JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Food and Beverage Chemistry/Biochemistry

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J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.0c01616 • Publication Date (Web): 12 May 2020

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Formation of Samin Diastereomers by Acid-catalyzed Transformation of Sesamolin with Hydrogen Peroxide

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ABSTRACT

The conversion of sesame lignans is of interest because the derived products may have potential applications. Here, in investigating the transformation of sesamin and sesamolin in both acidic aqueous and anhydrous systems, 7*R*,7'*S*-samin was identified as one of the major products of sesamolin in both systems catalyzed with common inorganic acids, but sesaminol was not generated. In investigating the effect of different oxidizing agents on the acid-catalyzed conversion of sesame lignans, 7*R*,7'*S*-samin was still the major product of sesamolin, whereas sesamolin stereo-selectively rendered 7*R*,7'*R*-samin in the presence of hydrogen peroxide. Hydrogen peroxide may play a role in stabilizing the transitional oxonium ions, derived from acid hydrolysis of sesamolin by forming a seven-membered ring intermediate through hydrogen bonding, to consequently produce 7*R*,7'*R*-samin as the final product.

KEYWORDS: sesame oil, furofuran lignans, sesamolin-derivative, acid-catalyzed transformation, samin diastereomers, hydrogen peroxide

INTRODUCTION

Sesame oil produced from seeds of *Sesamum indicum* is considered a nutritious and stable edible oil. Sesame seed is one of the major plant sources of furofuran lignans, which have numerous physiological activities. The main agluconic lignans in sesame seeds are sesamin and sesamolin. Sesamin and sesamolin have a variety of biological activities, such as anti-oxidation, hypocholesterolemic, hepatoprotective, antihypertension, and neuroprotective properties.[1-5] Meanwhile, sesame oil is also known for its superior oxidative stability. Despite its high level of unsaturated fatty acids, sesame oil is relatively stable against oil deterioration as compared with other dietary oils.[6-10] The resistance of sesame oil to oxidation was mainly attributed to the presence and synergy of minor components including lignans, lignan derivatives, tocopherols, and Maillard reaction products generated when sesame seeds are roasted.[6, 10-13] As a result, oil processing may affect the configuration of sesame lignans and play an important role in enhancing sesame oil's oxidative stability.

Several studies have reported the changes in lignans composition during each step of the manufacturing process of sesame oil.[14-17] During the processing, sesamolin was converted to sesamol under thermal conditions or rearranged to sesaminol under acid bleaching treatment.[15-16] The other main lignan, sesamin, was transformed to episesamin during acid bleaching and deodorization at high temperature.[15-16] The endogenous lignans, sesamin and sesamolin, are most abundant in sesame oils but possess relatively weak antioxidant activity.[10, 13, 18-19] In contrast, sesamol and sesaminol,

generated from sesamolin during processing, show strong *in vitro* antioxidant activity and were therefore considered responsible for the strengthened oxidative stability of roasted and refined unroasted sesame oils [10, 15, 18-19] Therefore, studies of the transformation of sesame lignans are of high interest because the relevant derivatives of lignans may possess better antioxidant activities than endogenous lignans in sesame oil or possess physiological activities. The chemical structures of sesame lignans and the derivatives sesamin, sesamolin, sesamol, sesaminol, and samin are illustrated in Figure 1.

Studies of the acid-catalyzed transformation of sesame lignans are limited. Most studies focused on the conversion of sesamolin to sesaminol in acidic anhydrous systems.[15-16, 20-21] In this work, strong acids and oxidizing agents were used to treat sesame lignans to generate derived compound(s) in aqueous or anhydrous systems. Reaction products of sesame lignans from acid-catalyzed reactions were isolated and identified to elucidate the transformation of sesame lignans. Also, the possible mechanism for formation of the major derivative, 7*R*,7'*R*-samin, from sesamolin treated with acidified hydrogen peroxide was clarified.

MATERIALS AND METHODS

Materials

The lignan standards sesamin, sesamolin, and sesamol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sesaminol and (+)-samin (7*R*,7'*S*-samin) were purchased from Nacalai tesque

(Kyoto, Japan). Solvents used in this study including acetic acid, acetonitrile, methanol, ethyl acetate were purchased from J. T. Baker (Phillipsburg, NJ, USA). Toluene was purchased from Merck (Darmstadt, Germany). Sulfuric acid and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Oxidizing agents including hydrogen peroxide 30%, potassium permanganate ($KMnO_4$), and potassium dichromate ($Kr_2Cr_2O_7$) were purchased from Echo Chemical (Miaoli, Taiwan), Hayashi Pure Chemical (Osaka, Japan), and Sigma-Aldrich (St. Louis, MO, USA), respectively. All chemicals and reagents used were of reagent or analytical grade.

Acid-catalyzed transformation of sesame lignans in aqueous solution

According to previous studies, the transformation of sesame lignans was severely affected by the presence of water in the reaction system. For example, sesamolin is decomposed and converted into samin in acidic aqueous solution but into sesaminol under the acidic anhydrous condition.[15-16, 20-21] Therefore, a preliminary test for transforming sesame lignans in acidic aqueous solution was first investigated. Briefly, a 10-mg reaction substrate, comprising 6.2 mg sesamin and 3.8 mg sesamolin, was added into 10 mL 2 M sulfuric acid at 60°C with occasional stirring for 0, 5, 15, 30, 60 min. Then, 10 mL ethyl acetate was added to the reaction mixture to extract the reaction products. The ethyl acetate layer was dried under reduced pressure for further lignan analysis.

Acid-catalyzed transformation of sesame lignans in anhydrous toluene

The transformation of sesame lignans in the anhydrous system was investigated. In brief, 2 mL of conc. sulfuric acid and formic acid were gently added into 2 mL toluene, containing 0.62 mg sesamin and 0.38 mg sesamolin as a reaction substrate, at room temperature with occasional stirring for 10 min. The organic layer was dried under reduced pressure for further lignan analysis.

Acid-catalyzed transformation of sesame lignans with oxidizing agents

Under certain conditions, sesame lignans could be transformed to their oxidized forms, which sometimes possess better antioxidant activities than their original forms.[22-23] In this work, sesamin and sesamolin were treated with different oxidizing agents to elucidate the effect of oxidizing agents on the transformation of lignans. In brief, a reaction substrate comprising 6.2 mg sesamin and 3.8 mg sesamolin was reacted with 10 mL with 10 mL 180 mM sulfuric acid solution containing oxidizing agents including 3 mM potassium permanganate, 3 mM potassium dichromate, or 30% hydrogen peroxide. The reaction mixture was incubated at 60°C with occasional stirring for 30 min and extracted with 10 mL ethyl acetate. The ethyl acetate layer was dried under reduced pressure for further lignan analysis.

Analysis of sesame lignans and related derivatives by HPLC

The lignan samples from the aforementioned processes were dissolved in methanol, then filtered by a 0.45-μm membrane filter to remove insoluble matters for HPLC analysis. An analytical Shimadzu LC-10AD HPLC system (Shimadzu, Kyoto, Japan) equipped with a YMC-Pack ODS-AM column

(250 x 4.6 mm, 5 μm) (YMC, Kyoto, Japan) and a L-2420 Hitachi UV/vis detector (Hitachi, Tokyo, Japan) was used. The linear mobile phase gradient was obtained with 0.1% (v/v) acetic acid in ultrapure water (solvent A) and 0.1% (v/v) acetic acid in methanol (solvent B). Following the injection of 20 μL sample, solvent B was increased from 45% to 90% over 25 min, then to 100% within 3 min, was isocratic for another 2 min, and finally decreased to 45% within 7 min. Before injection of the next sample, the column was equilibrated with solvent B at 45% for 10 min. The flow rate of 1 mL/min was used and the elute was detected at 290 nm. The data were processed by using the SISC chromatography data station v.3.1 (SISC, Taipei, Taiwan).

Isolation and identification of 7*R*,7'*S*-samin and 7*R*,7'*R*-samin

The separation and isolation of 7*R*,7'*S*-samin and 7*R*,7'*R*-samin were performed on a semi-preparative-scale HPLC system equipped with a Hypersil ODS C18 column (250 x 10 mm, 10 μm) (Thermo Fisher Scientific, Waltham, MA, USA). The linear mobile phase gradient was obtained with ultrapure water (solvent A) and methanol (solvent B). Following the injection of 100 μL of sample, solvent B was increased from 30 to 50% over 20 min, then to 75% in 1 min, isocratic for 4 min, decreased from 75 to 30% in another 3 min, and equilibrium for 5 min. The flow rate was 5.0 mL/min. The eluates were collected and monitored at 290 nm. Fractions containing the desired eluates were further concentrated and lyophilized to obtain a pale amorphous powder for further chemical structure identification.

106 7*R*,7'*S*-samin and 7*R*,7'*R*-samin were identified according to the data from electrospray ionization
107 mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy. The electrospray
108 ionization mass spectrometer (Thermo Q Exactive Plus, Thermo Finnigan LXQ Aadvantag, Thermo
109 Fisher Scientific, CA, USA) included a hybrid quadrupole-Orbitrap analyzer (Thermo Fisher
110 Scientific, CA, USA). The electrospray ionization source (HESI-II) was operated in the positive
111 ionization mode under the following specific conditions: capillary temperature, 262.5 °C; spray
112 voltage, 3.5 kV; sheath gas, 50 L/min; auxiliary gas, 12.5 L/min. The mass spectrometer performed a
113 full scan event (mass range m/z 100-1000) with a resolving power of 70,000 at full width at half
114 maximum (FWHM) at m/z 200 followed by 20 consecutive fragmentation events (variable data
115 independent acquisition [vDIA]) with a resolving power of 35000 at FWHM (m/z 200). NMR
116 spectra were recorded on a Bruker Avance 600 MHz NMR spectrometer (Bruker, Billerica, MA,
117 USA). Chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and Hz, respectively. ^{1}H
118 and ^{13}C chemical shifts were calibrated with $CDCl_3$ as internal standard at δ = 7.24 and 77.0,
119 respectively.

120 **Statistical analysis**

121 All data analyses involved using SPSS 19.0. Data are reported as mean ± standard deviation (SD).
122 All experiments were performed in triplicate (n=3).

RESULTS AND DISCUSSION

Acid-catalyzed transformation of sesame lignans in aqueous solution

Transformation of sesame lignans is influenced by the acid catalysts and solvents used. In previous studies, sesamolin was supposedly directly degraded into sesamol and samin via acid hydrolysis in the presence of water but converted to sesaminol via rearrangement of sesamol and the oxonium ion in acidic anhydrous systems.[15-16, 20-21] Epimerization of sesamin was also observed in acidic anhydrous systems,[15-16, 24] but the acidic transformation of sesamin in aqueous solution was not clear. In this work, we studied changes of sesame lignans during acid treatment in aqueous solution. Regardless of treatment with acids or not, peaks representing sesamin were unchanged (Figure 2a-c), so sesamin possesses good stability under acidic aqueous solutions, and no episesamin was observed under both conditions. In contrast, sesamolin was relatively unstable and depleted along with the generation of sesamol and an unidentified compound, designated compound **a** (peak 4 in Figure 2a-c). Fractions containing compound **a** were further isolated and identified by ESI-MS and NMR analysis.

Identification of compound a

The mass spectrum for compound **a** showed $[M+Na]^+$ at 273.29 m/z with close relation to samin. MS/MS spectrum for protonated ion of compound a was showed in Supplementary Figure 1a. Analysis of the NMR spectra using HSQC, HMBC, and COSY experiments gave the full

141 assignments of ^{1}H and ^{13}C signals. ^{1}H-NMR and ^{13}C-NMR spectral data for compound **a** were
142 corresponded with NMR spectral data for (+)-samin isolated from sesame seeds (Table 1).[25] From
143 the above spectral data, compound **a** was considered to be 7*R*,7'*S*-samin [(+)-samin], with a
144 methylenedioxyphenyl group on C7 and a hydroxyl group on C7' with *R* and *S* configurations,
145 respectively. These results agree with that sesamolin is known to be decomposed into sesamol and
146 samin in acidic aqueous solution. Moreover, the hydrolysis of sesamolin catalyzed with sulfuric acid
147 released sesamol and 7*R*,7'*S*-samin simultaneously in the aqueous system (Figure 2d).

148 **Acid-catalyzed transformation of sesame lignans in anhydrous toluene**

149 As previously described, the presence of water is one of the important determinants for the
150 transformation of lignans. Sesame lignans were treated with sulfuric acid and formic acid in
151 anhydrous toluene to investigate the conversion of lignans in anhydrous system. Sesamin remained
152 unchanged, and no epimerization was observed (Figure 3). Meanwhile, sesamol and 7*R*,7'*S*-samin
153 were still the major products of sesamolin in both cases, whereas no sesaminol was formed (Figure
154 3). According to previous studies, sesamolin should scission into sesamol and the transitional
155 oxonium ion and then undergo rearrangement to form sesaminol in anhydrous solvent systems, such
156 as acid bleaching, toluene with camphorsulfonic acid, and toluene with acidic cation exchange resin
157 which possesses a sulfonic functional group.[15-16, 20-21] However, sesaminol was not generated from
158 sesamolin treated with sulfuric acid or formic acid in toluene in our study, so the acid catalysts might
159 be crucial for the specific transformation of sesamolin into sesaminol. Otherwise, sesamolin was

prone to be decomposed directly into sesamol and 7*R*,7'*S*-samin with common inorganic acids as catalysts both in aqueous and anhydrous systems.

Acid-catalyzed transformation of sesame lignans with oxidizing agents

To generate derived compounds for further investigating the effect of oxidants on the conversion of sesame lignans, sesamin and sesamolin prepared in sulfuric acid aqueous solution were treated with the oxidizing agents potassium permanganate, potassium dichromate, and hydrogen peroxide. Sesamolin was degraded, but sesamin was relatively stable (Figure 4). Also, the main transformation products of sesamolin with different oxidizing agents varied. The main transformation products of sesamolin with acidified potassium permanganate were still sesamol and 7*R*,7'*S*-samin, whereas 7*R*,7'*S*-samin was the only major product of sesamolin with acidified potassium dichromate (Figure 4b,c). Previous studies have reported that sesamol was relatively heat unstable as compared with sesamin and sesaminol, especially with temperature above 200℃.[15, 17] Sesamol seemed to be also more unstable than 7*R*,7'*S*-samin with acidified potassium dichromate treatment, with 7*R*,7'*S*-samin as the main product of sesamolin. In contrast, Figure 4d shows that sesamol and a new compound, designated compound **b** (peak 5 in Figure 4d), were products after oxidation of sesamolin by acidified hydrogen peroxide. Fractions containing compound **b** were further isolated and identified by ESI-MS/MS and NMR analysis.

Identification of compound b (7R,7'R-samin)

178 Compound **b** was identified by ESI-MS/MS, UV-Vis and NMR spectra as 7*R*,7'*R*-samin, the
179 diastereomer of 7*R*,7'*S*-samin. Mass spectrum of compound **b** shows compound **b** has the same
180 molecular weight with 7*R*,7'*S*-samin. The MS/MS spectra for compound **b** and 7*R*,7'*S*-samin show
181 similar fragmentation patterns with 9 identical molecular-weight daughter ions (Supplementary
182 Figure 1). Also, UV-Vis absorption spectrum of compound **b** shows three major absorption peaks at
183 204, 235, 287 nm, which was close with that of 7*R*,7'*S*-samin (203, 238, 287 nm), indicating
184 compound b has a high structural similarity with 7*R*,7'*S*-samin (Supplementary Figure 2).[25] NMR
185 spectral analysis including ^{1}H, ^{13}C, HSQC, HMBC, and COSY experiments affords the full
186 assignments for compound **b,** revealing that compound **b** and 7*R*,7'*S*-samin might be diastereomers
187 (Table 1, Table 2 and Supplementary Figure 3-8). According to Table 1 and Table 2, compound b
188 shows many similar proton or carbon chemical shifts and proton splitting patterns with 7*R*,7'*S*-samin,
189 but with several different chemical shifts between compound **b** and 7*R*,7'*S*-samin (Table 1 and Table
190 2). Among these differences, the deviation of chemical shifts for C7' was the most marked with
191 102.2 ppm for 7*R*,7'*S*-samin and 111.7 ppm for compound **b** (Table 1 and Table 2)**.** Other protons
192 (H7', H8', H9'a, H9'b, H8, H9a and H9b) or carbons (C8' and C8) with slight different chemical
193 shifts for compound **b** and 7*R*,7'*S*-samin are all close to C7', implicating compound **b** may have a
194 different configuration of hydroxyl group on C-7' which causes these differences of chemical shifts
195 between compound **b** and 7*R*,7'*S*-samin (Table 1 and Table 2). Therefore, compound **b** was
196 concluded as 7*R*,7'*R*-samin. According to the above results, the major transformation product of

197 sesamolin under acidified potassium permanganate and potassium dichromate treatment was
198 7*R*,7'*S*-samin, whereas sesamolin was preferentially converted into 7*R*,7'*R*-samin in the presence of
199 hydrogen peroxide.

200 **Possible mechanism for formation of 7*R*,7'*R*-samin in acidified hydrogen peroxide**

201 This study revealed the specific formation of 7*R*,7'*R*-samin catalyzed by hydrogen peroxide for the
202 first time, so we explored the possible role of hydrogen peroxide in forming 7*R*,7'*R*-samin in
203 acidified hydrogen peroxide. Sesamolin was mainly converted to sesamol and 7*R*,7'*S*-samin in
204 sulfuric acid, whereas 7*R*,7'*R*-samin was the major reaction product of sesamolin with hydrogen
205 peroxide present in the same acid (Figure 2b,c and Figure 4d). Therefore, we speculate that hydrogen
206 peroxide might be related to the epimerization of 7*R*,7'*S*-samin under acidic conditions. To verify
207 whether hydrogen peroxide could catalyze the epimerization of 7*R*,7'*S*-samin to form 7*R*,7'*R*-samin,
208 the 7*R*,7'*S*-samin standard was directly reacted with 30% hydrogen peroxide only and 30% hydrogen
209 peroxide in 180 mM sulfuric acid. 7*R*,7'*R*-samin was formed under both conditions, which suggests
210 that hydrogen peroxide alone was capable of converting 7*R*,7'*S*-samin into 7*R*,7'*R*-samin (Figure 5).
211 However, hydrogen peroxide without sulfuric acid could only catalyze the partial conversion of
212 7*R*,7'*S*-samin into 7*R*,7'*R*-samin, so the presence of acid may enhance the epimerization. Sesamolin
213 and sesamin were treated with hydrogen peroxide under different concentrations of sulfuric acid to
214 investigate the effect of acid on the catalytic efficiency of hydrogen peroxide for epimerization of
215 7*R*,7'*S*-samin. As expected, the formation of 7*R*,7'*R*-samin was increased with increasing

concentration of sulfuric acid in the reaction system; furthermore, 7*R*,7'*S*-samin completely transformed to 7*R*,7'*R*-samin when the concentration of sulfuric acid reached 50 mM (Figure 6). Therefore, hydrogen peroxide was related to the epimerization of 7*R*,7'*S*-samin. In addition, increasing sulfuric acid concentration might accelerate the epimerization.

We further propose the possible formation mechanism of 7*R*,7'*R*-samin catalyzed by hydrogen peroxide according to the stereochemical relationship to elucidate why hydrogen peroxide causes the different configuration of the hydroxyl substituted group of samin. In the absence of hydrogen peroxide, the nucleophilic hydroxyl group was more likely to attack the transitional oxonium ion derived from sesamolin on the same side of the methylenedioxyphenyl group under acidic conditions owing to the steric hindrance caused by the cis-fused furofuran ring (Figure 7a). Therefore, the main product of sesamolin is usually 7*R*,7'*S*-samin under general acidic conditions. In contrast, hydrogen peroxide is prone to approach the oxonium ion intermediate derived from acid hydrolysis of sesamolin from the opposite site of the methylenedioxyphenyl group to stabilize a seven-membered ring intermediate structure by forming hydrogen bonds with the oxonium ion, thus resulting in the formation of 7*R*,7'*R*-samin as the final product (Figure 7b). Because hydrogen peroxide could catalyze the formation of 7*R*,7'*R*-samin both from sesamolin and 7*R*,7'*S*-samin (Figure 4d and Figure 5), we suggest that sesamolin and 7R,7'S-samin might be transformed into the transitional oxonium ions first, then both be accessible to form hydrogen bonds with hydrogen peroxide and then converted into 7*R*,7'*R*-samin. Although samin in racemic form has been synthesized via a radical

235 cyclization reaction or an Ireland-Claisen rearrangement of unsaturated oxa-macrolides,[26-29] the
236 stereo-selective formation of 7*R*,7'*R*-samin from sesamolin catalyzed with hydrogen peroxide was
237 first reported in this study.

238 Overall, sesamolin was mainly converted into 7*R*,7'*S*-samin and sesamol under acidic conditions in
239 both aqueous and anhydrous systems in the present study, whereas the formation of sesaminol, which
240 was reported to be formed from sesamolin in acidic anhydrous systems, was not observed. The acidic
241 conversion of sesamolin into sesaminol may require specific catalysts; otherwise, the formation of
242 sesaminol would be minor even in acidic anhydrous systems. Also, both sesamolin and 7*R*,7'*S*-samin
243 were found specifically transformed into 7*R*,7'*R*-samin under catalysis with hydrogen peroxide. The
244 acid-catalyzed transformation pathway for sesamolin is summarized in Figure 8.

245 Our findings broaden the current understanding of acid-catalyzed transformation of sesamolin under
246 different catalytic conditions. Moreover, the study proposes the possible mechanism for formation of
247 7*R*,7'*R*-samin via a stereo-selective reaction catalyzed by hydrogen peroxide under acidic conditions.
248 Characteristics such as the ability to delay oil deterioration and physiological activities of individual
249 stereoisomers of samin require further exploration. Recently, a series of furofuran lignans and
250 flavonolignans synthesized from 7*R*,7'*S*-samin as a starting material through a Friedel-Crafts–like
251 reaction were shown to possess β-glucosidase and free radical inhibition activities.[30-31] The present
252 study may also help in preparing stereoisomers of samin or samin derivatives to further discover their
253 potential applications.

FUNDING

The authors are grateful for the financial support from Flavor Full Foods Inc., Taipei (grant no. NTU102E32034).

ABBREVIATIONS USED

HPLC, high-performance liquid chromatography

ESI-MS, electrospray ionization mass spectrometry

NMR, nuclear magnetic resonance

HSQC, heteronuclear single quantum coherence

HMBC, heteronuclear multiple bond correlation

COSY, correlation spectroscopy

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TABLES

Table 1. 1-D and 2-D NMR results for compound a (7*R*,7'*S*-samin)

Proton	δ/ppm (m, *J*/Hz)	Correlated carbons		Correlated protons
		HSQC	HMBC	COSY
H8	2.87 (m)	52.8 (C8)	C1	H7, H8', H9a, H9b
H8'	3.04 (dd, 8.8, 16.4)	53.6 (C8')		H8, H9'a, H9'b
H9'a	3.54 (dd, 7.2, 9.2)	71.2 (C9')	C7, C8	H8, H9'b
H9a	3.88 (d, 9.2)	69.3 (C9)	C7, C8	H8, H9b
H9b	4.14 (dd, 6.0, 9.2)	69.3 (C9)	C7	H8, H9a
H7	4.32 (d, 7.0)	86.9 (C7)	C1, C2, C6, C8', C9	H8, H9'a
H9'b	4.35 (t, 7.0)	71.2 (C9')	C7	H8', H9'a
H7'	5.36 (s)	102.2 (C7')	C8, C9, C9'	
H10	5.93 (s)	101.0 (C10)	C3, C4	
H5	6.74 (d, 8.0)	108.2 (C5)	C1, C3, C4	
H6	6.78 (dd, 1.2, 7.8)	119.6 (C6)	C1, C4, C5, C7	
H2	6.83 (d, 1.2)	106.5 (C2)	C3, C4, C6, C7	

Table 2. 1-D and 2-D NMR results for compound b (7*R*,7'*R*-samin)

Proton	δ/ppm (m, *J*/Hz)	Correlated carbons		Correlated protons
		HSQC	HMBC	COSY
H8	2.76 (m)	52.5 (C8)	C1, C7, C7'	H7, H8', H9b
H8'	2.96 (dd, 8.4, 15.9)	49.5 (C8')	C7, C7'	H8, H9'a, H9'b
H9'a	3.62 (dd, 7.2, 9.6)	71.0 (C9')	C7', C8'	H8', H9'b,
H9a	3.95 (d, 9)	69.5 (C9)	C7, C7', C8, C8'	H9b
H9b	4.04 (dd, 6, 9.3)	69.5 (C9)	C7	H8, H9a
H7	4.33 (d, 7.8)	86.5 (C7)	C1, C2, C6, C8, C8', C9	H8
H9'b	4.38 (t, 9)	71.0 (C9')	C7, C7', C8	H8', H9'a,
H7'	5.43 (s)	111.7 (C7')	C8, C8', C9, C9'	
H10	5.93 (d, 10.8)	101.1 (C10)	C3, C4	
H5	6.75 (d, 8.4)	108.2 (C5)	C1	
H6	6.77 (dd, 1.2, 6.6)	119.6 (C6)	C1, C2, C4, C7,	
H2	6.82 (d, 1.2)	106.4 (C2)	C3, C4, C6, C7	

FIGURE LEGENDS

Figure 1. The chemical structures of sesame lignans and their derivatives, including sesamin, episesamin, sesamolin, sesaminol, sesamol, 7*R*,7'*S*-samin and 7*R*,7'*R*-samin.

Figure 2. HPLC chromatograms of the transformation of sesamin and sesamolin under treatment with 2 M sulfuric acid at 60℃ for 0 min (a), 30 min (b), 60 min (c), and the time course study of relative contents of sesamolin (■), sesamol (○) and 7*R*,7'*S*-samin (◇) under treatment with 2 M sulfuric acid at 60℃ (d). Peak assignment: 1. Sesamin; 2. Sesamolin; 3. 7*R*,7'*S*-samin; 4. Sesamol. The relative content (%) was defined as the peak area ratio of sesamolin, sesamol, or 7*R*,7'*S*-samin to sesamin (n = 3).

Figure 3. HPLC chromatograms of standards of sesamol, sesaminol, sesamin, and sesamolin (a) and the transformation of sesamin and sesamolin under treatment with conc. sulfuric acid (b) and formic acid (c) in toluene (1:1, v/v) at 60℃ for 30 min. Peak assignment: 1. Sesamol; 2. Sesaminol; 3. Sesamin; 4. Sesamolin; 5. 7*R*,7'*S*-samin.

Figure 4. HPLC chromatograms of the reaction substrate, sesamin and sesamolin (a), and the transformation of reaction substrate under treatment with 3 mM potassium permanganate (b), 3 mM potassium dichromate (c), and 30% hydrogen peroxide (d) in 180 mM sulfuric acid at 60℃ for 30 min. Peak assignment: 1. Sesamin; 2. Sesamolin; 3. Sesamol; 4. 7*R*,7'*S*-samin; 5. 7*R*,7'*R*-samin.

Figure 5. HPLC chromatograms of 7*R*,7'*S*-samin reacted with 30% hydrogen peroxide (a) and 30% hydrogen peroxide in 180 mM sulfuric acid (b) at 60℃ for 30 min. Peak assignment: 1. 7*R*,7'*S*-samin; 2. 7*R*,7'*R*-samin.

Figure 6. Effect of the concentration of sulfuric acid on the formation of 7*R*,7'*S*-samin and 7*R*,7'*R*-samin from sesamolin under treatment with 30% hydrogen peroxide in sulfuric acid (5, 10, 30, 50 mM). Data are mean ± SD (n=3). The relative peak area was defined as the peak area ratio of 7*R*, 7'*S*-samin or 7*R*, 7'*R*- samin to sesamin.

Figure 7. Proposed formation mechanisms of 7*R*,7'*S*-samin by acid-catalyzed transformation of sesamolin under general acidic conditions (a) and 7*R*,7'*R*-samin by acid-catalyzed transformation of sesamolin in the presence of hydrogen peroxide (b).

Figure 8. Transformation of sesamolin under general acidic conditions or catalyzed with hydrogen peroxide under acidic conditions.

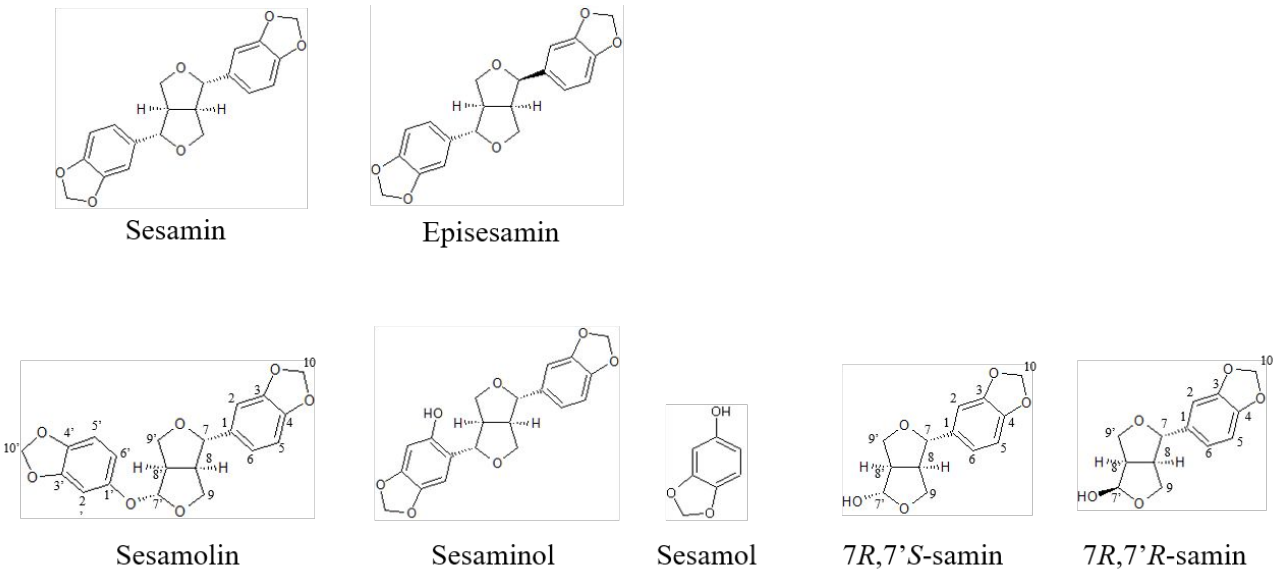

Sesamin
Episesamin
Sesamolin
Sesaminol
Sesamol
7R,7'S-samin
7R,7'R-samin

Figure 1.

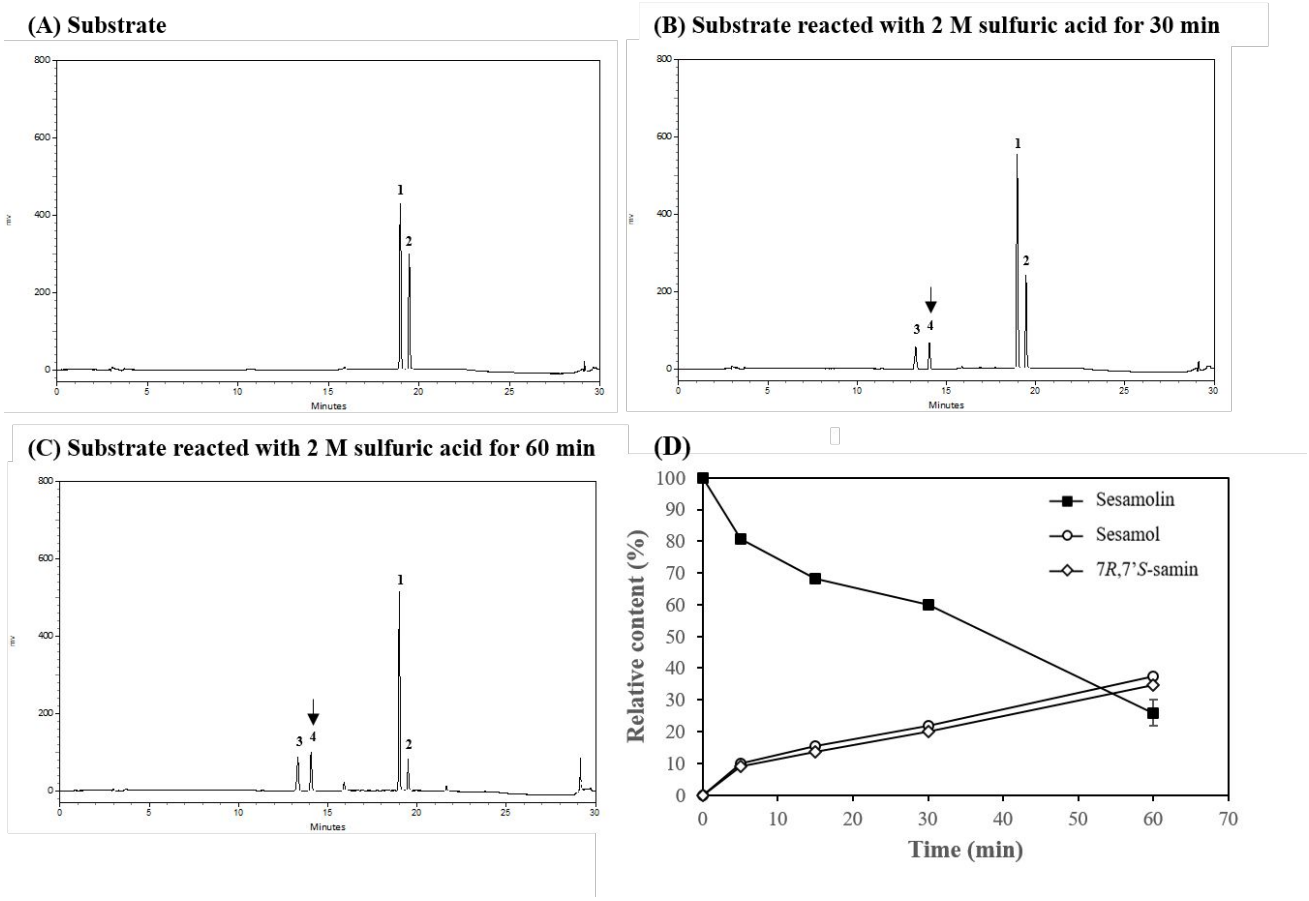

(A) Substrate
(B) Substrate reacted with 2 M sulfuric acid for 30 min
(C) Substrate reacted with 2 M sulfuric acid for 60 min
(D)
Minutes
Relative content (%)
Time (min)
Sesamolin
Sesamol
7R,7'S-samin

Figure 2.

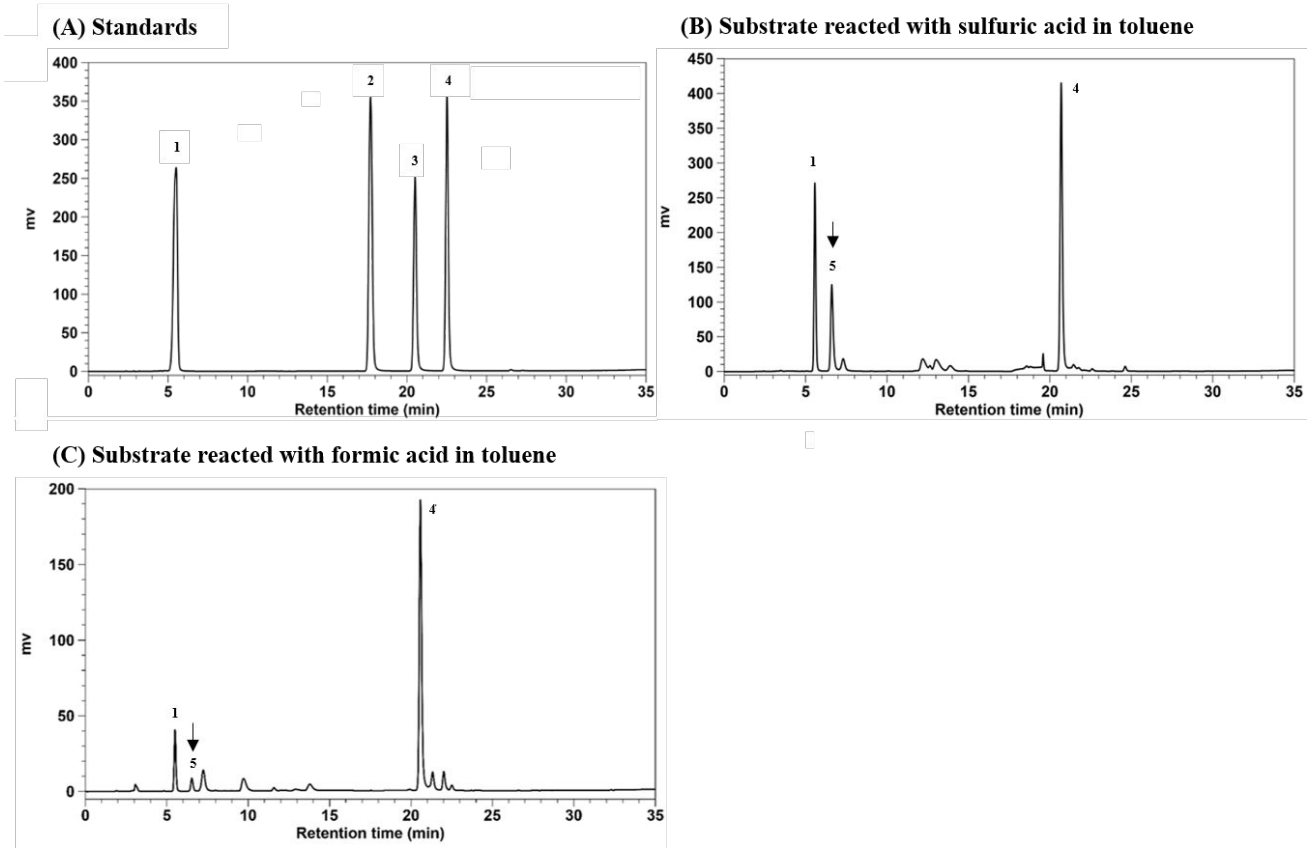

(A) Standards
(B) Substrate reacted with sulfuric acid in toluene
(C) Substrate reacted with formic acid in toluene
mv
Retention time (min)
1
2
3
4
5

Figure 3.

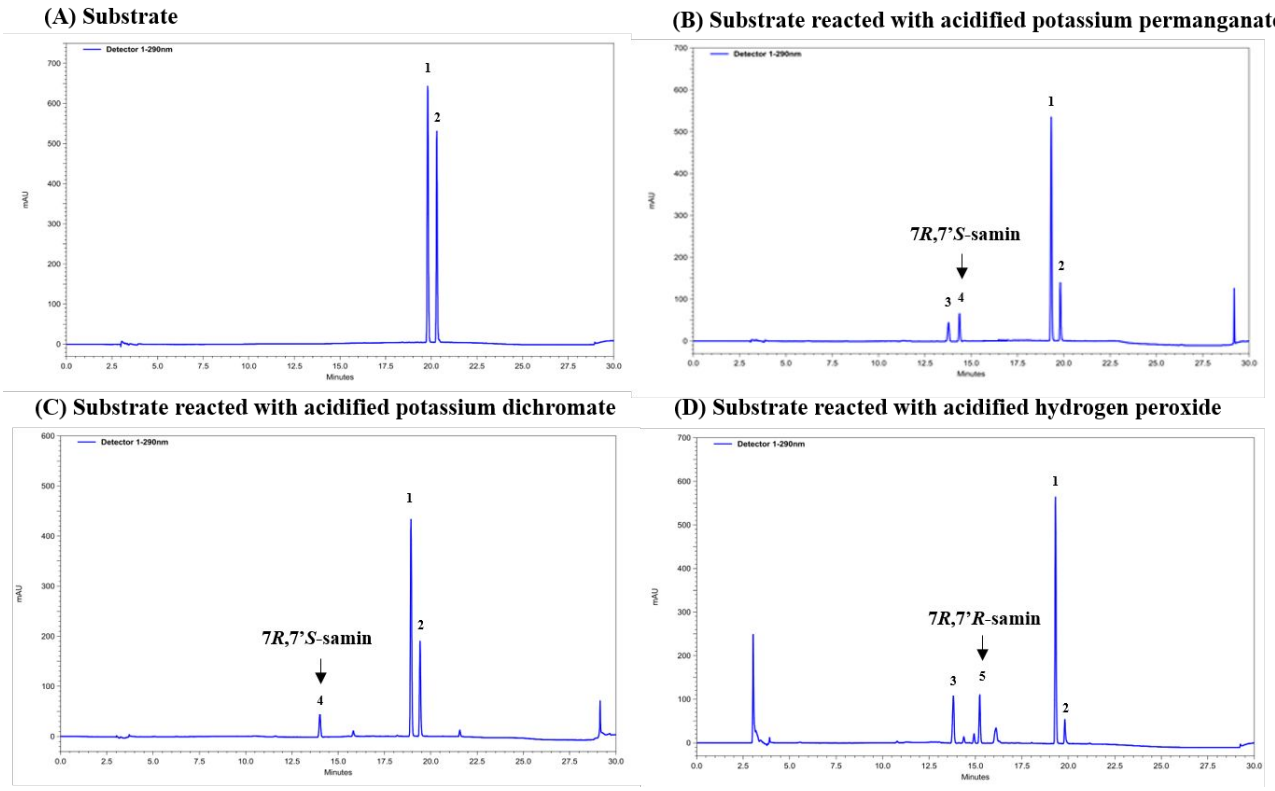

(A) Substrate
Detector 1-290nm
mAU
Minutes
1
2
(B) Substrate reacted with acidified potassium permanganate
Detector 1-290nm
mAU
Minutes
7R,7'S-samin
1
2
3
4
(C) Substrate reacted with acidified potassium dichromate
Detector 1-290nm
mAU
Minutes
7R,7'S-samin
1
2
4
(D) Substrate reacted with acidified hydrogen peroxide
Detector 1-290nm
mAU
Minutes
7R,7'R-samin
1
2
3
5

Figure 4.

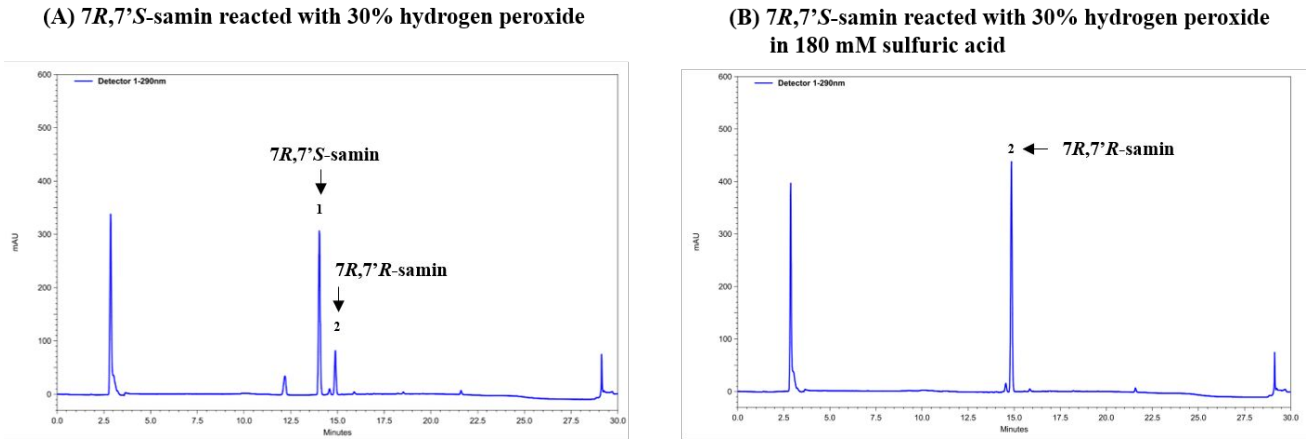

(A) 7R,7'S-samin reacted with 30% hydrogen peroxide
(B) 7R,7'S-samin reacted with 30% hydrogen peroxide in 180 mM sulfuric acid
Detector 1-290nm
7R,7'S-samin
1
7R,7'R-samin
2
mAU
Minutes

Figure 5.

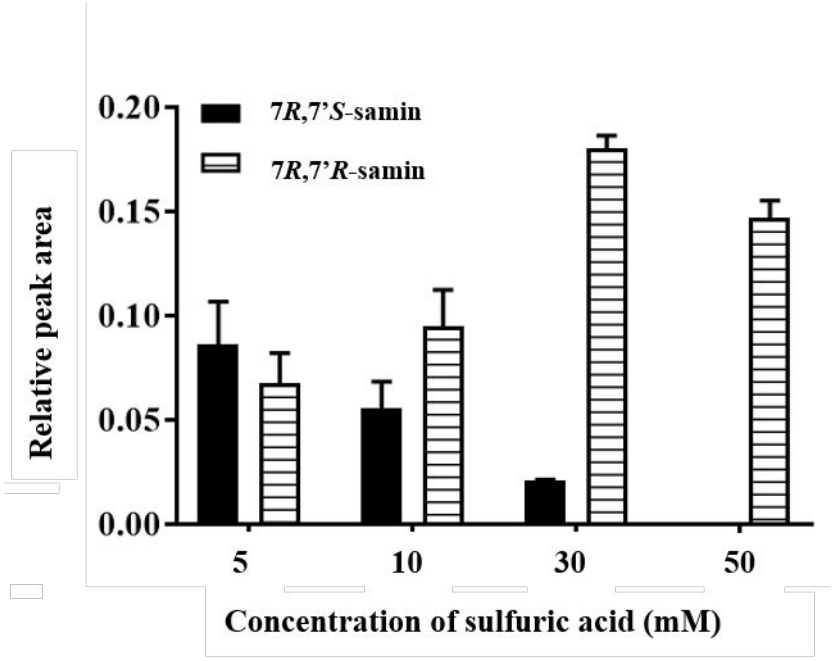
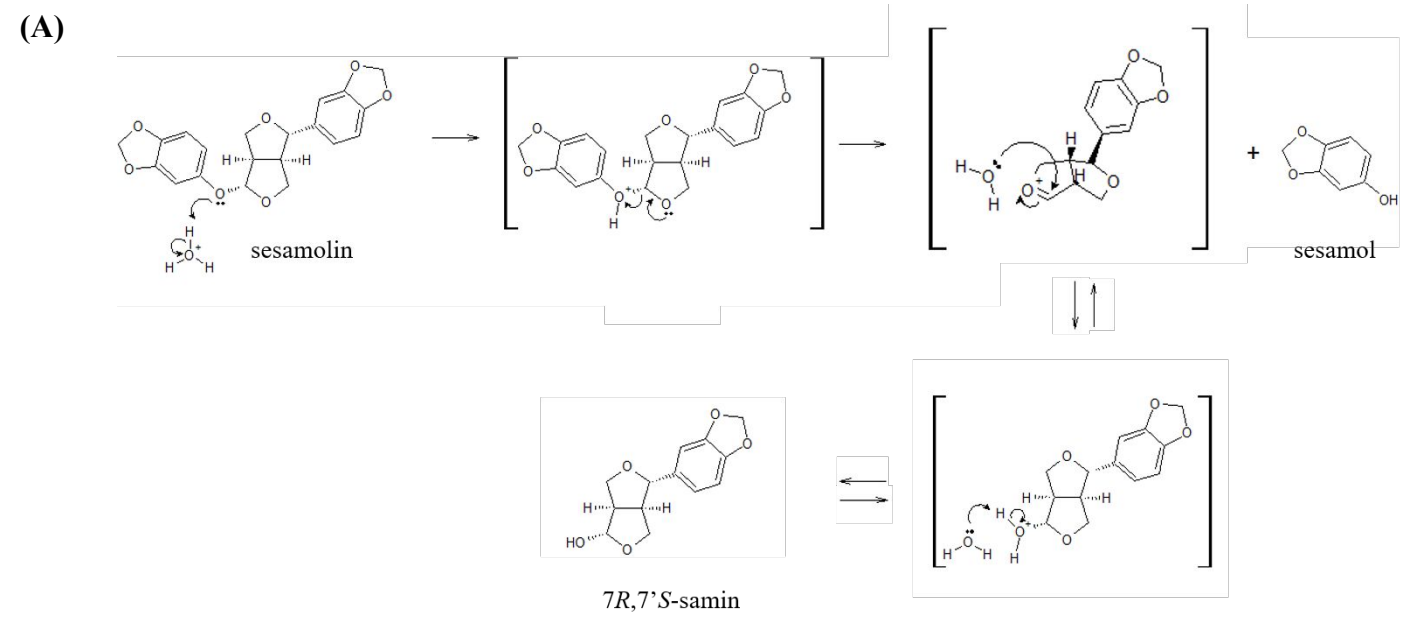

0.20
0.15
0.10
0.05
0.00
7R,7'S-samin
7R,7'R-samin
Relative peak area
5
10
30
50
Concentration of sulfuric acid (mM)

Figure 6.


(A)
sesamolin
sesamol
7R,7'S-samin

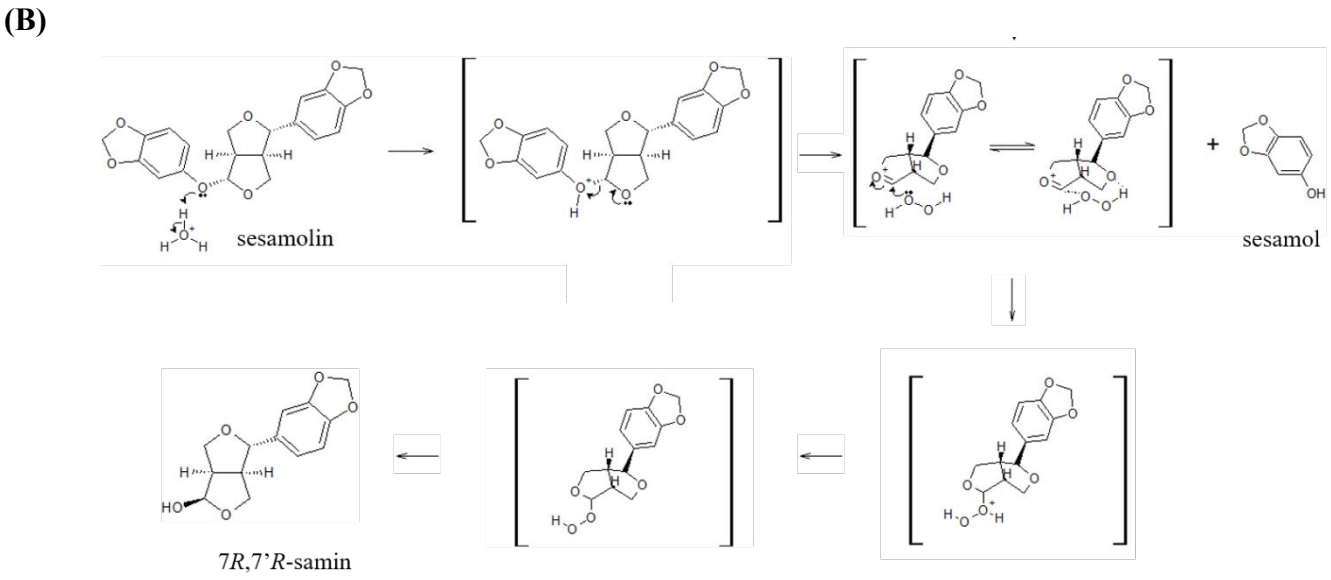

(B)
sesamolin
sesamol
7R,7'R-samin

Figure 7.

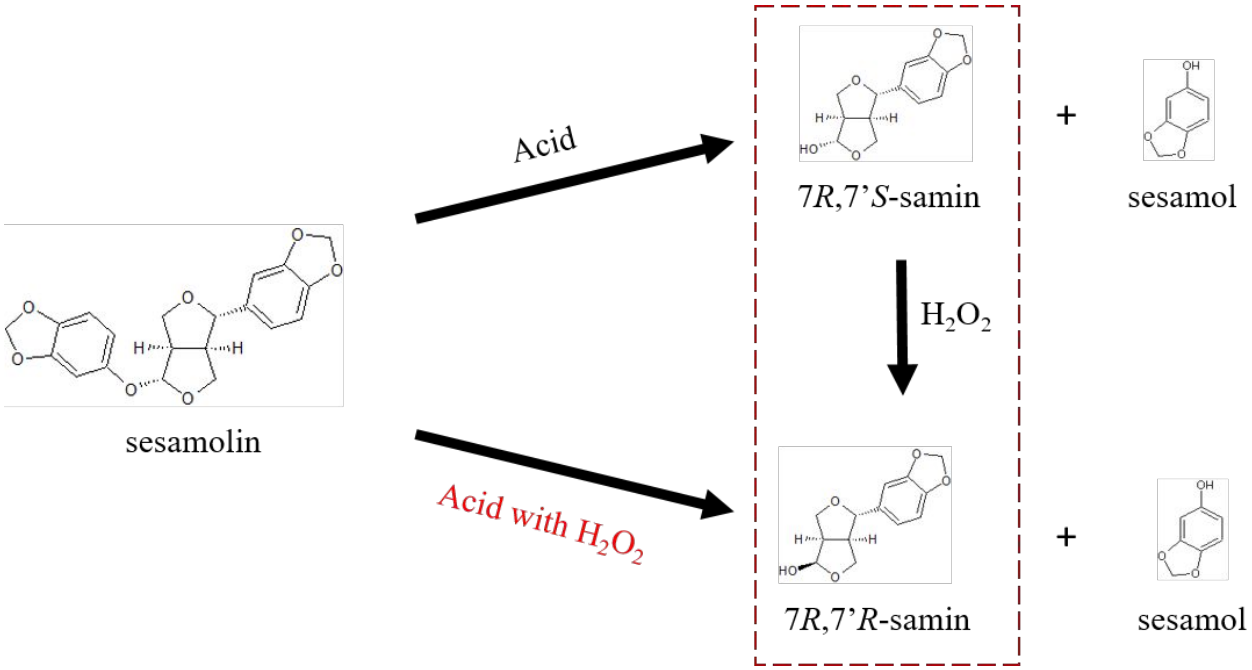
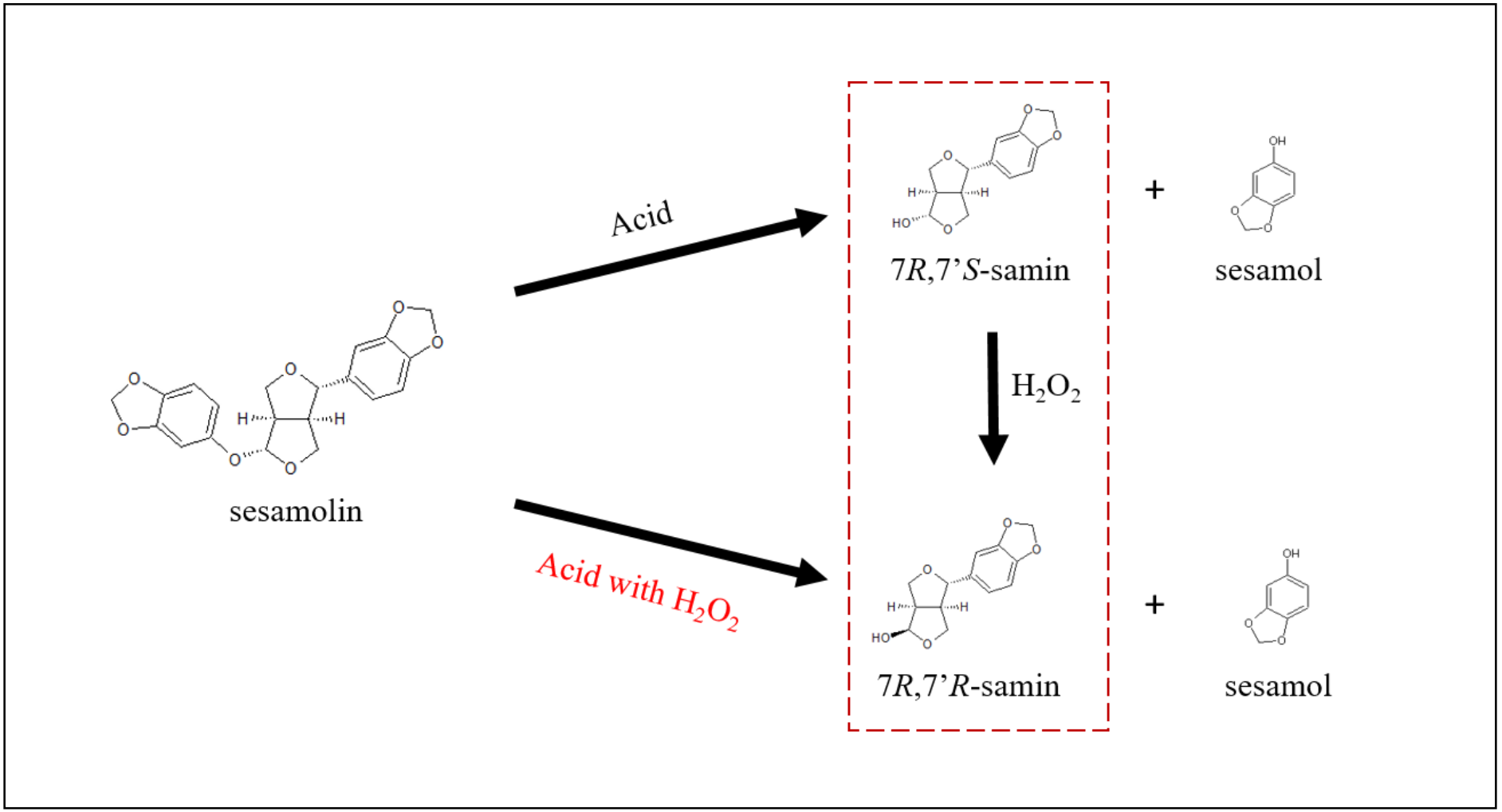

Acid
sesamolin
7R,7'S-samin
sesamol
H2O2
Acid with H2O2
7R,7'R-samin
sesamol

Figure 8.


Acid
sesamolin
7R,7'S-samin
sesamol
H2O2
Acid with H2O2
7R,7'R-samin
sesamol

TOC