

ABSOLUTE STEREOSTRUCTURES OF ACYLATED KHELLACTONE-TYPE COUMARINS FROM *ANGELICA FURCIJUGA*

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Abstract — Two new acylated khellactone-type coumarin hyuganoside I (**1**) and hyuganin F (**2**) were isolated from the leaves of *Angelica furcijuga*. Their absolute stereostructures were determined on the basis of chemical and physicochemical evidence. Furthermore, the absolute stereostructures of the acyl moieties in hyuganins A (**3**) and C (**4**) and isoeopoxypteryxin (**5**) were elucidated.

The Umbelliferae plant *Angelica furcijuga* is indigenous to Japan (Japanese name, hyugatouki) and the dried whole plant has been used for the treatment of hepatopathy, allergosis, inflammation, diabetes, and hypertension as a Japanese folk medicine. During the course of our characterization studies on *A. furcijuga*,¹⁻⁴ we have reported the structure elucidation of four acylated khellactone-type coumarins called hyuganins A–D and three glycosides, hyuganosides II, IIIa, and IIIb from the roots and hyuganosides IV and V from the flowers of *A. furcijuga*.²⁻⁴ The principal constituents were found to show nitric oxide (NO) production inhibitory,^{1,3} vasorelaxant,² and hepatoprotective activities,¹ and also the presence of the 3'- and 4'-acyl groups in acylated khellactone-type coumarins were essential for their strong activities. As a continuing study, we found that two new acylated khellactone-type coumarins, hyuganoside I (**1**, 0.013%) and hyuganin F (**2**, 0.0017%), were isolated from the dried leaves of this plant together with isoeopoxypteryxin^{1,2,4} (**5**, 0.11%), isopteryxin^{1,2,4} (**6**, 0.13%), hyuganosides II^{1,3} (**7**, 0.023%) and IV⁴ (**8**, 0.023%), chlorogenic acid⁴ (**9**, 0.0013%), kaempferol 3-*O*- β -D-glucopyranoside⁴ (**10**, 0.0063%), quercetin 3-*O*- β -D-glucopyranoside⁴ (**11**, 0.0049%), quercetin 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-galactopyranoside⁵ (**12**, 0.012%), and (*Z*)-3-hexenyl β -D-glucopyranoside⁶ (**13**, 0.0079%). In this paper, we describe the isolation and absolute stereostructure elucidation of hyuganoside I (**1**) and hyuganin F (**2**). In addition, the absolute stereostructures of the acyl moieties in hyuganins A² (**3**) and C² (**4**) and isoeopoxypteryxin (**5**) were also elucidated.

Hyuganoside I (**1**),⁷ a white powder, $[\alpha]_{\text{D}}^{25} -15.0^{\circ}$ ($c=0.38$, EtOH), C₂₇H₃₄O₁₄, showed absorption bands ascribable to hydroxyl, carbonyl, and ether functions and aromatic ring [IR (KBr): 3432, 1750–1720, 1608, 1560, 1148, 1078, 837 cm⁻¹]. Acid hydrolysis of **1** with 1.0 M hydrochloric acid (HCl) liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector.^{3,8} The ¹H-NMR (500 MHz, DMSO-*d*₆) and ¹³C-NMR (Table 1) spectra⁹ of **1** showed signals assignable to four methyls [δ 1.10 (3H, d, $J = 6.1$ Hz, 4''-H₃), 1.20 (3H, s, 5''-H₃), 1.40, 1.46 (3H each, both s, 2'-*gem*-CH₃)], an acetyl group [δ 2.02 (3H, s, 2''-H₃)], a coumarin moiety [δ 6.29 (1H, d, $J = 9.6$ Hz, 3-H), 6.87 (1H, d, $J = 8.5$ Hz, 6-H), 7.63 (1H, d, $J = 8.5$ Hz, 5-H), 7.97 (1H, d, $J = 9.6$ Hz, 4-H)], and three methines bearing an oxygen function [δ 4.10 (1H, q, $J = 6.1$ Hz, 3''-H), 5.23 (1H, d, $J = 5.0$ Hz, 3'-H), 6.45 (1H, d, $J = 5.0$ Hz, 4'-H)] together with a β -D-glucopyranosyl part { δ [3.38 (1H, dd, $J = 6.1, 10.8$ Hz), 3.64 (1H, br d, $J = ca. 11$ Hz), Glc-6-H₂] 4.21 (1H, d, $J = 7.6$ Hz, Glc-1-H)}. The aglycon of **1** named hyuganin E (**1a**)¹⁰ was obtained by enzymatic hydrolysis with naringinase, whose proton and carbon signals in the ¹H- and ¹³C-NMR spectra were superimposable on those of isoeopoxypteryxin (**5**), except for the signals due to the 3''-acyl group. Alkaline treatment of **1a** with 5.0% aqueous potassium hydroxide (KOH) liberated (+)-*cis*-khellactone (**14**) and (-)-*trans*-

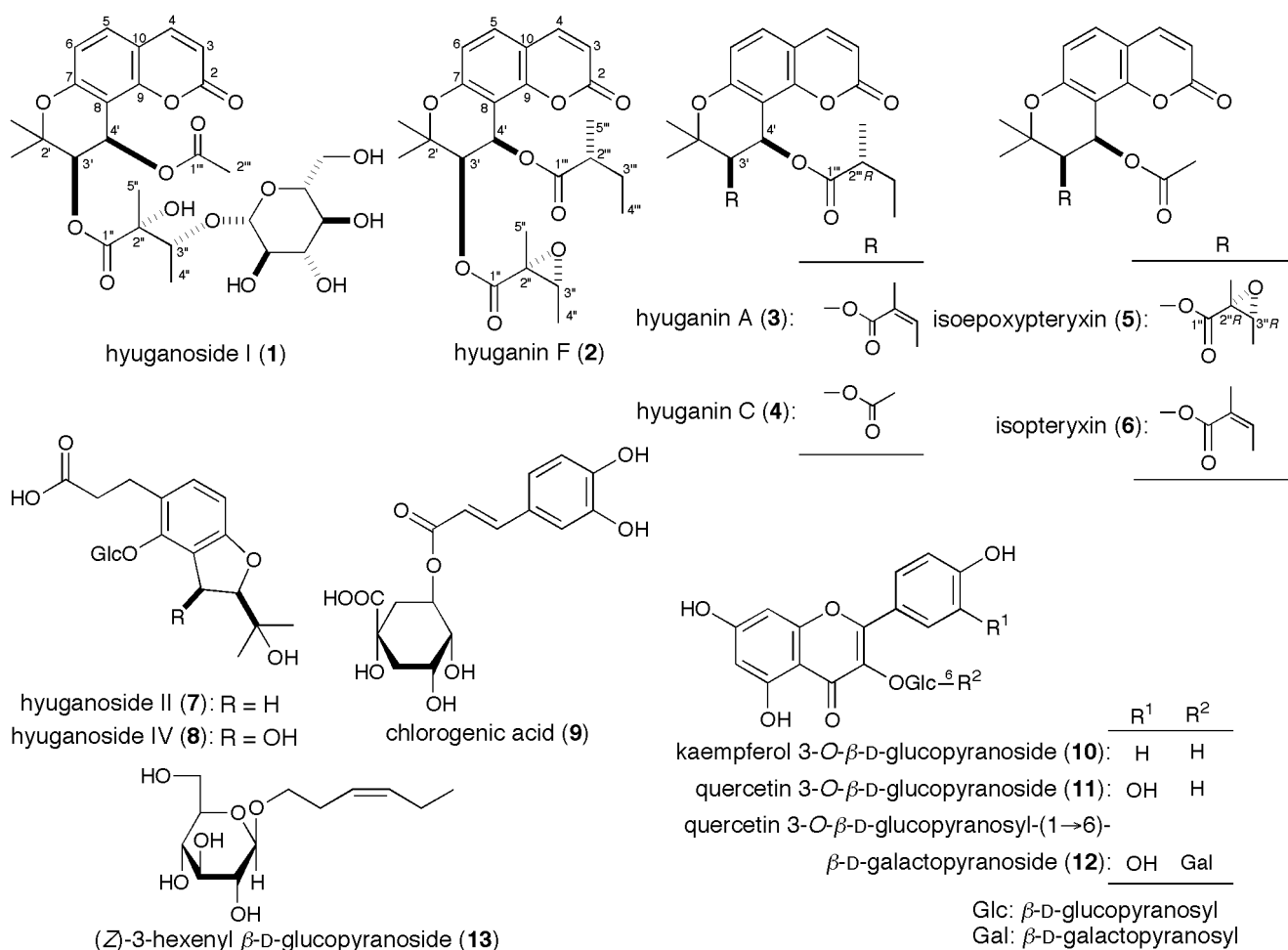


Chart 1

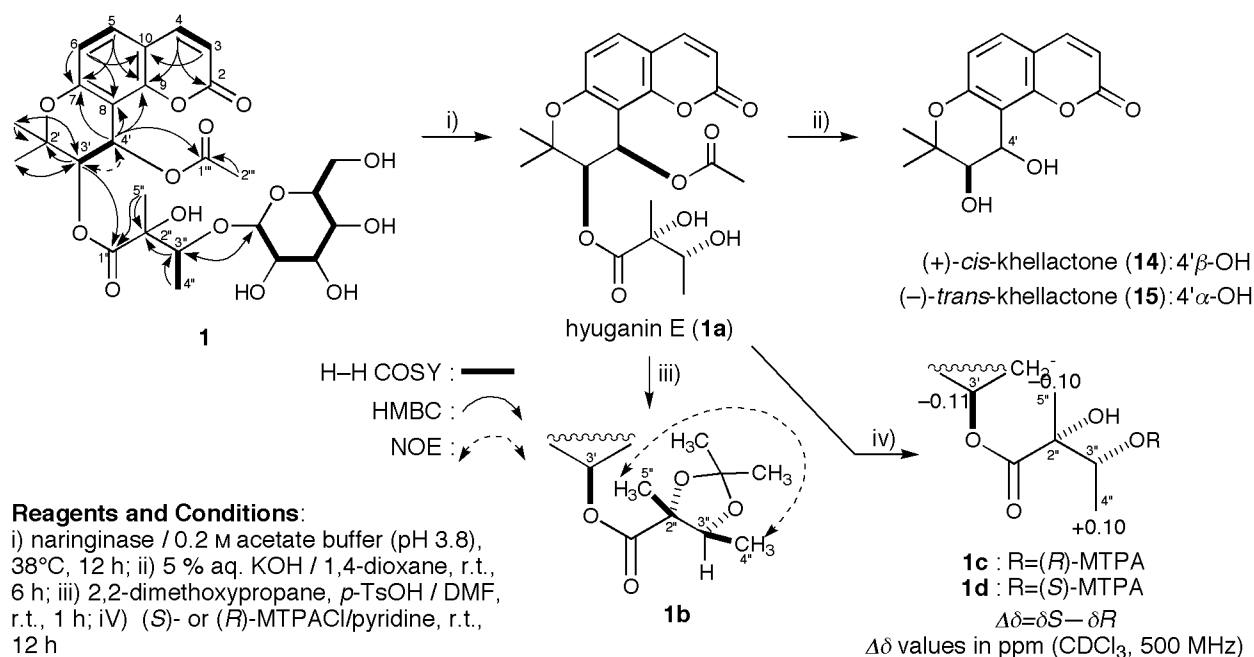


Figure 1

khellactone (15), which were known to be an epimerization product at the 4'-position of 14.^{2,11-13} The positions of the acyl groups and glucoside linkage in 1 were clarified by heteronuclear multiple-bond correlation (HMBC) experiment on 1 (Figure 1). Furthermore, in nuclear Overhauser enhancement spectroscopy (NOESY) experiment on 1, a nuclear Overhauser effect

(NOE) correlation was observed between the 3'- and 4'-proton pair, so that the absolute stereostructure of the (+)-*cis*-khellactone moiety in **1** was confirmed. In order to clarify the relative stereostructure of the diol part in 2'',3''-dihydroxy-2''-methylbutyryl group, the acetonide derivative (**1b**)¹⁴ was prepared from **1a**. In the NOESY experiment on **1b**, a NOE correlation was observed between the 4''-methyl and 5''-methyl protons. Furthermore, the absolute stereostructure of the 3''-position was confirmed by the application of modified Mosher's method¹⁵ to the (*R*)-MTPA (**1c**)¹⁶ and (*S*)-MTPA esters (**1d**),¹⁷ which were prepared by the treatment of **1a** with (*S*)- or (*R*)-MTPA chloride in pyridine (Figure 1). Consequently, the absolute stereostructure of **1** was elucidated as shown.

Hyuganin F (**2**),¹⁸ a white powder, C₂₄H₂₈O₈, ([α]_D²⁷ -34.8° in MeOH). The IR spectrum of **2** showed absorption bands at 1744, 1609, 1148, and 1115 cm⁻¹ ascribable to an ester carbonyl function and aromatic ring, while the UV spectrum showed absorption maxima at 245 (log ε 3.70) and 322 (4.20) nm suggestive of a characteristic coumarin skeleton.² The ¹H-NMR (CDCl₃) and ¹³C-NMR (Table 1) spectra⁹ of **2** showed signals assignable to six methyls [δ 0.95 (t, *J* = 7.3 Hz, 4'''-H₃), 1.21 (d, *J* = 7.0 Hz, 5'''-H₃), 1.43 (d, *J* = 5.5 Hz, 4''-H₃), 1.46, 1.50 (both s, 2'-*gem*-CH₃), 1.59 (s, 5''-H₃)], a methylene [δ 1.72 (m, 3'''-H₂)], a methine [δ 2.40 (dd, *J* = 6.6, 7.0 Hz, 2''-H)], three methines bearing an oxygen function [δ 3.08 (br d, *J* = *ca.* 6 Hz, 3''-H), 5.38 (d, *J* = 5.0 Hz, 3'-H), 6.59 (d, *J* = 5.0 Hz, 4'-H)], an *cis*-olefinic proton pairs [δ 6.24 (d, *J* = 9.5 Hz, 3-H), 7.60 (d, *J* = 9.5 Hz, 4-H)], and two aromatic protons [δ 6.81 (d, *J* = 8.6 Hz, 6-H), 7.38 (d, *J* = 8.6 Hz, 5-H)]. Alkaline hydrolysis of **2** with 5.0% aqueous KOH provided **14** and **15**.^{2,11-13} On the basis of above-mentioned evidence and the 2D-NMR experiments on **2** as shown in Figure 2, the stereostructure of **2** was clarified except for the acyl moieties.

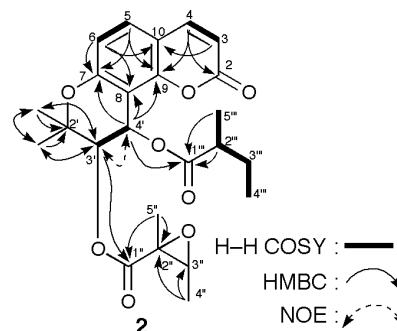


Figure 2

Table 1. ¹³C-NMR Data for Hyuganoside I (**1**) and Hyuganins E (**1a**) and F (**2**)

	1 ^(a)	1a ^(b)	2 ^(b)		1 ^(a)	1a ^(b)	2 ^(b)	
C-2	159.3	159.7	159.6	C-1''	174.1	174.9	C-1''	168.8
C-3	112.4	113.4	113.4	C-2''	77.3	77.6	C-2''	59.5
C-4	144.3	143.2	143.1	C-3''	76.0	71.0	C-3''	60.8
C-5	129.8	129.4	129.5	C-4''	11.7	15.9	C-4''	13.7
C-6	114.0	114.4	114.4	C-5''	21.8	21.4	C-5''	19.2
C-7	156.1	156.5	156.3	C-1'''	169.2	171.0	C-1'''	175.4
C-8	106.4	106.4	107.4	C-2'''	20.2	20.7	C-2'''	41.2
C-9	153.4	153.9	153.9	Glc-1	98.7		C-3'''	26.6
C-10	112.4	112.7	112.5	-2	73.4		C-4'''	11.6
C-2'	77.6	77.8	77.0	-3	76.2		C-5'''	16.7
C-3'	69.9	71.5	72.2	-4	70.3			
C-4'	60.2	61.4	59.8	-5	76.7			
2'- <i>gem</i> -CH ₃	22.3	22.8	21.4	-6	61.2			
	24.5	24.8	26.3					

Measured in ^(a)CD₃OD and ^(b)CDCl₃ at 125 MHz. Glc: β-D-glucopyranosyl

Many acylated khellactone-type coumarins were isolated from several Umbelliferae plants (e.g. *Peucedanum praeruptorum*,¹⁹ *Angelica keiskei*,²⁰ etc.) and they were known to show various biological activities such as platelet-aggregation inhibitory¹⁹ and anti-AIDS activities etc.²¹ However, the total absolute stereostructures including the acyl group were characterized only rarely. We previously reported the structures of hyuganins A (**3**) and C (**4**),² but the absolute configuration of the 2-methylbutyryl moiety in **3** and **4** was remained uncharacterized. To clarify the absolute configuration of **3**, we carried out X-Ray crystallographic analysis.²² As shown in Figure 3, the 2'''-methylbutyryl moiety in **3** was determined to be a *R*-orientation.²³ On the other hand, alkaline hydrolysis of isoeopoxypteryxin (**5**) with 10% aqueous KOH followed by purification using reversed-phase silica gel (ODS) column chromatography and finally HPLC gave (2*R*,3*R*)-(+)-2,3-epoxy-2-methylbutyric

acid²⁴ $\{[\alpha]_D^{30} +27.9^\circ (c\ 0.11, \text{CHCl}_3)\}$, so that the absolute configurations of the 2''- and 3''-positions in **5** were clarified to be in the 2''*R*, 3''*R* orientations. Finally, alkaline hydrolysis of **2** and **4** with 5% aqueous KOH and then subsection to reversed-phase silica gel column chromatography gave the organic acids fractions, which were analyzed with HPLC (optical rotation detector). As the result, (2*R*,3*R*)-(+)-2,3-epoxy-2-methylbutyric acid (**i**, 10.7 min, positive optical rotation) from **2** and (-)-(*R*)-2-methylbutyric acid (**ii**, 15.0 min, negative optical rotation) from **2** and **4** were identified by comparison of their retention times and optical rotation with those of authentic samples from **3** and **5**. On the basis of above-mentioned evidence, the absolute stereostructures of **2**–**5** were determined to be as shown.

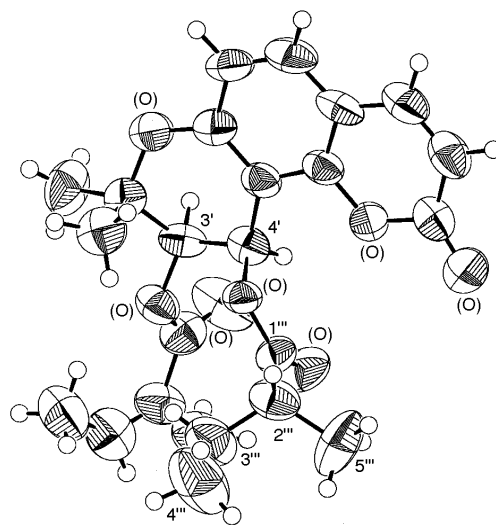


Figure 3. Perspective View of Hyuganin A (**3**)

REFERENCES AND NOTES

- H. Matsuda, T. Murakami, T. Kageura, K. Ninomiya, I. Toguchida, N. Nishida, and M. Yoshikawa, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2191.
- H. Matsuda, T. Murakami, N. Nishida, T. Kageura, and M. Yoshikawa, *Chem. Pharm. Bull.*, 2000, **48**, 1429.
- T. Morikawa, H. Matsuda, N. Nishida, T. Ohgushi, and M. Yoshikawa, *Chem. Pharm. Bull.*, submitted.
- H. Matsuda, T. Morikawa, T. Ohgushi, T. Ishiwada, N. Nishida, I. Toguchida, and M. Yoshikawa, *J. Nat. Prod.*, submitted.
- A. M. D. El-Mousallamy, *Phytochemistry*, 1998, **48**, 759.
- K. Mizutani, M. Yuda, O. Tanaka, Y. Saruwatari, T. Fuwa, M.-R. Jia, Y.-K. Ling, and X.-F. Pu, *Chem. Pharm. Bull.*, 1988, **36**, 2689.
- a) **1**: High-resolution positive-ion FAB-MS: Calcd for $\text{C}_{27}\text{H}_{34}\text{O}_{14}\text{Na}$ ($\text{M}+\text{Na}$)⁺: 605.1846. Found: 605.1859. UV [MeOH, nm (log ϵ)]: 218 (sh, 4.09), 242 (sh, 3.61), 251 (sh, 3.50), 298 (sh, 3.79), 322 (3.96). Positive-ion FAB-MS: m/z 605 ($\text{M}+\text{Na}$)⁺. Negative-ion FAB-MS: m/z 581 ($\text{M}-\text{H}$)⁻. b) Hyuganoside I (**1**) was also isolated from the fresh roots of this plant (0.001% from the fresh roots).
- M. Yoshikawa, T. Morikawa, Y. Kashima, K. Ninomiya, and H. Matsuda, *J. Nat. Prod.*, 2003, **66**, 922.
- The ¹H- and ¹³C-NMR spectra of new compounds were assigned on the basis of homo- and hetero-correlation spectroscopy (¹H–¹H, ¹³C–¹H COSY) and heteronuclear multiple bond correlation (HMBC) experiments.
- 1a**: A white powder, $[\alpha]_D^{23} +9.7^\circ (c=0.33, \text{CHCl}_3)$. High-resolution EI-MS: Calcd for $\text{C}_{25}\text{H}_{34}\text{O}_9$ (M^+): 420.1420. Found: 420.1400. UV [MeOH, nm, (log ϵ)]: 217 (sh, 4.08), 244 (sh, 3.56), 252 (sh, 3.44), 298 (sh, 3.74), 323 (3.91). IR (KBr): 3440, 1750–1718, 1608, 1560, 1490, 1236, 1118, 836 cm^{-1} . ¹H-NMR (500 MHz, CDCl_3) δ : 1.19 (3H, d, $J = 6.4$ Hz, 4''-H₃), 1.36 (3H, s, 5''-H₃), 1.42, 1.46 (3H each, both s, 2'-gem-CH₃), 2.17 (3H, s, 2'''-H₃), 3.83 (1H, q, $J = 6.4$ Hz, 3''-H), 5.43 (1H, d, $J = 4.9$ Hz, 3'-H), 6.26 (1H, d, $J = 9.6$ Hz, 3-H), 6.59 (1H, d, $J = 4.9$ Hz, 4'-H), 6.81 (1H, d, $J = 8.8$ Hz, 6-H), 7.38 (1H, d, $J = 8.8$ Hz, 5-H), 7.61 (1H, d, $J = 9.6$ Hz, 4-H). EI-MS m/z (%): 420 (M^+ , 15), 286 (27), 244 (61), 229 (100), 213 (76).
- E. Smith, N. Hosansky, W. G. Bywater, E. E. van Tamelen, *J. Am. Chem. Soc.*, 1957, **79**, 3534.
- Y. Yamada, C.-S. Hsu, K. Iguchi, M. Suzuki, *Tetrahedron Lett.*, 1974, **29**, 2513.
- M. Takata, S. Shibata, T. Okuyama, *Planta Med.*, 1990, **56**, 307.
- 1b**: ¹H-NMR (500 MHz, CDCl_3) δ : 1.17 (3H, d, $J = 6.3$ Hz, 4''-H₃), 1.33 (3H, s, 5''-H₃), 1.38, 1.45 (3H each, both s), 1.42, 1.46 (3H each, both s, 2'-gem-CH₃), 2.14 (3H, s, 2'''-H₃), 4.31 (1H, q, $J = 6.3$ Hz, 3''-H), 5.34 (1H, d, $J = 4.7$ Hz, 3'-H), 6.25 (1H, d, $J = 9.5$ Hz, 3-H), 6.60 (1H, d, $J = 4.7$ Hz, 4'-H), 6.80 (1H, d, $J = 8.7$ Hz, 6-H), 7.36 (1H, d, $J = 8.7$ Hz, 5-H),

- 7.60 (1H, d, $J = 9.5$ Hz, 4-H). EI-MS m/z (%): 460 (M^+ , 6), 445($M^+ - CH_3$, 18), 286 (15), 244 (34), 229 (68), 213 (23), 129 (100).
- 15 I. Ohtani, T. Kusumi, Y. Kashman, and H. Kakisawa, *J. Am. Chem. Soc.*, 1991, **113**, 4092.
- 16 **1c**: 1H -NMR (500 MHz, $CDCl_3$) δ : 1.26 (3H, d, $J = 6.1$ Hz, 4''-H₃), 1.36 (6H, s, 2'-gem-CH₃), 1.40 (3H, s, 5''-H₃), 2.12 (3H, s, 2'''-H₃), 5.33 (1H, d, $J = 4.8$ Hz, 3'-H), 5.33 (1H, q, $J = 6.1$ Hz, 3''-H), 6.26 (1H, d, $J = 9.6$ Hz, 3-H), 6.60 (1H, d, $J = 4.8$ Hz, 4'-H), 6.80 (1H, d, $J = 8.6$ Hz, 6-H), 7.36 (1H, d, $J = 8.6$ Hz, 5-H), 7.61 (1H, d, $J = 9.6$ Hz, 4-H).
- 17 **1d**: 1H -NMR (500 MHz, $CDCl_3$) δ : 1.36 (3H, d, $J = 6.4$ Hz, 4''-H₃), 1.30 (3H, s, 5''-H₃), 1.32, 1.34 (3H each, both s, 2'-gem-CH₃), 2.14 (3H, s, 2'''-H₃), 5.22 (1H, d, $J = 4.7$ Hz, 3'-H), 5.33 (1H, q, $J = 6.4$ Hz, 3''-H), 6.26 (1H, d, $J = 9.5$ Hz, 3-H), 6.58 (1H, d, $J = 4.7$ Hz, 4'-H), 6.80 (1H, d, $J = 8.5$ Hz, 6-H), 7.36 (1H, d, $J = 8.5$ Hz, 5-H), 7.60 (1H, d, $J = 9.5$ Hz, 4-H).
- 18 **2**: High-resolution EI-MS: Calcd for $C_{24}H_{28}O_8$ (M^+): 444.1784. Found: 444.1796. IR (KBr): 1744, 1609, 1148, 1115 cm^{-1} . EI-MS m/z (%): 444 (M^+ , 4), 229 (100).
- 19 N. Takeuchi, T. Kasama, K. Mayuzumi, K. Tamaru, H. Fukaya, T. Ohno, and S. Tobinaga, *Chem. Pharm. Bull.*, 1988, **36**, 4221.
- 20 K. Baba, T. Kido, Y. Yoneda, M. Taniguchi, and M. Kozawa, *Shoyakugaku Zasshi*, 1990, **44**, 235.
- 21 L. Xie, D. Yu, C. Wild, G. Allaway, J. Turpin, P. C. Smith, and K.-H. Lee, *J. Med. Chem.*, 2004, **47**, 756.
- 22 Crystal data for **3**: Colorless prismatic crystals, mp 133–134 °C (from *n*-hexane–EtOAc), $C_{24}H_{28}O_7$, $M = 428.48$, crystal dimensions: 0.15 × 0.09 × 0.23 mm, crystal system: orthorhombic, lattice type: primitive, lattice parameters: $a = 15.051(2)$, $b = 15.679(2)$, $c = 9.704(2)$ Å, $V = 2289.9(6)$ Å³, space group: $P2_12_12_1$ (#19), $Z = 4$, $\mu(CuK\alpha) = 7.54$ cm^{-1} , temperature: 23.0 °C, structure solution: direct methods (SHELXS-86), residuals: $R = 0.095$, $Rw = 0.152$, $RI = 0.048$, goodness of fit indicator: 1.16. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-K α ($\lambda = 1.54178$ Å) radiation and a rotating anode generator.
- 23 D. A. Evans and J. M. Takacs, *Tetrahedron*, 1980, **21**, 4233.
- 24 J. M. Torres-Valencia, C. M. Cerda-García-Rojas, and P. Joseph-Nathan, *Tetrahedron Asymmetry*, 1998, **9**, 757.