First Chemical Synthesis of Antioxidative Metabolites of Sesamin

Hidehito URATA,*[,a] Yuka NISHIOKA,[a] Takafumi TOBASHI,[a] Yasuo MATSUMURA,[a] Namino TOMIMORI,[b] Yoshiko ONO,[b] Yoshinobu KISO,[b] and Shun-ichi WADA[a]

[a] *Osaka University of Pharmaceutical Sciences; 4–20–1 Nasahara, Takatsuki, Osaka 569–1094, Japan: and* [b] *Institute for Health Care Science, Suntory Ltd.; 1–1–1 Wakayamadai, Shimamoto-cho, Mishima-gun, Osaka 618–8503, Japan.*
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The first chemical synthesis of two metabolites ((1*R*,2*S*,5*R*,6*S*)-6-(3,4-dihydroxyphenyl)-2-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (SC-1) and (1*R*,2*S*,5*R*,6*S*)-2,6-bis(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (SC-2)) of sesamin was achieved by a simple two-step approach from sesamin. The approach consists of acetoxylation of the methylenedioxy moiety(ies) with lead(IV) tetraacetate and acid hydrolysis of the resulting hemiorthoester to SC-1 and SC-2.

Key words sesamin; antioxidant; metabolite; lead(IV) tetraacetate; dioxirane

Sesamin (**1**) is the major lignan found in sesame seed and oil. It has been reported that dietary sesamin suppresses the development of hypertension in deoxycorticosterone acetate (DOCA)-salt-induced hypertensive,[1] two-kidney, one-clip renal hypertensive,[2] and salt-loaded stroke-prone spontaneously hypertensive rats.[3] The anti-hypertensive effect of sesamin on DOCA-salt-induced hypertension was reported to be related to its inhibitory effect on aortic superoxide anion radical production in hypertensive animals,[4] although sesamin itself shows little antioxidative effect *in vitro*. Based on the hypothesis that the metabolites of sesamin in rat liver are responsible for the observed antioxidative effects, Nakai *et al.* isolated four metabolites, including (1*R*,2*S*,5*R*,6*S*)-6-(3,4-dihydroxyphenyl)-2-(3,4-methylenedioxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (**2**, SC-1) and (1*R*,2*S*,5*R*,6*S*)-2,6-bis(3,4-dihydroxyphenyl)-3,7-dioxabicyclo[3,3,0]octane (**3**, SC-2), of sesamin from a reaction mixture with rat liver homogenate *in vitro* and with rat bile *in vivo*, and showed that some of the metabolites possess radical scavenging activity.[5] However, synthetic approaches are essential for extensive biological studies of the metabolites. Here, we report the first chemical synthesis of **2** and **3**, which were also detected in human urine as metabolites of sesamin.[6]

The structures of **1**, **2**, and **3** are shown in Fig. 1. **1** has two methylenedioxyphenyl moieties and the synthesis of **2** and **3** can be achieved by hydrolysis of the methylene acetal group(s). Deprotection of the methylene acetal group of catechols is usually conducted by treatment with Lewis acids, such as $AlBr_3$ and BCl_3.[7,8] However, sesamin has benzyl ether moieties as well, which are thought to be sensitive to these conditions. Indeed, treatment of sesamin with $BCl_3 \cdot S(CH_3)_2$ did not afford the desired products at all, and instead, decomposition took place.

In this study, we employed lead(IV) tetraacetate [$Pb(OAc)_4$] as the acetoxylating agent at the carbon atom of the methylenedioxy group.[9] We found that the reagent selectively acetoxylated the methylene carbon atoms of the methylenedioxy moieties of **1** and the acetoxylated products were readily converted into catechols (Chart 1). Treatment of **1** with $Pb(OAc)_4$ in dry benzene at 70 °C for 2 h gave mono- (**4**, SAc-1) and bis- (**5**, SAc-2) acetoxylated sesamin derivatives. However, these acetoxylated compounds could not be separated from each other or from **1** on TLC. Nevertheless, the acetoxylation could be confirmed by ^{1}H-NMR measurement of the reaction mixture, in which proton signals for the acetoxy group at 2.09 ppm and the hemiorthoformate group at 7.86 ppm appeared, while the methylene proton signal at 5.99 ppm for the methylenedioxy moiety(ies) diminished. The acetoxylated products were also characterized by LC-MS analysis (**1**: t_R: 4.12 min; Calcd for $C_{20}H_{18}O_6 \cdot NH_4^+$ = 372.14; Found 371.9. **4**: t_R: 4.58 min; Calcd for $C_{22}H_{20}O_8 \cdot NH_4^+$ = 430.15; Found 429.7. **5**: t_R: 5.00 min; Calcd for $C_{24}H_{22}O_{10} \cdot NH_4^+$ = 488.15; Found 487.8). The acetoxylated products (**4**, **5**) should have diastereomeric isomers. However, we could not detect the existence of such isomers by reversed-phase HPLC and ^{1}H-NMR analyses.

The acetoxylated methylenedioxyphenyl moiety of the

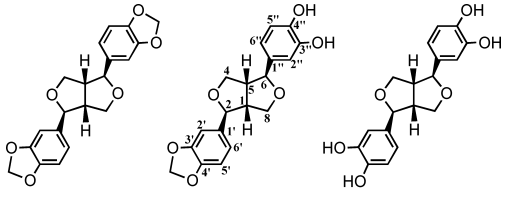

Fig. 1. Structures of Sesamin and Its Metabolites

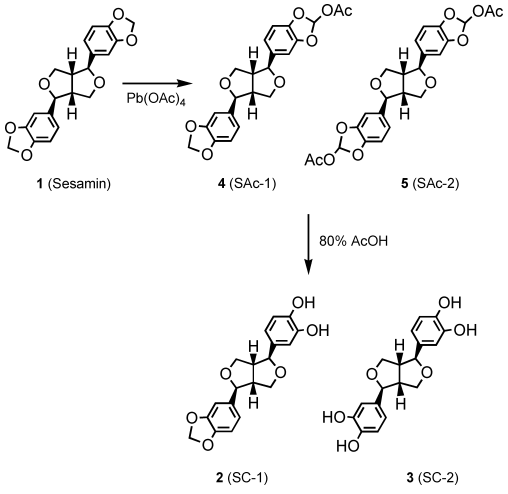

Chart 1

* To whom correspondence should be addressed. e-mail: urata@gly.oups.ac.jp

Table 1. Yields of Acetoxylation of Sesamin and Subsequent Hydrolysis

Entry	Scale (mmol)	Acetoxylation[a]				Hydrolysis[c]	
		$Pb(OAc)_4$ (eq)	Sesamin (**1**) (%)[b]	SAc-1 (**4**) (%)[b]	SAc-2 (**5**) (%)[b]	SC-1 (**2**) (%)[d]	SC-2 (**3**) (%)[d]
1	0.5	1.5	23.3	46.7	30.0	43.4	29.1
2	0.5	2.0	14.9	44.1	41.1	38.4	35.2
3	0.5	2.5	0.0	21.0	79.0	22.6	59.9
4	0.5	3.0	0.0	3.7	96.3	9.2	62.0
5	2.0	1.5	19.8	46.2	33.9	43.6	28.6
6	5.0	1.5	16.6	45.2	38.2	43.1	31.7

a) Acetoxylation was carried out by treatment with lead(IV) tetraacetate at 70 °C for 2 h. *b*) Ratios of HPLC peak area at 280 nm. Reversed-phase HPLC analyses were performed on a μBondasphere 5C18 100 Å column with a linear gradient of acetonitrile (40—60%/20 min) in distilled water. The t_R (min) of **1**, **4**, and **5** was 16.8, 18.3 and 19.6, respectively. *c*) Hydrolysis was carried out by treatment with 80% AcOH at room temperature for 15 min. *d*) Isolated yields from sesamin.

products was readily converted into the catechol by treatment with 80% AcOH for 15 min at room temperature to give a mixture of **2** and **3**. After isolation of **2** and **3** by silica gel column chromatography, structural assignment was achieved by ^1H–^1H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and nuclear Overhauser and exchange spectroscopy (NOESY) experiments. The results were in good agreement with literature data.[5] The yields of the acetoxylation and the acid hydrolysis are summarized in Table 1. The monoacetoxylated product (**4**, SAc-1) was the major product when sesamin was reacted with 1.5 eq of $Pb(OAc)_4$ (Entry 1). As the amount of $Pb(OAc)_4$ increased, the yield of **4** was lowered and that of **5** was raised (Entries 1—4). The results were reproducible in large-scale reactions (Entries 5, 6).

In conclusion, we achieved the first chemical synthesis of **2** and **3**, the antioxidative metabolites of sesamin (**1**), of which selectivity for the formation of **4** and **5** can be controlled by adjusting the amount of $Pb(OAc)_4$. This simple method allows us to continue with the biological evaluation of **2** and **3** and the synthesis of other metabolites.[5]

Experimental

Thin-layer chromatography and column chromatography were carried out on Merck coated plates $60F_{254}$ and Wako-gel C-400HG silica gel, respectively. ^1H- and ^{13}C-NMR spectra were obtained with a Varian UNITY INOVA-500 spectrometer and are reported as parts per million (ppm) relative to tetramethylsilane as an internal standard. EI-MS spectra were obtained with a JEOL JMS-700 spectrometer. Reversed-phase HPLC analyses were performed on a μBondasphere 5C18 100 Å column (Waters) with a linear gradient of acetonitrile (40—60%/20 min) in distilled water, using a Shimadzu LC-6A system. LC-MS analyses were performed using a Quattro micro triple-quadrupole LC-MS (Waters/Micromass) equipped with an ESI source coupled to an Alliance HT Waters 2795 separation module. LC analyses were performed on a C30-UG-5 column (2.0×50 mm, Nomura Chemica, Japan), at 0.25 ml/min flow rate. The elution was carried out with a linear gradient of acetonitrile (45—80%/10 min) in 10 mM ammonium acetate (column temperature, 45 °C). ESI source conditions were as follows: capillary voltage, 3.5 kV; cone voltage, 15 V; source temperature, 80 °C; desolvation temperature, 250 °C.

General Procedure for Acetoxylation of Sesamin To a solution of sesamin (5.0 mmol) and dry benzene (50 ml) was added lead(IV) tetraacetate (1.5 eq) and the mixture was heated at 70 °C for 2 h. After cooling, the mixture was diluted with benzene and filtered through a Celite pad. The filtrate was washed with distilled water (×3), dried with anhydrous sodium sulfate, and concentrated under reduced pressure to give a mixture (2.22 g) of sesamin, **4** and **5** as a pale brown foam: ^1H-NMR (500 MHz, DMSO-d_6) δ: 2.09 (s, $-COCH_3$), 3.02 (m), 3.78 (m), 4.13 (m), 4.65 (2d), 4.72 (2d), 5.99 (s, O–CH_2-O), 6.80—7.20 (m, aromatic), 7.86 (s, hemiorthoformate).

Acid Hydrolysis of Acetoxylated Sesamin Derivatives The resulting residue from the above procedure was treated with 80% AcOH (50 ml) at room temperature for 15 min. After evaporation of the solvents, residual acetic acid was removed by co-evaporation with H_2O–EtOH several times, and then with only EtOH. The residue was purified by silica gel column chromatography. Elution was performed with MeOH/chloroform=2—3% to give **2** (737 mg, 43.1% from sesamin) as pale brown foam: **2**: ^1H-NMR (500 MHz, DMSO-d_6) δ: 2.96 (2H, m, H-1, H-5), 3.71 (1H, dd, J=9.0, 3.9 Hz, H-4b), 3.73 (1H, dd, J=9.0, 3.7 Hz, H-8b), 4.07 (1H, dd, J=9.0, 6.9 Hz, H-8a), 4.11 (1H, dd, J=9.0, 6.9 Hz, H-4a), 4.54 (1H, d, J=4.3 Hz, H-6), 4.62 (1H, d, J=4.6 Hz, H-2), 5.99 (2H, s, O–CH_2–O), 6.59 (1H, dd, J=8.4, 2.1 Hz, H-6″), 6.68 (1H, d, J=8.4 Hz, H-5″), 6.73 (1H, d, J=2.1 Hz, H-2″), 6.83 (1H, dd, J=8.2, 1.6 Hz, H-6′), 6.86 (1H, d, J=8.2 Hz, H-5′), 6.92 (1H, d, J=1.6 Hz, H-2′), 8.84 (2H, br s, –OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 53.43 (C-5), 53.75 (C-1), 70.61 (C-8), 70.97 (C-4), 84.80 (C-6), 84.84 (C-2), 100.76 (O–CH_2–O), 106.44 (C-2′), 107.85 (C-5′), 113.47 (C-2″), 115.16 (C-5″), 116.91 (C-6″), 119.24 (C-6′), 132.16 (C-1″), 135.44 (C-1′), 144.49 (C-4″), 145.01 (C-3″), 146.36 (C-4′), 147.28 (C-3′). HR-EI-MS m/z: 342.1098 (Calcd for $C_{19}H_{18}O_6$: 342.1104).

Elution was performed with MeOH/chloroform=4—5% to give **3** (523 mg, 31.7% from sesamin) as pale brown foam: **3**: ^1H-NMR (500 MHz, DMSO-d_6) δ: 2.93 (2H, m, H-1), 3.68 (2H, dd, J=9.0, 3.5 Hz, H-4b), 4.07 (2H, dd, J=9.0, 7.1 Hz, H-4a), 4.53 (2H, d, J=4.3 Hz, H-2), 6.59 (2H, dd, J=8.1, 1.9 Hz, H-6′), 6.68 (2H, d, J=8.1 Hz, H-5′), 6.73 (2H, d, J=1.9 Hz, H-2′), 8.84 (4H, br s, –OH). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 53.52 (C-1), 70.72 (C-4), 84.90 (C-2), 113.47 (C-2′), 115.17 (C-5′), 116.92 (C-6′), 132.24 (C-1′), 144.49 (C-4′), 145.01 (C-3′). HR-EI-MS m/z: 330.1106 (Calcd for $C_{18}H_{18}O_6$: 330.1104).

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