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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 isoepoxypteryxin (**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 isoepoxypteryxin[1,2,4] (**5**, 0.11%), isopteryxin[1,2,4] (**6**, 0.13%), hyuganosides II[1,3] (**7**, 0.023%) and IV[4] (**8**, 0.023%), chlorogenic acid[4] (**9**, 0.0013%), kaempferol 3-*O*-β-D-glucopyranoside[4] (**10**, 0.0063%), quercetin 3-*O*-β-D-glucopyranoside[4] (**11**, 0.0049%), quercetin 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-galactopyranoside[5] (**12**, 0.012%), and (*Z*)-3-hexenyl β-D-glucopyranoside[6] (**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 A[2] (**3**) and C[2] (**4**) and isoepoxypteryxin (**5**) were also elucidated.

Hyuganoside I (**1**),[7] a white powder, $[\alpha]_D^{25}$ –15.0° (*c*=0.38, EtOH), $C_{27}H_{34}O_{14}$, 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 ^{1}H-NMR (500 MHz, DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[9] of **1** showed signals assignable to four methyls [δ 1.10 (3H, d, *J* = 6.1 Hz, 4''-H_3), 1.20 (3H, s, 5''-H_3), 1.40, 1.46 (3H each, both s, 2'-*gem*-CH_3)], an acetyl group [δ 2.02 (3H, s, 2'''-H_3)], 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-H_2] 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 ^{1}H- and ^{13}C-NMR spectra were superimposable on those of isoepoxypteryxin (**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*-

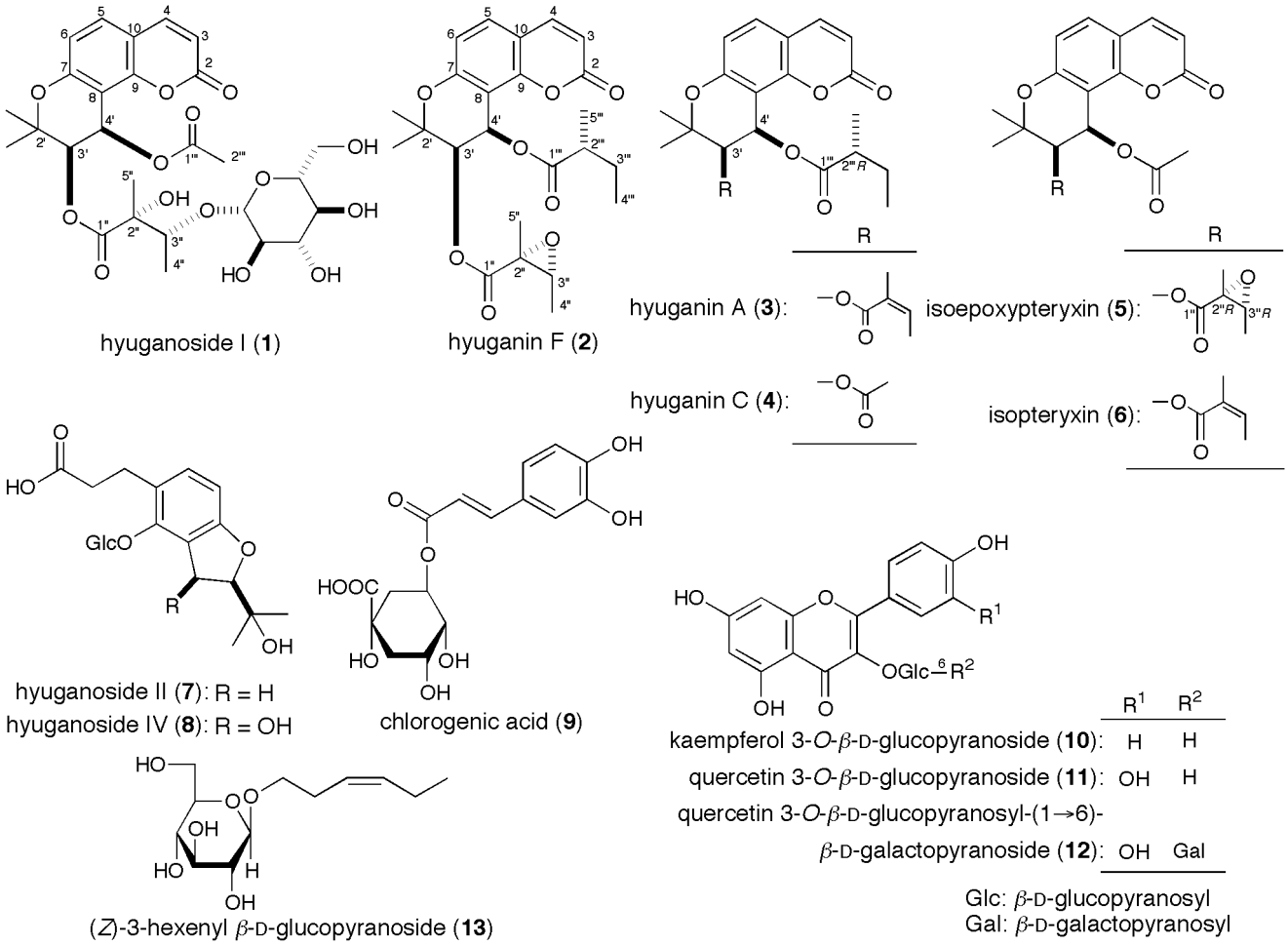

Chart 1

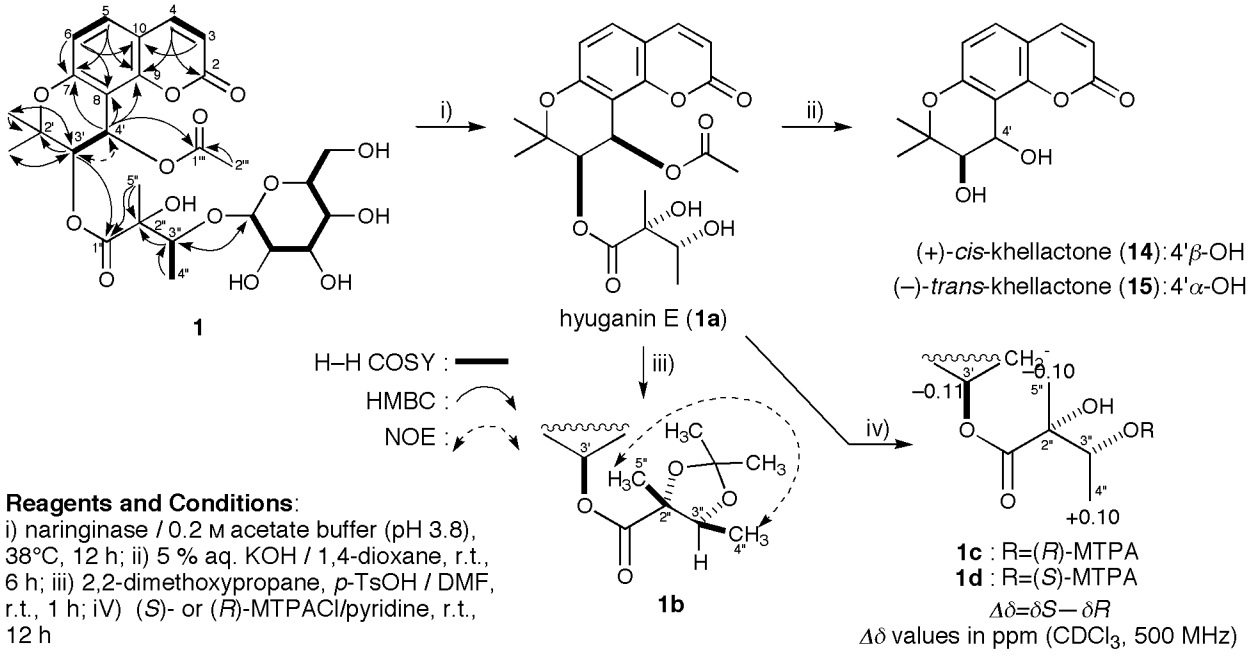

Figure 1

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 method[15] 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, $C_{24}H_{28}O_8$, ($[\alpha]_D^{27}$ –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 ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[9] of **2** showed signals assignable to six methyls [δ 0.95 (t, J = 7.3 Hz, 4'''-H_3), 1.21 (d, J = 7.0 Hz, 5'''-H_3), 1.43 (d, J = 5.5 Hz, 4"-H_3), 1.46, 1.50 (both s, 2'-*gem*-CH_3), 1.59 (s, 5"-H_3)], a methylene [δ 1.72 (m, 3'''-H_2)], 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.

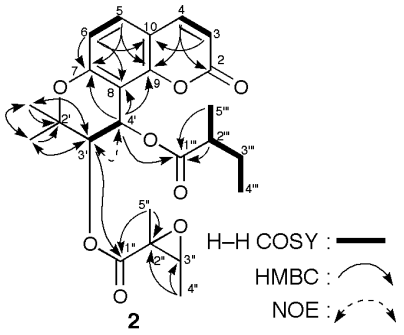

Figure 2

Table 1. ^{13}C-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'-*gem*-CH_3	22.3	22.8	21.4	-6	61.2			
	24.5	24.8	26.3					

Measured in [a)]CD_3OD and [b)]$CDCl_3$ 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 inhibitory[19] 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 isoepoxypteryxin (**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

acid[24] {$[\alpha]_D^{30}$ +27.9° (*c* 0.11, $CHCl_3$)}, 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 subjection 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.

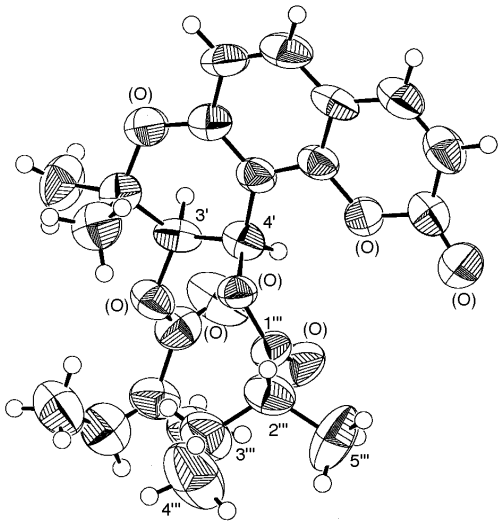

Figure 3. Perspective View of Hyuganin A (**3**)

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7 a) **1**: High-resolution positive-ion FAB-MS: Calcd for $C_{27}H_{34}O_{14}Na$ $(M+Na)^+$: 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 $(M+Na)^+$. Negative-ion FAB-MS: *m/z* 581 $(M–H)^–$. b) Hyuganoside I (**1**) was also isolated from the fresh roots of this plant (0.001% from the fresh roots).

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9 The ^1H- and ^{13}C-NMR spectra of new compounds were assigned on the basis of homo- and hetero-correlation spectroscopy (^1H–^1H, ^{13}C–^1H COSY) and heteronuclear multiple bond correlation (HMBC) experiments.

10 **1a**: A white powder, $[\alpha]_D^{23}$ +9.7° (*c*=0.33, $CHCl_3$). High-resolution EI-MS: Calcd for $C_{25}H_{34}O_9$ (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''-H_3), 1.36 (3H, s, 5''-H_3), 1.42, 1.46 (3H each, both s, 2'-*gem*-CH_3), 2.17 (3H, s, 2'''-H_3), 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).

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14 **1b**: ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.17 (3H, d, *J* = 6.3 Hz, 4''-H_3), 1.33 (3H, s, 5''-H_3), 1.38, 1.45 (3H each, both s), 1.42, 1.46 (3H each, both s, 2'-*gem*-CH_3), 2.14 (3H, s, 2'''-H_3), 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).

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16 **1c**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.26 (3H, d, J = 6.1 Hz, 4''-H_3), 1.36 (6H, s, 2'-*gem*-CH_3), 1.40 (3H, s, 5''-H_3), 2.12 (3H, s, 2'''-H_3), 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**: ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.36 (3H, d, J = 6.4 Hz, 4''-H_3), 1.30 (3H, s, 5''-H_3), 1.32, 1.34 (3H each, both s, 2'-*gem*-CH_3), 2.14 (3H, s, 2'''-H_3), 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).

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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) $Å^3$, space group: $P2_12_12_1$ (#19), Z = 4, μ(CuKα) = 7.54 cm^{-1}, temperature: 23.0 °C, structure solution: direct methods (SHELXS-86), residuals: R = 0.095, Rw = 0.152, $R1$ = 0.048, goodness of fit indicator: 1.16. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu-Kα (λ=1.54178 Å) radiation and a rotating anode generator.

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