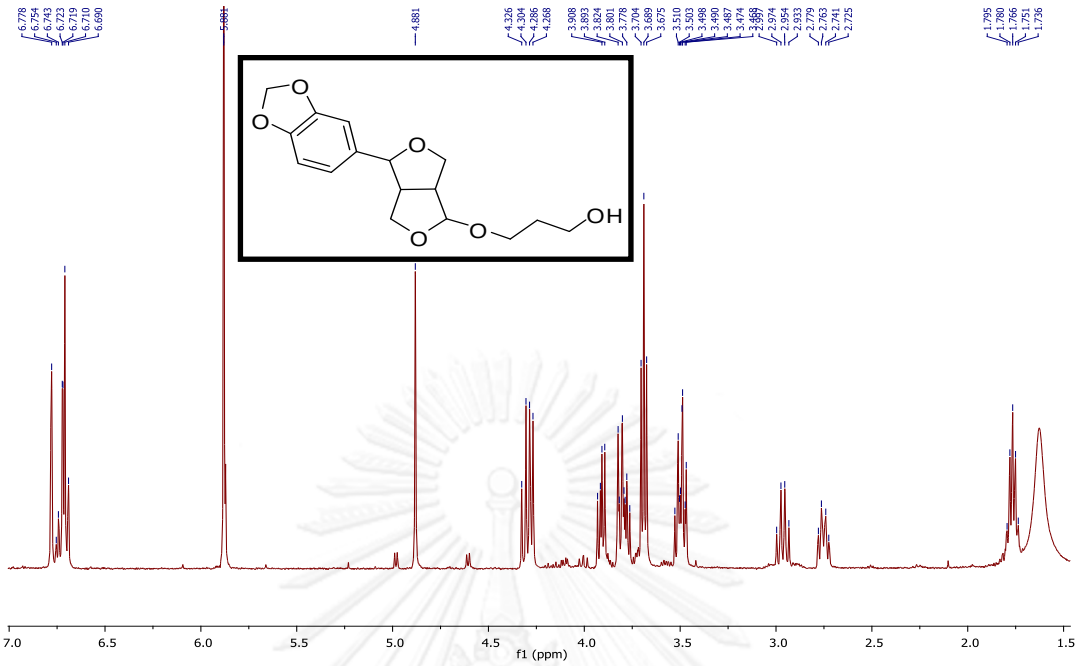

Figure 17 The ^{1}H NMR ($CDCl_3$) spectrum of **propane-1-ol-3-oxy samin (2i)**

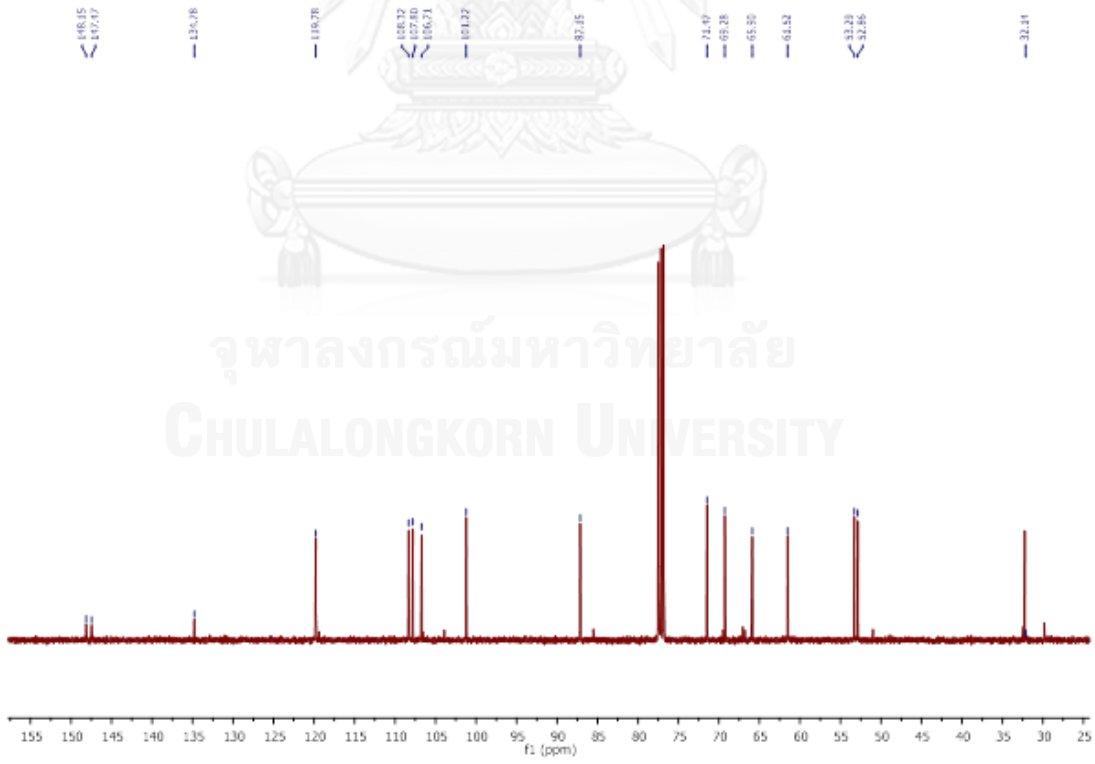

Figure 18 The ^{13}C NMR ($CDCl_3$) spectrum of **propane-1-ol-3-oxy samin (2i)**

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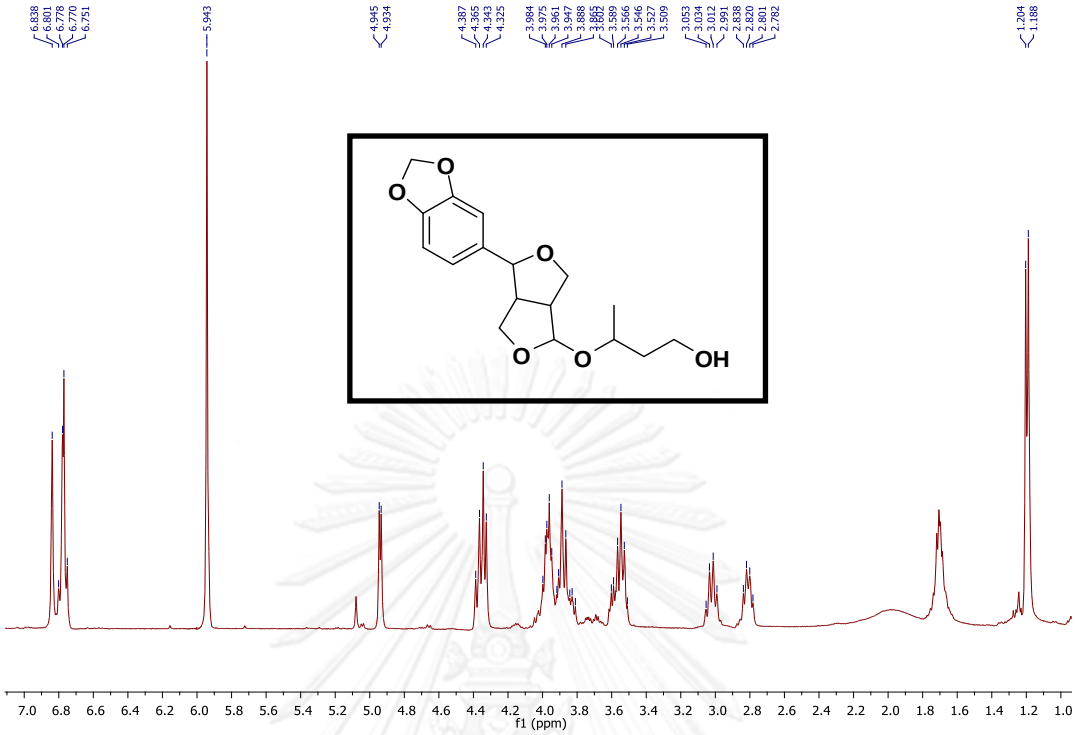

Figure 19 The ^{1}H NMR ($CDCl_3$) spectrum of **butane-1-ol-3-oxy samin (2j)**

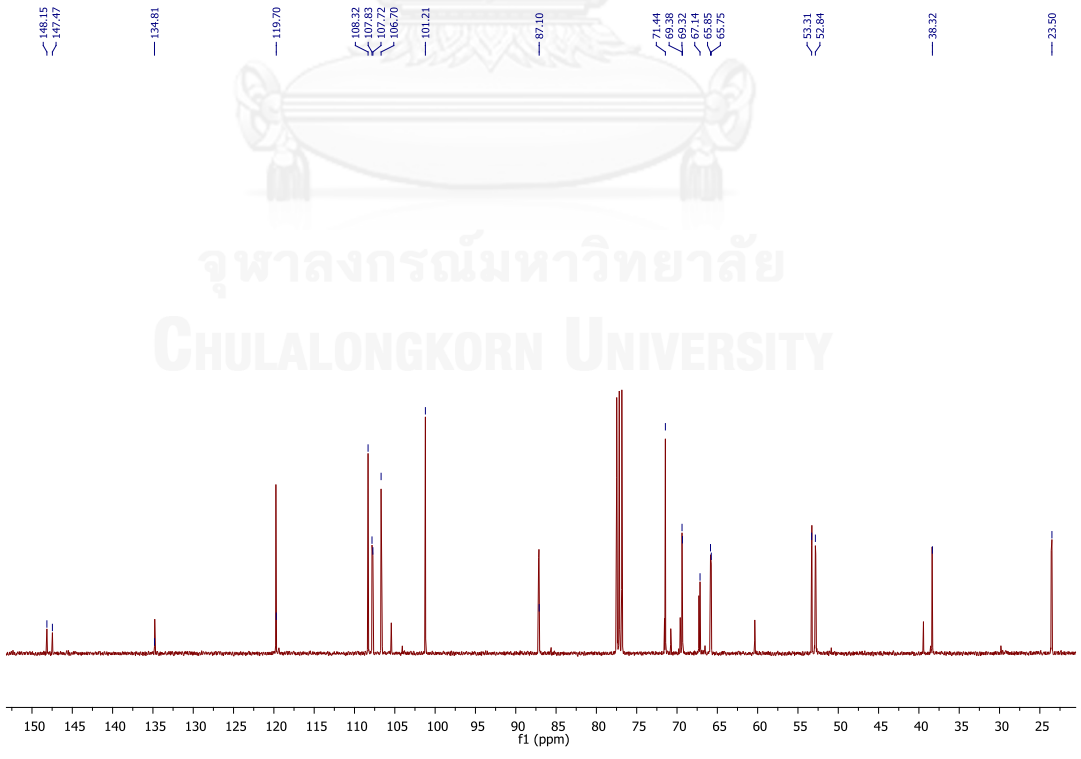

Figure 20 The ^{13}C NMR ($CDCl_3$) spectrum of **butane-1-ol-3-oxy samin (2j)**

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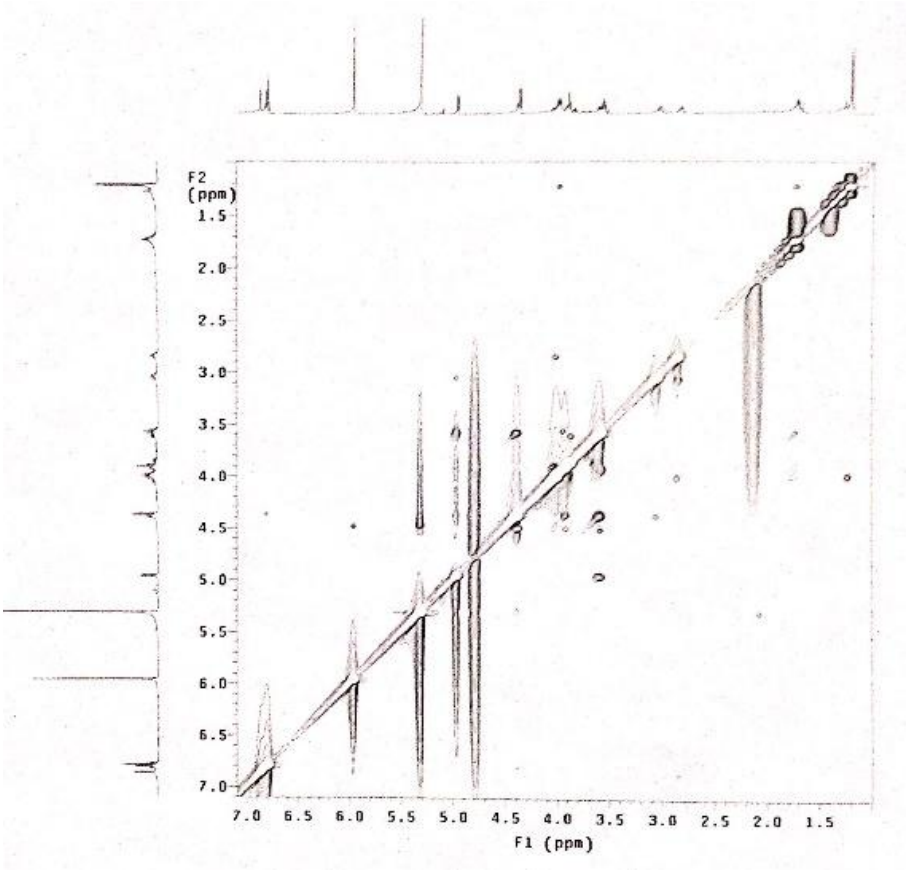

Figure 21 The NOESY spectrum (500, in $CDCl_3$) of **2j**

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VITA

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