Regular Article

Chemical Structures of Novel Maillard Reaction Products under Hyperglycemic Conditions

Daisuke Imahori, Takahiro Matsumoto, Naoto Kojima, Tomohiro Hasei, Megumi Sumii, Taishi Sumida, Masayuki Yamashita, and Tetsushi Watanabe*

Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan.
Received October 7, 2017; accepted January 19, 2018

Two novel and two known compounds, 4-quinolylaldoxime and indole-3-aldehyde, were isolated from a reaction mixture consisting of D-glucose and L-tryptophan at physiological temperature and pH. The chemical structures of the two novel compounds were elucidated by spectroscopic analysis such as X-ray crystallography. One of the novel compound and the indole-3-aldehyde showed mutagenicity toward *Salmonella typhimurium* YG1024 with S9 mix. Furthermore, 4-quinolylaldoxime was detected from streptozotocin-induced diabetic rat plasma by LC-MS/MS analysis; however, the isolated compounds were not detected in rat diet extracts. To our knowledge, this is the first report in which 4-quinolylaldoxime was detected in rat plasma. These results suggest that amino-carbonyl reaction products may be formed in diabetic condition and induce genetic damage.

Key words Maillard reaction; structure elucidation; mutagenicity; LC-MS/MS analysis

The Maillard reaction comprises of a series of complex non-enzymatic reactions between carbonyl groups of a reducing sugar and amino groups of amino acids, peptides, or proteins.[1–3] In diabetic patients, accelerated amino-carbonyl reaction that occur between reducing sugars and amino acids to form reaction products is manifested as hyperglycemia and is proposed to play a significant role in the complications of diabetes. Epidemiological studies have shown that diabetic patients have increased incidence of cancer in certain organs including the liver, pancreas and kidney.[4–6] Previously, we have identified a novel mutagen, 5-amino-6-hydroxy-8*H*-benzo[6,7]-azepino[5,4,3-*de*]quinolin-7-one (ABAQ), that was a Maillard reaction product between D-glucose and L-tryptophan at physiological temperature and pH.[7] We reported that ABAQ showed genotoxicity *in vitro* and *in vivo* and tumor-initiating activity in mouse colon. The mutagenicity of ABAQ was accounting for 18% of the reaction mixture.[7–9] Therefore, to determine whether other mutagens are formed under physiological conditions, we continued to isolate various products from the reaction mixture. This paper reports the chemical structure, the mutagenic activities, and the detection of reaction products from diabetic rat plasma.

Results and Discussion

To identify mutagens that are formed under physiological conditions, we isolated reaction products from previously reported reaction mixtures. Briefly, D-glucose and L-tryptophan were dissolved in phosphate buffer, and $FeSO_4$ and H_2O_2 (Fenton reagent) was subsequently added to the mixture. In previous study, we described the mutagenicity of reaction mixture with and without Fenton reagent, and we found Fenton reagent induce eight times stronger mutagenicity. Then, the mixture was incubated at 37°C for 3 d and extracted with $CHCl_3$. The $CHCl_3$ soluble fraction was subjected to normal- and reversed-phase silica-gel column chromatography and repeated HPLC. From the $CHCl_3$-soluble fraction, two new compounds (**1** and **2**) were isolated together with two known compounds 4-quinolylaldoxime (**3**)[10] and indole-3-aldehyde (**4**)[11] (Fig. 1).

Compound **1** was isolated as yellow crystals with negative specific rotations ($[\alpha]_D^{25}$ −51.2 in $CHCl_3$). For the electron ionization (EI)-MS of **1**, a molecular ion peak $[M]^+$ was observed at *m*/*z* 291 and the molecular formula $C_{17}H_{13}N_3O_2$ was determined by high resolution (HR)-MS measurements of the molecular ion peak. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments, showed signals associated with two aryl groups [δ 8.24 (d, J=6.8 Hz, H-1), 7.45 (t, J=6.8 Hz, H-2), 7.73 (t, J=6.8 Hz, H-3), 7.60 (d, J=6.8 Hz, H-4), 6.66 (d, J=8.3 Hz, H-16), 7.02 (t, J=8.3 Hz, H-17), 6.86 (t, J=8.3 Hz, H-18), and 7.42 (d, J=8.3 Hz, H-19)], methine groups bearing two nitrogen functions [δ 5.97 (s, H-13)], and a methylene group [δ 3.70 (d, J=14.5 Hz, H-11) and 3.76 (d, J=14.5 Hz, H-11)]. As shown in Fig. 2, the double quantum filtered (DQF) correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity (HMBC) experiments of **1** indicated the presence of certain structures. Specifically, long-range correlations were observed between the following proton and carbon pairs: H-1 and C-5, 7, H-11 and C-9, 12, 13, 20, H-13 and C-9, 20, 15, and H-19 and C-12. Since single crystals of **1** were obtained from an EtOH–H_2O solution, X-ray diffraction was performed to determine the relative configuration of **1** (Fig. 3). The relative configuration was determined to be 12*R**, 13*S**. According to the results from various spectroscopic analysis, including X-ray crystallographic analysis diffraction, the chemical structure of compound **1** was clarified as shown in Fig. 1.

Compound **2** was also isolated as yellow crystals. For the EI-MS of **2**, a molecular ion peak $[M]^+$ was observed at *m*/*z* 170 and the molecular formula $C_{10}H_6N_2O$ was determined by HR-MS measurements of the molecular ion peak. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **2**, which were assigned by various NMR experiments, showed signals associated with a quinoline group [δ 9.24 (d, J=4.1 Hz, H-2), 7.95 (d, J=4.1 Hz, H-3), 7.04 (d, J=6.9 Hz, H-7), 7.71 (t, J=6.9 Hz, H-8), 7.67 (d, J=6.9 Hz, H-9)]. As shown in Fig. 2,

* To whom correspondence should be addressed. e-mail: watanabe@mb.kyoto-phu.ac.jp

the DQF COSY and HMBC experiments of **2** indicated the presence of certain structures. Specifically, long-range correlations were observed between the following proton and carbon pairs: H-2 and C-4, 10, H-3 and C-5, 11, H-7 and C-5, 9, and H-8 and C-6, 10. However, we could not confirm the correlation between the C-6 position and amide moiety by two-dimensional NMR (2D-NMR). Therefore, X-ray diffraction was performed to confirm the chemical structure of **2** (Fig. 4). Based on the results from X-ray diffraction, the chemical structure of compound **2** was clarified as shown in Fig. 1. The compounds with this three rings skeleton were previously isolated as the cell cycle modulators reported from a marine-derived *Streptomyces* species.[12)]

Mutagenicity of the Isolated Compounds (1–4) The mutagenic effects of the isolated compounds (**1–4**) were tested on *Salmonella typhimurium* YG1024 with S9 mix. *S. typhimurium* YG1024 is sensitive to mutagens that require metabolic activation by *O*-acetyltransferase, such as amino- and/or nitroaromatic compounds. Accordingly, compound **1** and indole-3-aldehyde (**4**) showed mutagenicity toward YG1024 in a dose-dependent manner with S9 mix (Table 2). This is the first report to evaluate the mutagenic effect of compound **1**. The mutagenic effect of indole-3-aldehyde (**4**) in the Ames test was reported in previous report.[13)]

Identification of the Isolated Compounds (1–4) in Diabetic Rats To confirm that the isolated reaction products **1–4** are formed *in vivo*, we analyzed rat plasma samples by LC-MS/MS. For analysis, a Multiple Reaction Monitoring (MRM) method was established. The daughter ions were selected for MRM analysis by product ion scan using *m/z* 274.00, 101.00, 129.00, and 91.00 for **1–4**, respectively. These ions were formed from protonated molecular ions $[M+H]^+$ of **1–4**. The chromatographic run time for the samples was 75 min. A mobile phase consisting of methanol with 0.1% formic acid and water with 0.1% formic acid was used in a

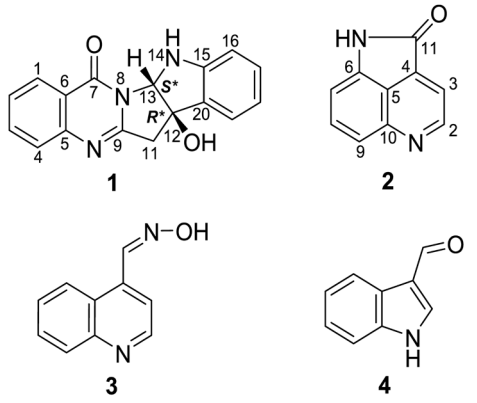

Fig. 1. Chemical Structures of Isolated Compounds

Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data for Compounds **1** and **2**

Position	**1**		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	126.4	8.24 (d, 6.8)		
2	126.7	7.45 (t, 6.8)	153.8	9.24 (d, 4.1)
3	134.8	7.73 (t, 6.8)	117.6	7.95 (d, 4.1)
4	127.1	7.60 (d, 6.8)	134.8	
5	149.1		120.2	
6	121.3		139.6	
7	161.5		107.3	7.04 (d, 6.9)
8			133.4	7.71 (t, 6.9)
9	156.2		120.8	7.67 (d, 6.9)
10			146.1	
11	44.0	3.70 (d, 14.5)	168.8	
		3.76 (d, 14.5)		
12	82.8			
13	83.4	5.97 (s)		
14				
15	148.2			
16	110.7	6.66 (d, 8.3)		
17	131.5	7.02 (t, 8.3)		
18	120.4	6.86 (t, 8.3)		
19	124.3	7.42 (d, 8.3)		
20	128.9			

Measured in $CDCl_3$.

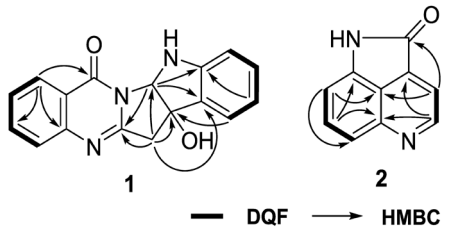

Fig. 2. Important 2D-NMR Correlations of Compounds **1** and **2**

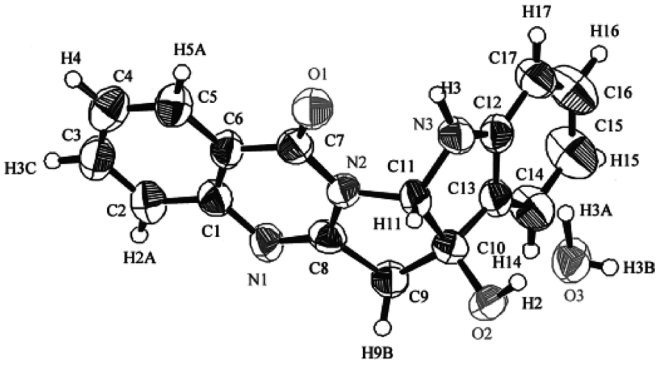

Fig. 3. X-Ray Crystal Structure of **1**

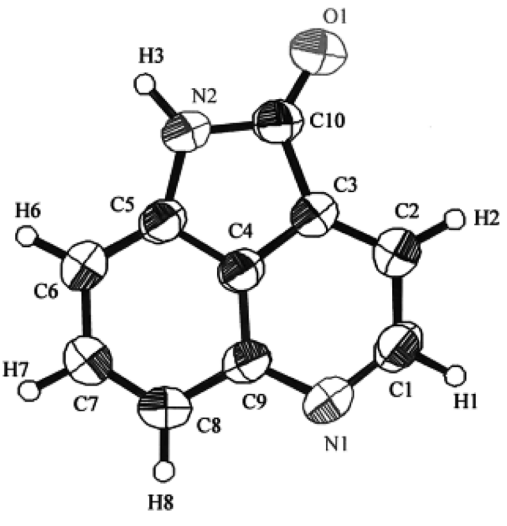

Fig. 4. X-Ray Crystal Structure of **2**

Table 2. Mutagenicity of the Isolated Reaction Products toward *S. typhimurium* YG1024 with S9 Mix

Dose (μg/plate)	Revertants (plate)					
	Control	25	50	100	200	400
1	91±2.5	138±15.5	142±0.5	173±1.5	243±21.0	
2	83±2.5		101±0.5	96±8.5	111±13.5	96±2.0
3	83±2.5		108±12.0	103±2.5	125±8.0	138±0.5
4	83±2.5		115±13.5	128±1.5	183±10	249±2.0
	Control	0.5	1	2		
Benzo[*a*]pyrene	64±1.5	106±15.5	133±9.5	193±12.0		

COSMOSIL 5C18-AR-II (5.0 μm, 250×4.6 mm i.d.) column. To prepare the rat plasma samples, the blood samples were deproteinized by centrifuging at 5000 rpm for 10 min after adding MeOH. The plasma samples were subsequently stored at −20°C until LC-MS/MS analysis. By using this method, **1**–**4** in the plasma samples from diabetic rats yielded by intraperitoneal injection of streptozotocin (STZ) (Sigma, St. Louis, MO, U.S.A.), healthy rat and the $CHCl_3$ extract of rat diet were analyzed. As the result, compound **3** was successfully detected from three independent diabetic rat plasma and healthy rat plasma (Supplementary materials). The concentration of compound **3** in diabetic rat plasma samples were caliculated as 3.56±1.24 ng/mL using caliburation curb (n=3). On the other hand, the concentration of compound **3** in healthy rat plasma samples was caliculated as 2.20 ng/mL (n=1). In addition, compound **3** was not detected in the $CHCl_3$ extract of rat diet. For the quantitative analysis of the compounds identified in this study, further study is necessary.

Conclusion

In this study, we determined two novel and two known compounds, 4-quinolylaldoxime and indole-3-aldehyde, produced from a reaction of D-glucose and L-tryptophan at physiological temperature and pH. The chemical structures of the novel compounds were clarified by spectroscopic analysis such as X-ray crystallography. Compounds **1** and **3** showed mutagenicity toward YG1024 in a dose-dependent manner with S9 mix. In addition, we detected one of the isolated reaction products **3** in rat plasma. These results suggest that isolated compounds may be formed in plasma and induce genetic damage. Further studies regarding the biological activities of reaction products, such as genotocixity *in vivo*, and the quantification of isolated compounds from biological samples of diabetic individuals are important to estimate the risk of these products.

Experimental

General Experimental Procedures The following instruments were used to obtain the physical data: specific rotations, JASCO P-2200 polarimeter; EI-MS, JEOL JMS-GC MATE mass spectrometer; ^{1}H-NMR spectra, JNM-ECA 600 K (600 MHz,) spectrometers; ^{13}C-NMR spectra, JNM-ECA 600 K (150 MHz,) spectrometers; X-ray crystallography RIGAKU R-AXIS RAPID diffractometer; HPLC, a Shimadzu (Kyoto, Japan) SPD-M10AVP UV-VIS detector. COSMOSIL 5C18-MS-II (250×4.6 mm i.d. and 250×10 mm i.d.) columns were used for analytical and preparative procedures. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel 60-N (Kanto Chemical Co., Inc. (Tokyo, Japan), 163–210 μm); reversed-phase silica gel CC, Cosmosil 75C_{18}-OPN (Nacalai Tesque Inc. (Kyoto, Japan), 75–140 μm); TLC, pre-coated TLC plates with silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase high performance thin-layer chromatography (HPTLC), pre-coated TLC plates with silica gel RP-18 WF_{254S} (Merck, 0.25 mm).

Preparation of the Reaction Products Mixture D-Glucose (12.5 mmol), L-tryptophan (25 mmol), and $FeSO_4$ (2.5 mmol) were dissolved in 0.5 M phosphate buffer (pH 7.4, 500 mL), and 30% H_2O_2 (10 mL) was added to the solution. The mixture was incubated at 37°C for 3 d. The mixture was extracted with 500 mL of $CHCl_3$. Evaporation of the organic layer under reduced pressure was due to a $CHCl_3$ soluble fraction (20.0 g).

Isolation of the Reaction Products A $CHCl_3$ soluble fraction (20.0 g) was subjected to normal phase silica gel CC [600 g, n-hexane : $CHCl_3$ (1 : 1→1 : 3→1 : 5 v/v)→$CHCl_3$→$CHCl_3$: MeOH (50 : 1→20 : 1→10 : 1→2 : 1→1 : 1→1 : 2→1 : 4 v/v)→MeOH] to give eight fractions [fr. 1, fr. 2, fr. 3, fr. 4 (4.5 g), fr. 5 (3.6 g), fr. 6, and fr. 7]. Fraction 4 was separated by HPLC {mobile phase: H_2O–MeCN (80 : 20, v/v) [COSMOSIL 5C18-MS-II (250×10 mm i.d.)]} to give **4** (1167 mg). Fraction 5 was further separated by reversed phase silica gel CC [100 g, MeOH : H_2O (2 : 8→3 : 7→4 : 6→5 : 5→6 : 4→7 : 3→8 : 2→9 : 1 v/v)→MeOH] to give twelve fractions [fr. 5-1, fr. 5-2 (348 mg), fr. 5-3, fr. 5-4, fr. 5-5, fr. 5-6 (802 mg), fr. 5-7, fr. 5-8, fr. 5-9, fr. 5-10, fr. 5-11, and fr. 5-12]. Fraction 5-2 was separated by HPLC {mobile phase: H_2O : MeCN (85 : 15, v/v) [COSMOSIL 5C18-MS-II (250×20 mm i.d.)]} to give **2** (12.7 mg), and **3** (2.9 mg). Fraction 5-6 was separated by HPLC {mobile phase: H_2O : MeCN (70 : 30, v/v) [COSMOSIL 5C18-MS-II (250×10 mm i.d.)]} to give **1** (25.5 mg).

Compound **1**: Yellow crystals; $[\alpha]_D^{25}$ −51.2 (c 0.84, $CHCl_3$); For ^{1}H-NMR ($CDCl_3$, 600 MHz,) and ^{13}C-NMR (150 MHz) spectroscopic data, see Table 1; UV (MeOH) λ_{max} 240.8 nm (logε 4.29); EI-MS: m/z 291 $[M]^+$; HR-EI-MS m/z 291.1013 (Calcd for $C_{17}H_{13}N_3O_2$ $[M]^+$: m/z 291.1008).

Compound **2**: Yellow crystals; For ^{1}H-NMR ($CDCl_3$, 600 MHz,) and ^{13}C-NMR (150 MHz) spectroscopic data, see Table 1; UV (MeOH) λ_{max} 249.8 nm (logε 4.07); EI-MS: m/z 170 $[M]^+$ HR-EI-MS m/z 170.0477 (Calcd for $C_{10}H_6N_2O$ $[M]^+$: m/z 170.0480).

X-Ray Diffraction Analysis of Compound 1 The yellow crystals of $C_{17}H_{13}N_3O_2 \cdot H_2O$ had approximate dimensions of 0.250×0.100×0.050 mm and was mounted

on a glass fiber. All measurements were performed using a Rigaku R-AXIS RAPID diffractometer using graphite monochromated Mo-K_α radiation. Monoclinic, space group $P2_1/c$ (#14), a=8.33540 Å, b=23.22490 Å, c=14.79704 Å, β=91.34600°, V=2863.75127 Å^3, Z=8, D_{calcd}=1.435 g/cm^3, μ(Mo-K_α)=1.008 cm^{-1}, F(000)=1296.00, No. of reflections measured: total, 27452; unique, 6514 (R_{int}=0.2175). For other crystallographic data regarding the compound structure, see Supplementary materials.

X-Ray Diffraction Analysis of Compound 2 The yellow crystals of $C_{10}H_6N_2O$ had approximate dimensions of 0.300×0.300×0.100 mm and were mounted on a glass fiber. All measurements were performed using a Rigaku R-AXIS RAPID diffractometer with graphite monochromated Mo-K_α radiation. The crystal-to-detector distance was 127.40 mm. Molecular weight (MW) 170.05, monoclinic, space group $P2_1/c$ (#14), a=5.7523(7)Å, b=15.5021(17)Å, c=8.7414(10) Å, β=92.320(3)°, V=778.85(16)Å^3, Z=2, D_{calcd}=0.726 g/cm^3, μ(Mo-K_α)=0.489 cm^{-1}, F(000)=176.00, No. of reflections measured: total, 7502; unique, 1775 (R_{int}=0.0322). For other crystallographic data regarding the compound structure, see Supplementary materials.

Mutagenicity Assay The mutagenicity of the Maillard reaction products was assayed by the Ames test using *S. typhimurium* YG1024 with S9 mix. Each sample was dissolved in dimethyl sulfoxide. *S. typhimurium* YG1024, which was kindly provided by Dr. Nohmi of the National Institute of Health, is an *O*-acetyltransferase-overproducing derivative of *S. typhimurium* TA98 and is sensitive to the mutagenicity of Maillard reaction products. A mammalian metabolic system (S9 mix) was prepared using the livers of male Sprague-Dawley rat (SLC Inc., Shizuoka, Japan) treated with phenobarbital and β-naphthoflavone. Benzo[*a*]pyrene and 2-acetylaminofluorene were used as positive controls. The slope of the dose–response curve obtained with four doses and duplicate plates at each dose was adopted as the mutagenic potency. Samples were considered positive when they induced twofold increases compared to the average of spontaneous revertants and showed well-behaved concentration–response patterns.

Animals Used for the Identification of Compounds (1–4) Diabetes was induced by intraperitoneal injections of freshly prepared STZ (90 mg/kg of body mass) in 0.36 mL saline in 5 healthy 6-week-old male albino rats that weighed between 160–180 g. Wistar rat were purchased from Japan SLC. The animals were housed in a temperature- and light-controlled room (12 h light/dark cycles) and were given food and water *ad libitum* (MF, Oriental Yeast, Tokyo, Japan). Blood samples for the daily analysis of glucose levels were collected from the tail veins of the diabetic rats, and the blood glucose levels were measured using a One-Touch glucometer (Johnson & Johnson, U.S.A.). The intake of solid food and water for each mouse was monitored daily throughout the experiment. Diabetic rats with blood glucose levels ≥500 mg/dL (27.8 mM) 11 weeks after the first STZ administration were used in the experiments. Blood samples were collected by heart puncture from the rats anaesthetized with ether and were subsequently centrifuged at 5000 rpm for 10 min at 4°C. From the resulting rat plasma samples, 500 μL aliquots were subsequently deproteinized by adding MeOH (2 mL) followed by mixing for 1 min and centrifugation at 5400 rpm for 15 min. The supernatant was evaporated until dry, and MeOH (1 mL) was added to the residue. The experiments were conducted according to the "Guidelines for Animal Experiments at Kyoto Pharmaceutical University," and the animal studies were approved by the Experimental Animal Research Committee.

LC-MS/MS Analysis of Compounds (1–4) in Rat Plasma LC-MS/MS analysis were performed using a Shimadzu LC-20 AD Nexera Liquid Chromatograph, a Shimadzu SIL-30 AC Nexera autosampler, and Shimadzu CTO-20 AC Prominence Column Oven that were connected to a Shimadzu LCMS-8030 Tandem Quadrupole LC-MS/MS system (Shimadzu). Coupling was through the electrospray ionization (ESI) probe that was equipped with the instrument. A COSMOSIL 5C18-AR-II (5.0 μm, 250×4.6 mm i.d.) column maintained at 40°C was used. The mobile phases were water with 0.1% formic acid (A) and MeOH (B), and the flow rate was 1 mL/min. The gradient elution was performed with the following program: 15% B, increasing to 100% B at 45 min, maintaining this condition for 10 min, ramping back down to 15% B at 5 min and holding at this condition for a further 15 min for a total runtime of 75 min. The column oven and the autosampler temperatures were kept constant at 40 and 15°C, respectively, and the sample injection volume was 50 μL. Mass spectrometry was performed by positive mode electrospray ionization. The conditions of the interface were: DL temperature, 280°C; nebulizing gas flow, 3 L/min; heat block temperature, 400°C; drying gas flow, 15 L/min; and Interface Voltage, 4.5 kV. MRM was used; the dwell time for each transition was 100 ms and the collision energy was 35 V. The MRM transitions were 146.00/91.00, 171.00/101.00, 173.00/129.00, and 292.00/274.00 (*m/z*) for **1**–**4**, respectively. The MS parameters consisted of: collision-induced dissociation (CID) gas pressure, 230 kPa; conversion dynode, −6 kV; detector voltage, −1.9 kV; PG vacuum, 8.2e; and IG vacuum, 1.7e^{-3}. The data generated was processed using LabSolutions version 5.6 (Shimadzu). For quantitative analysis, the calibration graph was drawn from 0.1, 1, and 10 ng/mL of compound **3**. The blood sugar levels of diabetic mice were more than 500 mg/dL, while those of normal mice were less than 200 mg/dL.

Acknowledgment This research was supported in part by a Grant-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science (JSPS) Grant Number JP17K15473.

Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

Experimental details: ^{1}H- and ^{13}C-NMR spectrums of compounds **1** and **2**, LC-MS/MS chromatogram for the identification of compound **3** in diabetic rat plasma, X-ray experimental details of compounds **1** and **2**.

References

1) Singh R., Barden A., Mori T., Beilin L., *Diabetologia*, **44**, 129–146 (2001).
2) Thornalley P. J., Langborg A., Minhas S., *Biochem. J.*, **344**, 109–116 (1999).
3) Glomb M. A., Monnier V. M., *J. Biol. Chem.*, **270**, 10017–10026

(1995).
4) Aronson D., *Br. J. Pharmacol.*, **142**, 1055–1058 (2004).
5) Nagaraj R. H., Shipanova I. N., Faust F. M., *J. Biol. Chem.*, **271**, 19338–19345 (1996).
6) McCance D. R., Dyer D. G., Dunn J. A., Bailie K. E., Thorpe S. R., Baynes J. W., Lyons T. J., *J. Clin. Invest.*, **91**, 2470–2478 (1993).
7) Nishigaki R., Watanabe T., Kajimoto T., Tada A., Takamura-Enya T., Enomoto S., Nukaya H., Terao Y., Muroyama A., Ozeki M., Node M., Hasei T., Totsuka Y., Wakabayashi K., *Chem. Res. Toxicol.*, **22**, 1588–1593 (2009).
8) Kochi T., Shimizu M., Totsuka Y., Shirakami Y., Nakanishi T., Watanabe T., Tanaka T., Nakagama H., Wakabayashi K., Moriwaki H., *Toxicol. Reports*, **1**, 69–73 (2014).
9) Totsuka Y., Watanabe T., Coulibaly S., Kobayashi S., Nishizaki M., Okazaki M., Hasei T., Wakabayashi K., Nakagama H., *Mutat. Res.*, **760**, 48–55 (2014).
10) Boehlendorf B., Forche E., Bedorf N., Gerth K., Irschik H., Jansen R., Kunze B., Trowitzsch K. W., Reichenbach H., Hoefle G., *Liebigs Annalen*, **1**, 49–53 (1996).
11) Nakajima E., Nakano H., Yamada K., Shigemori H., Hasegawa K., *Phytochemistry*, **61**, 863–865 (2002).
12) Hughes C. C., MacMillan J. B., Gaudencio S. P., Jensen P. R., Fenical W., *Angew. Chem. Int. Ed. Engl.*, **48**, 725–727 (2009).
13) Sasagawa C., Matsushima T., *Mutat. Res.*, **250**, 169–174 (1991).