New Phenolic Components from *Daphne giraldii*

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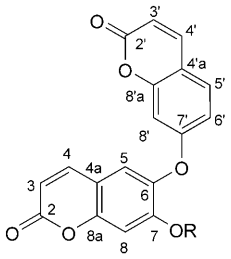
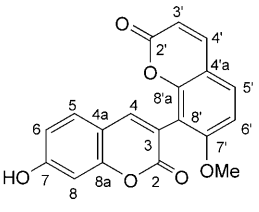
Two new bis-coumarins, namely daphnogitin (**1**), daphnogirin (**2**), and a flavan-3-ol derivative, 5-*O*-methylafzelechin (**3**), together with eleven known compounds, daphnetoxin, 1,2-dihydrodaphnetoxin, daphnetin, daphnoretin, daphneticin, isodaphneticin, *trans*-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-9*H*-pyrano[*2*,*3*-*f*]-1,4-benzodioxin-9-one (=demethoxyisodaphneticin; **4b**), (+)-pinoresinol, acuminatin, (−)-dihydrosesamin, and isosalicifoline, were isolated from the leaves and stems of *Daphne giraldii*. Compound **4b** was isolated for the first time as a natural product, and 1,2-dihydrodaphnetoxin, daphneticin, isodaphneticin, (+)-pinoresinol, acuminatin, (−)-dihydrosesamin, and isosalicifoline were isolated for the first time from *Daphne giraldii*. The structures of the three new compounds were elucidated by spectroscopic analysis.

Introduction. – The genus *Daphne* is distinguished for producing a wide variety of biologically active secondary metabolites including diterpenoids [1], coumarins [1e][2], flavonoids [3], lignans [1e][2b][4], and coumarinolignans [5]. *Daphne giraldii* NITSCHE (Thymelaeaceae) is a tiny shrub distributed mainly in central and western China [6]. The stems and roots of this plant used historically as an abortifacient in the traditional Chinese medicine (TCM) are applied in the treatment of trauma, rheumatoid arthritis, and bronchitis [6]. Previous chemical studies on this plant reported the isolation of diterpenoids [7], monomeric coumarins [8], bis-coumarins [8], flavonoids [8b], bis-flavonoids [9], and other phenolic compounds [10]. In the current investigation, two new bis-coumarins, daphnogitin (**1**) and daphnogirin (**2**), and one new flavan-3-ol derivative, 5-*O*-methylafzelechin (**3**), together with eleven known compounds including daphnetoxin [11], 1,2-dihydrodaphnetoxin [12], daphnetin [13], daphnoretin [14], daphneticin [15], isodaphneticin (**4a**) [16], *trans*-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-9*H*-pyrano[*2*,*3*-*f*]-1,4-benzodioxin-9-one (=demethoxyisodaphneticin; **4b**) [17], (+)-pinoresinol [18], acuminatin [19], (−)-dihydrosesamin [20], and isosalicifoline [21] were isolated from the leaves and stems of *D. giraldii*. Compound **4b** was obtained for the first time from a natural source, and 1,2-dihydrodaphnetoxin, daphneticin, isodaphneticin, (+)-pinoresinol, acuminatin, (−)-dihydrosesamin, and isosalicifoline were isolated for the first time from *D. giraldii*. We report herein the isolation and structural elucidation of the three new compounds by spectroscopic analysis.

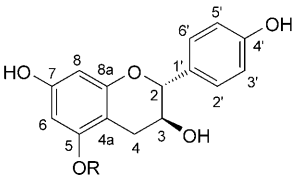
Results and Discussion. – The EtOH extract of the leaves and stems of *D. giraldii* was suspended in H_2O and was successively partitioned with petroleum ether, AcOEt, and BuOH. The AcOEt-soluble fraction was subjected to column chromatography and prep. TLC to give three new compounds **1**–**3** and eleven known compounds.



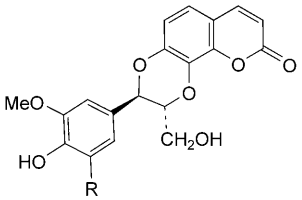
1 R = H daphnogitin
1a R = Me lasiocephalin



2 daphnogirin



3 R = Me 5-*O*-methylafzelechin
3a R = H afzelechin



4a R = MeO
b R = H

Daphnogitin (**1**) was isolated as an amorphous white powder, and was determined to have the molecular formula $C_{18}H_{10}O_6$ on the basis of HR-EI-MS. IR Absorptions at 3433, 1726, 1684, 1618, and 1560 cm^{-1} were indicative of the existence of OH, C=O, and aromatic groups, respectively. The typical UV absorptions at 327 and 293 nm suggested the presence of a coumarin analogue [22]. Analysis of the 1D and 2D NMR data enabled us to elucidate the structure of **1** as 7-hydroxy-6-[(2-oxo-2*H*-1-benzopyran-7-yl)oxy]-2*H*-1-benzopyran-2-one.

In the ^{1}H-NMR spectrum of **1** (*Table 1*), two characteristic spin systems at δ 6.26 and 7.91 (2*d*, $J=9.4$ Hz) and at δ 6.34 and 8.01 (2*d*, $J=9.5$ Hz) were assigned to H–C(3) and H–C(4), respectively, of two coumarin moieties, suggesting that **1** is a bis-coumarin derivative. The remaining aromatic-proton signals in the ^{1}H-NMR, an *ABX* spin system (δ 7.67 (*d*, $J=8.6$ Hz), 6.91 (*dd*, $J=8.6$, 1.8 Hz), and 6.84 (*d*, $J=1.8$ Hz) and 2*s* (δ 7.51 and 6.92), indicated a 6,7-disubstitution pattern for one of the two coumarin moieties and a 6′- or 7′-substitution pattern for the other. Analysis of the ^{13}C-NMR data (*Table 1*) and the molecular formula suggested the presence of a 6- or 7-OH group and the connection of the two coumarin moieties *via* an ether bond. Comparison of the ^{1}H-NMR data of **1** with those of the known bis-coumarin lasiocephalin (**1a**) ($\Delta\delta$(H–C(5))$=-0.07$, $\Delta\delta$(H–C(8))$=-0.36$, $\Delta\delta$(H–C(6′))$=+0.01$, and $\Delta\delta$(H–C(8′))$+0.01$, in the same solvent) [23], which was isolated from the root bark of *Lasiosiphon eriocephalus* (Thymelaeaceae), suggested the presence of a 7-OH group and a C(6)–O–C(7′) ether linkage between the two coumarin moieties of **1**. Extensive analysis of the HMQC, HMBC (*Table 2* and *Fig. 1*), and NOESY (*Fig. 2*) data further confirmed the structural assignment of **1**, and enabled a complete assignment of the ^{1}H- and ^{13}C-NMR data for **1**. In particular, HMBC correlations of both H–C(5′) and H–C(6′) with C(7′) (*Fig. 1*) were indicative of a 7′-substitution pattern for one coumarin moiety, while the NOESY correlation between H–C(5) and H–C(8′) (*Fig. 2*) revealed a 7-OH group and a C(6)–O–C(7′) ether linkage of the two coumarin moieties.

Daphnogirin (**2**), a white amorphous powder, had the molecular formula $C_{19}H_{12}O_6$ as derived from its HR-EI-MS. The UV and IR spectra showed that **2** possessed the

Table 1. *^{1}H- and ^{13}C-NMR Data for Compounds* **1**–**3**. δ in ppm, J in Hz.

	Daphnogitin (**1**)[a])		Daphnogirin (**2**)[a])		5-*O*-Methylafzelechin (**3**)[b])	
	δ(H)[c])	δ(C)[d])	δ(H)[c])	δ(C)[d])	δ(H)[c]	δ(C)[d])
H–C(2)		160.3		159.1	4.59 (*d*, *J* = 7.8)	83.3
H–C(3)	6.26 (*d*, *J* = 9.4)	112.0		114.5	3.97 (*ddd*, *J* = 8.4, 7.8, 5.5)	69.2
H–C(4) or CH_2(4)	7.91 (*d*, *J* = 9.4)	144.0	7.98 (*s*)	144.9	2.48 (*dd*, *J* = 16.3, 5.5, H_α), 2.84 (*dd*, *J* = 16.3, 8.4, H_β)	29.2
C(4a)		110.9		111.3		102.3
H–C(5)	7.51 (*s*)	121.2	7.58 (*d*, *J* = 8.5)	129.9		160.6
H–C(6)		138.8	6.85 (*dd*, *J* = 8.5, 2.2)	113.5		93.3
C(7)		154.0		161.6	6.02 (*d*, *J* = 2.0)	158.7
H–C(8)	6.92 (*s*)	104.2	6.81 (*d*, *J* = 2.2)	102.0		96.9
C(8a)		152.6		155.2	5.93 (*d*, *J* = 2.0)	157.2
C(1′)						131.9
H–C(2′)		160.0		159.9		130.1
H–C(3′)	6.34 (*d*, *J* = 9.5)	113.4	6.31 (*d*, *J* = 9.5)	112.6	7.20 (*d*, *J* = 8.5)	116.6
H–C(4′)	8.01 (*d*, *J* = 9.5)	144.1	8.06 (*d*, *J* = 9.5)	144.6	6.78 (*d*, *J* = 8.5)	158.9
C(4′a)		113.7		112.6		
H–C(5′)	7.67 (*d*, *J* = 8.6)	129.8	7.79 (*d*, *J* = 8.7)	129.7	6.78 (*d*, *J* = 8.5)	116.6
H–C(6′)	6.91 (*dd*, *J* = 8.6, 1.8)	112.9	7.20 (*d*, *J* = 8.7)	108.4	7.20 (*d*, *J* = 8.5)	130.1
H–C(7′)		160.9		160.1		
H–C(8′)	6.84 (*d*, *J* = 1.8)	103.0		111.6		
H–C(8′a)		155.0		152.4		
MeO			3.85 (*s*)	56.5	3.75 (*s*)	56.3

[a]) In (D_6)DMSO. [b]) In CD_3OD. [c]) At 500 MHz. [d]) At 125 MHz.

Table 2. *^{1}H,^{13}C-HMBC Correlations for Compounds* **1**–**3**[a]).

	Daphnogitin (**1**)[b])	Daphnogirin (**2**)[b])	5-*O*-Methylafzelechin (**3**)[c])
H–C(2)			C(3), C(4), C(8a), C(1′), C(2′), C(6′)
H–C(3)	C(2), C(4a)		C(2), C(4), C(4a), C(1′)
H–C(4) or C*H*$_2$(4)	C(2), C(5), C(8a)	C(2), C(5), C(8a), C(8′)	C(2), C(3), C(4a), C(5), C(8a)
H–C(5)	C4), C(6), C(7), C(8a)	C(4), C(6), C(7), C(8a)	
H–C(6)		C(4a), C(7), C(8)	C(4a), C(5), C(7), C(8)
H–C(8)	C(4a), C(6), C(7), C(8a)	C(6), C(7), C(8a)	C(4a), C(6), C(7), C(8a)
H–C(2′)			C(2), C(1′), C(3′), C(4′), C(6′)
H–C(3′)	C(2′), C(4a′)	C(2′), C(4a′)	C(1′), C(4′), C(5′)
H–C(4′)	C(2′), C(5′), C(8a′)	C(2′), C(5′), C(8a′)	
H–C(5′)	C(4′), C(7′), C(8a′)	C(4′), C(7′), C(8a′)	C(1′), C(4′), C(6′)
H–C(6′)	C(7′), C(8′), C(4a′)	C(4a′), C(8′)	C(2), C(1′), C(2′), C(4′), C(5′)
H–C(8′)	C(4a′), C(6′), C(7′), C(8a′)		
C*H*$_3$O		C(7′)	C(5)

[a]) At 500 MHz. [b]) In (D_6)DMSO. [c]) In CD_3OD.

same functionalities as **1**. Analysis of the ^{1}H-NMR data (*Table 1*) suggested the structure of a bis-coumarin derivative also for **2**, which was supported by the presence of ^{13}C-NMR signals (*Table 1*) for 18 sp^2 C-atoms and 1 MeO group (δ 56.5). The structure of **2** was finally elucidated as 7-hydroxy-3-(7-methoxy-2-oxo-2*H*-1-benzopyran-8-yl)-2*H*-1-benzopyran-2-one by spectral methods, especially 1D and 2D NMR.

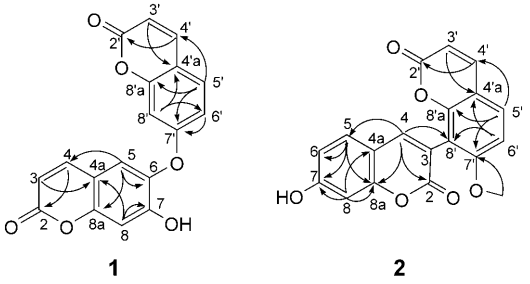


Fig. 1. *Selected HMBC correlations* (→) *of* **1** *and* **2**

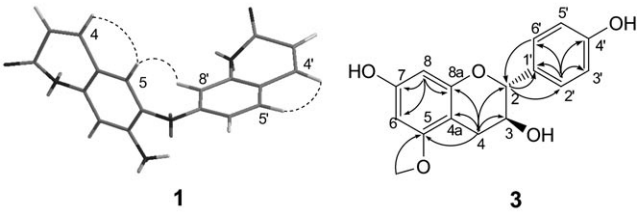


Fig. 2. *Key NOESY correlations* (···) *of* **1** *and selected HMBC correlations* (→) *of* **3**

In the ^{1}H-NMR spectrum of **2** (*Table 1*), two *d* at δ 6.31 and 8.06 ($J = 9.5$ Hz, each 1 H) were assigned to H–C(3′) and H–C(4′), respectively, of one coumarin moiety, and a *s* at δ 7.98 was attributed to H–C(4) of another 3-substituted coumarin moiety, indicating that **2** possessed a bis-coumarin skeleton. An *ABX* spin system (δ 7.58 (*d*, $J = 8.5$ Hz), 6.85 (*dd*, $J = 8.5$, 2.2 Hz), and 6.81 (*d*, $J = 2.2$ Hz)) suggested that one coumarin moiety was 6- or 7-substituted, and the 2*d* at δ 7.79 and 7.20 (each $J = 8.7$ Hz) indicated a 5,6- or 7,8-disubstitution pattern for the other one. An aromatic MeO group at δ 3.85 was also observed. Two relatively upfield-shifted signals of quaternary C-atoms at δ 114.5 (C(3)) and 111.6 (C(8′)) indicated that the two coumarin moieties were linked through a C(3)–C(8′) bond. A down-field-shifted quaternary-C signal at δ 161.6, assigned to C(7), was only compatible with the presence of an OH–C(7), in accord with the molecular formula. The structure of **2** was confirmed by the HMBC experiment (*Fig. 1*). Thus the correlations of H–C(4) and H–C(6′) with C(8′) established the existence of a C(3)–C(8′) bond, while the correlation between CH_3 and C(7′) was in accord with the location of the MeO group at C(7′). The presence of OH–C(7) was also verified by the correlations of H–C(5) and H–C(8) with C(7).

The 5-*O*-methylafzelechin (**3**), an optically active amorphous powder, had a molecular formula $C_{16}H_{16}O_5$ as determined by HR-EI-MS (M^+ at *m/z* 288.0993). The IR spectrum of **3** showed absorptions for OH groups (3396 cm^{-1}) and aromatic rings (1618 and 1520 cm^{-1}). Analysis of the ^{1}H- and ^{13}C-NMR data (*Table 1*) suggested that **3** was an *O*-methylated derivative of afzelechin (**3a**), its 16 C-atoms giving rise to ^{13}C-NMR signals of 12 aromatic and 3 aliphatic C-atoms (2 OCH and 1 CH_2) besides that of an aromatic MeO group. The location of the MeO group was determined by a HMBC experiment, and the configuration of **3** was established as (2*R*,3*S*)-3,4-dihydro-2-(4-hydroxyphenyl)-5-methoxy-2*H*-1-benzopyran-3,7-diol by its CD spectrum.

In the ^{1}H-NMR spectrum of **3**, a 1,4-disubstituted (δ 7.20, (*d*, $J = 8.5$ Hz, 2 H), and 6.78 (*d*, $J = 8.5$ Hz, 2 H)) and a 1,2,3,5-tetrasubstituted benzene ring (δ 6.02 (*d*, $J = 2.0$ Hz, 1 H) and 5.93 (*d*, $J = 2.0$ Hz, 1 H)) were distinguished, besides a MeO group (δ 3.75) and a spin system $CH_2CH(OH)CH(O)$– (δ 2.48 (*dd*, $J = 16.3$, 8.4 Hz),

2.84 (*dd*, *J* = 16.3, 5.5 Hz), 3.97 (*ddd*, *J* = 8.4, 7.8, 5.5 Hz), and 4.59 (*d*, *J* = 7.8 Hz)). The aforementioned spectral data clearly indicated that **3** possessed a 5,7,4′-trioxygenated flavan-3-ol skeleton. The large coupling constant (*J* = 7.8 Hz) between H–C(2) and H–C(3) was in accordance with their *trans* configuration. The above observations were consistent with the structure of an *O*-methylated derivative of afzelechin [24] for **3**. The HMBC correlations (*Fig. 2*) not only allowed to locate the MeO group at C(5) by the key correlation between CH_3 and C(5) but were also useful for the complete assignment of the ^{1}H- and ^{13}C-NMR data. A negative *Cotton* effect at 274 nm of **3**, which is very similar to that of afzelechin (**3a**) [24], established the (2*R*)-configuration.

Among the known compounds, daphnetin [13] and daphnoretin [14] were identified by their ^{1}H-NMR and MS data and comparison with authentic samples (co-TLC). Daphnetoxin [11] and 1,2-dihydrodaphnetoxin [12] were identified by comparison of their ^{1}H- and ^{13}C-NMR and MS data with those reported, and the structure of *trans*-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-9*H*-pyrano[*2*,*3-f*]-1,4-benzodioxin-9-one (**4b**) was determined by comparison of its ^{1}H-NMR, MS, and optical rotation data with those of the synthetic compound [17]. Daphneticin [15], isodaphneticin [16], (+)-pinoresinol [18], acuminatin [19], (−)-dihydrosesamin [20], and isosalicifoline [21] were identified by comparison of their ^{1}H-NMR, ^{13}C-NMR, MS, and optical rotation data with the reported ones.

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Experimental Part

General. All solvents used were of anal. grade (*Shanghai Chemical Plant*, Shanghai, P. R. China). Column chromatography (CC): silica gel (200–300 mesh), silica gel *H60*, *C18* reversed-phase silica gel (250 mesh, *Merck*), and *MCI* gel (*CHP20P*, 75–150 μ; *Mitsubishi Chemical Industries, Ltd.*). TLC: precoated silica gel *GF254* plates (*Qingdao Haiyang Chemical Plant,* Qingdao, P. R. China). Optical rotation: *Perkin-Elmer-341* polarimeter. UV Spectra: *Shimadzu UV-210A*; λ_{max} (log ε) in nm. CD Spectrum: *Jasco-J-810* instrument; λ_{max} ($\Delta\varepsilon$) in nm. IR Spectra: *Perkin-Elmer-577* spectrometer; in cm^{-1}. NMR Spectra: *Avance-Unity-Inova-500* spectrometer; $SiMe_4$ as internal standard. EI-MS (70 eV): *Finnigan-MAT-95* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The leaves and stems of *Daphne giraldii* NITSCHE were collected in September 2003 in the area of Shanxi province of the Qinling Mountains, P. R. China. The plant was authenticated by Professor *Xiao-An Wang*, School of Biology, Shanxi Normal University. A voucher specimen has been deposited in the Shanghai Institute of Materia Medica (accession number: Daphne-gir-2003–1Y).

Extraction and Isolation. The powder of the leaves and stems of *D. giraldii* (1.7 kg) was percolated with 95% EtOH. Evaporation of the EtOH left a greenish extract (175.5 g), which was subsequently suspended in H_2O (1 l) and extracted successively with petroleum ether, AcOEt, and BuOH. The AcOEt-soluble fraction (51.5 g) was fractionated by CC (silica gel, petroleum ether/Me_2CO 20:1 → 0:1) to give *Fractions 1–3*. *Fr. 1* (2.1 g) was subjected to CC (silica gel, petroleum ether/AcOEt 4:1) and purified by prep. TLC (silica gel, $CHCl_3$/MeOH 100:1): *(−)-dihydrosesamin* (7 mg). *Fr. 2* (14.5 g) was fractionated by CC (*MCI* gel, 25% → 100% MeOH/H_2O): *Fr. 2a–2c*. *Fr. 2a* (3.5 g) was subjected to CC (silica gel, $CHCl_3$/MeOH 80:1 → 20:1): *daphnetin* (20 mg), *daphnoretin* (205 mg), and *5*-O-*methylafzelechin* (**3**; 9 mg). *Fr. 2b* (496 mg) was separated by CC (silica gel, $CHCl_3$/MeOH 100:1 → 20:1): *Fr. 2b.1–2b.3*. *Fr. 2b.1* (136 mg) was further separated by CC (silica gel, petroleum ether/AcOEt 3:1 → 1:1): *(+)-pinoresinol* (20 mg) and *isosalicifoline* (9 mg). *Fr. 2b.2* (65 mg) was purified by CC (silica gel, $CHCl_3$/AcOEt 8:1): *daphnogitin* (**1**; 5 mg) and *acuminatin* (6 mg). Separation of *Fr. 2b.3* (116 mg) by CC (silica gel, $CHCl_3$/Me_2CO 8:1 and 6:1) provided *isodaphneticin* (**4a**; 24 mg), *trans*-2,3-dihydro-3-(4-hydroxy-3-methoxyphenyl)-2-(hydroxymethyl)-9*H*-pyrano[*2*,*3-f*]-1,4-benzodioxin-9-one (**4b**; 13 mg), and *daphnogirin* (**2**; 6 mg). *Fr. 2c* (652 mg) was subjected to CC (silica gel, $CHCl_3$/MeOH 100:1 → 50:1): *daphnetoxin* (20 mg) and *1,2-dihydrodaphnetoxin* (3 mg). *Fr. 3* (2.6 g) was first decolorized by CC (*MCI* gel, MeOH/H_2O 7:3) and then purified by CC (*Sephadex LH-20*, MeOH): *daphneticin* (8 mg).

Daphnogitin (=*7-Hydroxy-6-[(2-oxo-2*H*-1-benzopyran-7-yl)oxyl]-2*H*-1-benzopyran-2-one*; **1**): White powder. UV (MeOH): 327 (4.54), 293 (4.31), 202 (4.96). IR (KBr): 3433, 2920, 2850, 1726, 1684, 1618, 1560, 1394, 1286, 1259, 1157, 1132, 995, 835. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 322 (100, M^+), 294 (40), 266 (8), 149 (14), 118 (13). HR-EI-MS: 322.048 (M^+, $C_{18}H_{10}O_6^+$; calc. 5322.0477).

Daphnogirin (=*7-Hydroxy-3-(7-methoxy-2-oxo-2*H*-1-benzopyran-8-yl)-2*H*-1-benzopyran-2-one*; **2**). White powder. UV (MeOH): 325 (3.97), 247 (3.64), 202 (4.37). IR (KBr): 3427, 2918, 2850, 1711, 1605, 1560, 1495, 1460, 1294, 1277, 1240, 1092, 1049, 837. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 336 (100, M^+), 319 (22), 308 (49), 279 (29), 265 (56), 237 (27). HR-EI-MS: 336.0643 (M^+, $C_{19}H_{12}O_6^+$; calc. 336.0634).

*5-*O*-Methylafzelechin* (=*(2*R*,3*S*)-3,4-Dihydro-2-(4-hydroxyphenyl)-5-methoxy-2*H*-1-benzopyran-3,7-diol*; **3**): White powder. $[\alpha]_D^{20} = -15$ ($c = 0.13$, MeOH). UV (MeOH): 310 (2.58), 274 (3.43), 202 (4.61). CD (MeOH): (274) (−0.76). IR (KBr): 3396, 2922, 2850, 1618, 1520, 1475, 1437, 1350, 1207, 1124, 1026, 820. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 289 (5), 288 (13, M^+), 154 (18), 153 (100), 136 (18), 107 (24). HR-EI-MS: 288.0993 (M^+, $C_{16}H_{16}O_5^+$; calc. 288.0998).

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