

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/8158341>

Isolation of Cytochrome P450 Inhibitors from Strawberry Fruit, *Fragaria ananassa*

ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · DECEMBER 2004

Impact Factor: 3.8 · DOI: 10.1021/np0400104 · Source: PubMed

CITATIONS

48

READS

66

7 AUTHORS, INCLUDING:



Hiroyuki Onuki

Tokyo Chemical Industry Co., Ltd.

21 PUBLICATIONS 407 CITATIONS

SEE PROFILE

Isolation of Cytochrome P450 Inhibitors from Strawberry Fruit, *Fragaria ananassa*

Sachiko Tsukamoto,[†] Kyoko Tomise,[†] Maki Aburatani,[†] Hiroyuki Onuki,^{‡,§} Hiroshi Hirorta,^{‡,§} Eiji Ishiharajima,[⊥] and Tomihisa Ohta^{*,†}

Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920-0934, Japan, RIKEN Genomic Sciences Center (GSC), 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan, Graduate School of Integrated Sciences, Yokohama City University, 1-7-29 Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan, and Tochigi Prefectural Institute of Public Health and Environmental Science, 2145-13 Shimookamoto, Kawachi-cho, Tochigi 329-1196, Japan

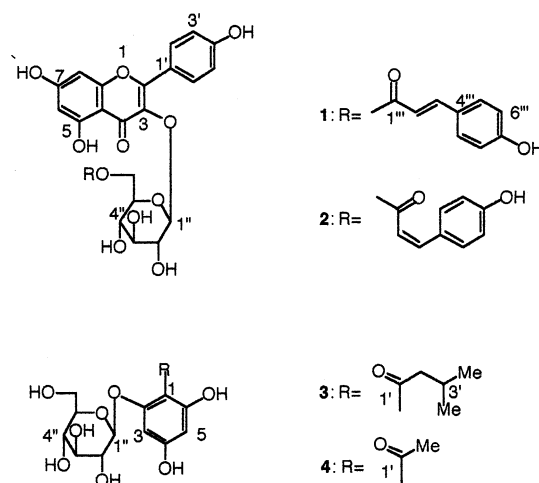
Received January 15, 2004

A new glycoside, 2- β -D-glucopyranosyloxy-4,6-dihydroxyisovalerophenone (**3**), was isolated from strawberry fruit along with kaempferol-3- β -D-(6-*O*-*trans*-*p*-coumaroyl)glucopyranoside (**1**) and kaempferol-3- β -D-(6-*O*-*cis*-*p*-coumaroyl)glucopyranoside (**2**). Compounds **1** and **2** inhibited activity of a drug-metabolizing enzyme, CYP3A4.

Cytochrome P450 (CYP) enzymes are heme-containing monooxygenases and constitute three families, CYP1, CYP2, and CYP3.¹ These enzymes have been mainly expressed in liver microsomes and are recognized to be responsible for drug metabolism, carcinogenesis, and degradation of xenobiotics. CYPs are also important in the biosynthesis of lipids, steroids, and other secondary metabolites. CYP3A4 is the most abundant enzyme in human liver microsomes; approximately 30% of the total CYP was suggested to be CYP3A4.² Recent investigations have shown that more than 50% of clinically used drugs are oxidized by CYP3A4.^{3,4} It is reported that concomitant oral administration of several foods and herbs affects drug metabolism in humans by inhibiting CYP3A4 activity and that grapefruit juice alters the pharmacokinetics of various drugs, including cyclosporine,^{5,6} midazolam,⁷ dihydropyridine-type calcium channel blockers,⁸ and triazolam.⁹ In the course of our study of CYP inhibitors from foods, we have reported the isolation and structure elucidation of furanocoumarins from grapefruit juice^{10–12} and bisalkaloids, dipiperamides A–E, from the white pepper *Piper nigrum*.^{13,14} This paper reports the isolation, structure elucidation, and CYP inhibitory activity of glycosides from the strawberry *Fragaria ananassa* Duch. cv. Tochtotome.

Results and Discussion

The strawberries (2.0 kg) were obtained at a market in Tochigi Prefecture and kept frozen until extraction with MeOH. After evaporation of the solvent, the resulting aqueous residue was extracted with EtOAc. The EtOAc layer was partitioned between hexane and 90% MeOH–H₂O, and the more polar fraction showed CYP inhibitory activity. The fraction was subjected to ODS chromatography followed by reverse-phase HPLC to furnish a new glycoside, **3** (2.6 mg), together with kaempferol-3- β -D-(6-*O*-*trans*-*p*-coumaroyl)glucopyranoside (**1**, 7.5 mg)¹⁵ and its *cis* isomer (**2**, 1.0 mg).¹⁶ Although **1** and **2** were obtained as a mixture (2:1) from the seeds of *Rosa canina*¹⁶ previously, in this paper we report the isolation and characterization of **2** for the first time.



The molecular formula of **2** was established as C₁₇H₂₄O₉ by HRFABMS and was identical with that of **1**. The ¹H NMR spectrum (Table 1) showed the signals for a *cis*-coumaroyl moiety, an AA'XX' system for H-5'', 9'' and H-6'', 8'' (δ 7.50, 6.67, each 2H, d, J = 8.8 Hz), and an AX system for H-2'' and H-3'' (δ 5.51, 6.71, each 1H, d, J = 13.2 Hz). In addition, the ¹H NMR spectrum indicated another AA'XX' system (δ 7.95, 6.80, each 2H, d, J = 8.8 Hz, H-2', 6' and H-3', 5'), two doublets (δ 6.14, 6.26, each 1H, d, J = 2.2 Hz, H-6 and H-8), and an anomeric signal (δ 5.14, d, J = 7.1 Hz, H-1''). These data were closely related to those of **1** except for the chemical shifts and magnitude of the coupling constants for H-2'' and H-3'' (δ 6.06, 7.39, each 1H, d, J = 16.0 Hz for **1**), indicating that **2** bears a *cis*-coumaroyl moiety instead of the *trans*-coumaroyl moiety in **1**. The ¹³C NMR spectrum of **2** was closely related to that of **1**. Therefore, the structure of **2** was determined to be kaempferol-3- β -D-(6-*O*-*cis*-*p*-coumaroyl)glucopyranoside, which was unambiguously confirmed by COSY, HMQC, and HMBC data.

Compound **3** has the molecular formula C₁₇H₂₄O₉ as established by HRFABMS and ¹³C NMR data. The ¹H NMR spectrum showed two methyl doublets (δ 0.92, d, J = 6.8 Hz, H-4'; δ 0.96, d, J = 6.8 Hz, H-5'), a methine signal (δ 2.24, heptet, J = 6.8 Hz, H-3'), and a geminally coupled methylene spin system (δ 2.87, dd, J = 15.6, 6.8 Hz, H-2'; δ 3.16, dd, J = 15.6, 6.8 Hz, H-2'). This indicated a 2-methylpropyl moiety, as established by COSY data.

* To whom correspondence should be addressed. Tel: +81-76-234-4468. Fax: +81-76-234-4417. E-mail: ohta@p.kanazawa-u.ac.jp.

[†] Kanazawa University.

[‡] RIKEN.

[§] Yokohama City University.

[⊥] Tochigi Prefectural Institute of Public Health and Environmental Science.

Table 1. NMR Data for **1** and **2** in CD₃OD

no.	1			2		
	δ_{H}	δ_{C}	HMBC	δ_{H}	δ_{C}	HMBC
2		160.1 s			159.9 s	
3		136.0 s			135.9 s	
4		180.1 s			179.9 s	
5		163.7 s			163.7 s	
6	6.11 br s	101.1 d	C-5, C-7, C-10	6.14 d 2.2	101.7 d	C-10
7		167.6 s			166.3 s	
8	6.28 br s	95.8 d	C-6, C-7, C-9, C-10	6.26 d 2.2	96.3 d	C-6, C-9, C-10
9		159.3 s			159.5 s	
10		106.2 s			105.1 s	
1'		123.6 s			123.6 s	
2'	7.98 d 8.8	133.0 d	C-2, C-3', C-4', C-5', C-6'	7.95 d 8.8	133.0 d	C-2, C-4', C-6'
3'	6.80 d 8.8	116.8 d	C-1', C-4', C-5'	6.80 d 8.8	116.8 d	C-1', C-4', C-5'
4'		162.3 s			162.3 s	
5'	6.80 d 8.8	116.8 d	C-1', C-3', C-4'	6.80 d 8.8	116.8 d	C-1', C-3', C-4'
6'	7.98 d 8.8	133.0 d	C-2, C-9, C-2', C-3', C-4', C-5'	7.95 d 8.8	133.0 d	C-2, C-2', C-4'
1''	5.22 d 7.1	104.9 d	C-3, C-2'', C-3''	5.14 d 7.1	105.1 d	C-3
2''	3.45 m	78.8 d	C-1'', C-3''	3.41 dd 9.9, 7.1	78.9 d	C-1'', C-3'', C-4''
3''	3.44 m	76.5 d	C-2''	3.44 m	76.5 d	C-1'', C-2''
4''	3.31 m	72.5 d	C-3'', C-5'', C-6''	3.27 t 9.9	72.4 d	C-2''
5''	3.45 m	76.6 d	C-3''	3.39 m	76.4 d	C-3''
6''	4.18 dd 11.6, 6.6 4.29 dd 11.6, 2.2	65.1 t	C-4'', C-5'', C-1''' C-4'', C-1'''	4.15 dd 12.5, 6.0 4.19 dd 12.5, 2.2	64.9 t	C-5'', C-1''' C-5'', C-1'''
1'''		169.6 s			168.6 s	
2'''	6.06 d 16.0	115.6 d	C-1''', C-4'''	5.51 d 13.2	117.0 d	C-4'''
3'''	7.39 d 16.0	147.4 d	C-1''', C-2''', C-4''' C-5''', C-9'''	6.71 d 13.2	146.2 d	C-5'''
4'''		127.9 s			128.4 s	
5'''	7.30 d 8.8	132.0 d	C-3''', C-6''', C-7''' C-8'''	7.50 d 8.8	134.5 d	C-3''', C-7''', C-9'''
6'''	6.79 d 8.8	117.6 d	C-4''', C-7''', C-8'''	6.67 d 8.8	116.5 d	C-4''', C-8'''
7'''		162.0 s			160.9 s	
8'''	6.79 d 8.8	117.6 d	C-4''', C-6''', C-7'''	6.67 d 8.8	116.5 d	C-4''', C-6'''
9'''	7.30 d 8.8	132.0 d	C-3''', C-5''', C-6''' C-7''', C-8'''	7.50 d 8.8	134.5 d	C-3''', C-5''', C-7'''

HMBC correlations between the methine and methylene signals of H-3' and H₂-2' and a keto carbon signal of C-1' (δ 207.9, s), and hence the presence of a 3-methylbutanone group. The ¹H and ¹³C NMR spectra revealed anomeric signals (δ 5.01, d, J = 7.7 Hz; δ 102.7, d) of a sugar moiety. Analysis of the COSY data showed the sequential correlation of signals at δ 5.01 (d, J = 7.7 Hz, H-1''), 3.53 (dd, J = 9.0, 7.7 Hz, H-2''), 3.46 (t, J = 9.0 Hz, H-3''), 3.39 (t, J = 9.0 Hz, H-4''), 3.45 (m, H-5''), 3.71 (dd, J = 12.2, 5.6 Hz, H-6''), and 3.91 (dd, J = 12.2, 2.1 Hz, H-6''), which indicated the presence of a β -glucopyranosyl moiety by judging from the coupling constants. The ¹H NMR spectrum revealed two *meta*-coupled aromatic doublets (δ 5.93, 6.15, each 1H, J = 2.1 Hz, H-5 and H-3). The HMQC data suggested that these hydrogens were connected to carbons at δ 99.3 (C-5) and 96.4 (C-3). HMBC correlations between the aromatic signal of H-3 and carbons C-1 (δ 107.7, s), C-2 (δ 162.9, s), C-4 (δ 167.3), and C-5 (δ 99.3) and between H-5 and carbons C-1, C-3, C-4, and C-6 (δ 168.4, s) implied that **3** contained a phloroglucinol moiety linked with a substituent at C-1, which was supported by the upfield resonances of C-3 and C-5. An HMBC cross-peak for the anomeric signal of H-1'' and C-2 suggested that the β -glucopyranosyl moiety was linked to an oxygen atom at C-2. The HMBC spectrum measured at an optimized J value of 3.3 Hz exhibited a correlation of H-2' (δ 2.86)/C-1, which revealed the 3-methylbutanone group was attached to C-1. Thus, the structure of **3** was established as 2- β -D-glucopyranosyloxy-4,6-dihydroxyisovalerophenone. Previously, 2- β -D-glucopyranosyloxy-4,6-dihydroxyacetophenone (**4**), showing choleric activity, was isolated from the rhizome of *Curcuma comos*,¹⁷ and the NMR data of **3** were almost superimposable on

those of **4** except for the 2-methylpropyl group in **3**. Although several compounds have been isolated from strawberries, e.g., pelargonidin 3-glucoside¹⁸ as a pigment, 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone¹⁹ as an aroma compound, and 1-*O*-*trans*-cinnamoyl- β -D-glucopyranose,²⁰ this is the first paper on the study of CYP inhibitors in strawberry fruit.

CYP3A4 activity was monitored by nifedipine oxidation with expressed human CYP3A4. Compounds **1** and **2** exhibited significantly potent inhibition of CYP3A4 with IC₅₀ values of 0.7 μ M, while the IC₅₀ value of **3** was 120 μ M.

Experimental Section

General Experimental Conditions. Optical rotations were determined with a Horiba SEPA-300 high-sensitive polarimeter. UV spectra were measured on a Shimadzu UV-1600 UV–visible spectrometer. IR spectra were recorded on a Shimadzu IR-460 infrared spectrophotometer. NMR spectra were recorded on a JEOL GSX500 and a Bruker Avance500 NMR spectrometers in CD₃OD. Chemical shifts were referenced to the residual solvent peaks (δ_{H} 3.30 and δ_{C} 49.8). Mass spectra were measured on a JEOL SX-102 mass spectrometer.

Extraction and Isolation. The frozen strawberries (2.0 kg) were extracted with MeOH. The extract was concentrated under reduced pressure and extracted with EtOAc. The EtOAc layer (18.4 g) was partitioned between hexane and 90% MeOH–H₂O. The aqueous MeOH fraction (3.35 g) was subjected to ODS chromatography with a stepwise gradient of MeOH–H₂O. The fraction (0.13 g) eluted with 60% MeOH–H₂O was purified by reverse-phase HPLC with 50% MeOH–H₂O to afford **1** (7.5 mg, $3.8 \times 10^{-4}\%$), **2** (1.0 mg, $5.0 \times 10^{-5}\%$), and **3** (2.6 mg, $1.3 \times 10^{-4}\%$).

Table 2. NMR Data for **3** in CD₃OD

no.	δ_{H}	δ_{C}	HMBC ^a
1		107.7 s	
2		162.9 s	
3	6.15 d 2.1	96.4 d	C-1, C-2, C-4, C-5
4		167.3 s	
5	5.93 d 2.1	99.3 d	C-1, C-3, C-4, C-6
6		168.4 s	
1'		207.9 s	
2'	2.87 dd 15.6, 6.8	54.9 t	C-1', C-3', C-4', C-5'
	3.16 dd 15.6, 6.8		C-1', C-3', C-4', C-5'
3'	2.24 heptet 6.8	27.1 d	C-2', C-4', C-5'
4'	0.92 (3H) d 6.8	23.7 q	C-2', C-3'
5'	0.96 (3H) d 6.8	24.2 q	C-2', C-3'
1''	5.01 d 7.7	102.7 d	C-2
2''	3.53 dd 9.0, 7.7	75.7 d	C-1'', C-3''
3''	3.46 t 9.0	79.4 d	C-2'', C-4''
4''	3.39 t 9.0	72.0 d	C-2'', C-3'', C-5'', C-6''
5''	3.45 m	79.2 d	C-4''
6''	3.71 dd 12.2, 5.6	63.3 t	C-4'', C-5''
	3.91 dd 12.2, 2.1		C-4'', C-5''

^a Measured at an optimized *J* value of 7.6 Hz.

Kaempferol-3- β -D-(6-*O*-*trans*-*p*-coumaryl)glucopyranoside (1): [α]_D²⁶ -62° (c 0.28, MeOH); NMR data, see Table 1; FABMS (positive) *m/z* 595 [M + H]⁺.

Kaempferol-3- β -D-(6-*O*-*cis*-*p*-coumaryl)glucopyranoside (2): [α]_D²⁶ -57° (c 0.045, MeOH); UV (MeOH) λ_{max} (log ϵ) 315.5 (4.5), 267.5 (4.4), 227.0 (4.3), 208.0 nm (4.4, sh); IR (film) ν_{max} 3300, 1700, 1660 cm⁻¹; NMR data, see Table 1; HRFABMS (positive) *m/z* 595.1084 [M + H]⁺ (calcd for C₂₉H₂₃O₁₄, Δ -0.4 mmu).

2- β -D-Glucopyranosyloxy-4,6-dihydroxyisovalerophenone (3): [α]_D²⁶ -55° (c 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 285.5 (4.2), 225.0 nm (4.0); IR (film) ν_{max} 3400, 1630 cm⁻¹; NMR data, see Table 2; HRFABMS (positive) *m/z* 373.1508 [M + H]⁺ (calcd for C₁₇H₂₅O₉, Δ +1.0 mmu).

Assay of CYP Inhibition. CYP activity was based on nifedipine oxidation. Various amounts (0–10 μ M, final concentration) of samples in 1 μ L of DMSO were added to 192 μ L of solution containing 100 mM phosphate buffer (pH 7.4) containing 50 μ M nifedipine (Wako Pure Chemical Industries, Ltd. (Osaka, Japan)), 5 mM glucose-6-phosphate (Oriental Yeast Co., Ltd. (Tokyo, Japan)), 0.5 mM β -NADP⁺ (Oriental Yeast Co., Ltd.), 0.5 mM MgCl₂, and 4.3 μ g/mL glucose-6-phosphate dehydrogenase (Oriental Yeast Co., Ltd.) and incubated at 37 °C for 5 min. CYP3A4 (Gentest Co. (Woburn, MA)) was also preincubated in 7 μ L of the buffer at 37 °C for 5 min and added to the sample solution. After the incubation at 37 °C for 1 h, the reaction was quenched by the addition of 100 μ L of MeOH. After adding 3.7 μ g of 6-methoxycarbonyl-

5-methyl-7-(2-nitrophenyl)-4,7-dihydrofuro[3,4-*b*]pyridin-1-(3*H*)-one in 1 μ L of DMSO as an internal standard, the reaction mixture was extracted with 1 mL of ether, and the ether layer was evaporated. The residue was dissolved in 100 μ L of MeOH, and an aliquot (20 μ L) was analyzed by reverse-phase HPLC (column, TSK-gel ODS-120T, 4.6 mm i.d. \times 150 mm; mobile phase, 64% MeOH–H₂O; flow rate, 1.0 mL/min; detection, UV 254 nm); retention times: 2.9 min for the internal standard, 4.0 min for the nifedipine metabolite (nifedipine pyridine), and 5.5 min for nifedipine. The value of IC₅₀, the concentration required for 50% inhibition of CYP3A4 activity, was calculated from the data of duplicate measurements.

Acknowledgment. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References and Notes

- (1) Nelson, D. R.; Koymans, L.; Kamataki, T.; Stegeman, J. J.; Feyereisen, R.; Waxman, D. J.; Waterman, M. R.; Gotoh, O.; Coon, M. J.; Estabrook, R. W.; Gunsalus, I. C.; Nebert, D. W. *Pharmacogenetics* **1996**, *6*, 1–42.
- (2) Shimada, T.; Yamazaki, H.; Mimura, M.; Inui, Y.; Guengerich, F. P. *J. Pharmacol. Exp. Ther.* **1994**, *270*, 414–423.
- (3) Guengerich, F. P. *Adv. Pharmacol.* **1997**, *43*, 7–35.
- (4) Rendic, S.; DiCarlo, F. J. *Drug Metab. Rev.* **1997**, *29*, 413–580.
- (5) Ducharme, M. P.; Warbasse, L. H.; Edwards, D. J. *Clin. Pharmacol. Ther.* **1995**, *57*, 485–491.
- (6) Yee, G. C.; Stanley, D. L.; Pessa, L. J.; Dalla, C. T.; Beltz, S. E.; Ruiz, J. *Lancet* **1995**, *345*, 955–956.
- (7) Kupferschmidt, H. H.; Ha, H. R.; Ziegler, W. H.; Meier, P. J.; Krahenbuhl, S. *Clin. Pharmacol. Ther.* **1995**, *58*, 20–28.
- (8) Bailey, D. G.; Arnold, J. M.; Spence, J. D. *Clin. Pharmacokinet.* **1994**, *26*, 91–98.
- (9) Hukkinen, S. K.; Varhe, A.; Olkkola, K. T.; Neuvonen, P. J. *Clin. Pharmacol. Ther.* **1995**, *58*, 127–131.
- (10) Ohta, T.; Nagahashi, M.; Hosoi, S.; Tsukamoto, S. *Bioorg. Med. Chem.* **2002**, *10*, 969–973.
- (11) Ohta, T.; Maruyama, T.; Nagahashi, K.; Miyamoto, Y.; Hosoi, S.; Kiuchi, F.; Yamazoe, Y.; Tsukamoto, S. *Tetrahedron* **2002**, *58*, 6631–6635.
- (12) Ohta, T.; Miyamoto, Y.; Maruyama, T.; Kiuchi, F.; Tsukamoto, S. *Nat. Med.* **2002**, *56*, 264–267.
- (13) Tsukamoto, S.; Cha, B.-C.; Ohta, T. *Tetrahedron* **2002**, *58*, 1667–1671.
- (14) Tsukamoto, S.; Tomise, K.; Miyakawa, K.; Cha, B.-C.; Abe, T.; Hamada, T.; Hirota, H.; Ohta, T. *Bioorg. Med. Chem.* **2002**, *10*, 2981–2985.
- (15) Higuchi, R.; Donnelly, D. M. X. *Phytochemistry* **1978**, *17*, 787–791.
- (16) Kumarasamy, Y.; Cox, P. J.; Jaspars, M.; Rashid, M. A.; Sarker, S. D. *Pharm. Biol.* **2003**, *41*, 237–242.
- (17) Suksamrarn, A.; Eiamong, S.; Piyachaturawat, P.; Byrne, L. T. *Phytochemistry* **1997**, *45*, 103–105.
- (18) Sondheimer, E.; Kertesz, Z. I. *J. Am. Chem. Soc.* **1948**, *70*, 3476–3478.
- (19) Pickenhagen, W.; Velluz, A.; Paserar, J.-P.; Ohloff, G. *J. Sci. Food Agric.* **1981**, *32*, 1132–1134.
- (20) Latza, S.; Gansser, D.; Berger, R. G. *J. Agric. Food Chem.* **1996**, *44*, 1367–1370.

NP0400104