New Neolignans from *Spiraea formosana*

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Phytochemical investigation on the ethanol extract from the stems of *Spiraea formosana* has resulted in the isolation of four new neolignans, named spiraformin-A, -B, -C and -D (1—4), together with thirty five known compounds. Their structures were established primarily on the basis of 1D and 2D NMR spectral and chemical transformation methods.

Key words *Spiraea formosana*; Rosaceae; neolignan; methylation; Gibb's test

Spiraea formosana HAYATA is an endemic shrub of Rosaceae distributed widely in high altitudes of forests in central Taiwan.[1)] The young leaves, fruits and roots of *Spiraea* species have been used as diuretic, detoxicant, and analgesic agents and for the treatment of inflammation, cough, headache and toothache in traditional Chinese medicine.[2,3)] Roots of *Spiraea* species are also known to use traditionally for the treatments of malaria, fever and emetic conditions.[4,5)] Some of *Spiraea* species reported to exhibit potent antiplatelet aggregation activities induced by PAF and AA,[6)] and also inhibition of generation of nitric oxide and superoxide in RAW 264.7 cells.[7,8)] Several *Spiraea* species of Japan origin were reported to have high allelopathic potential.[9)] Plants in this genus are known to produce various diterpene alkaloids of atisine and hetisine types,[10—17)] and some of them significantly inhibited rabbit platelet aggregation induced by PAF and AA *in vitro* and *ex vivo*.[6)] Some of these diterpene alkaloids also exhibited protective effects on cerebral ischemia-reperfusion injury in gerbils.[18)] The members of *Spiraea* are also shown to contain benzaldehydes, cinnamoyl glucosides, flavonoids, terpenoids and terpenoid glycosides.[19—23)] An acetylated derivative of a unique terpene glycoside, prunioside A from *Spiraea* species is reported to inhibit nitric oxide production in murine macrophage-like RAW 264.7 cells.[24)] However, little is known concerning the chemical constituents of *S. formosana*. As a part of serial studies on the Formosan endemic plants, we have undertaken the investigation of the chemical components of the stems of *S. formosana* and isolated thirty nine compounds including four new neolignans. Details of the isolation and structural determination of new neolignans (**1**—**4**) are presented here.

Results and Discussion

Fresh stems of *S. formosana* were extracted with hot ethanol. The water suspension of the original ethanol extract was subjected to a liquid–liquid partition to obtain $CHCl_3$, *n*-BuOH and water subfractions. Four new biphenyl ether neolignans together with thirty five known compounds were separated from these subfractions by a combination of chromatographic techniques. New neolignans were characterized as dimers of *trans*-4-hydroxycinnamic acid, an important lignin precursor, which were isolated for the first time from the natural sources. However, Katase *et al.* reported the transformation of *trans*-4-hydroxycinnamic acid by a laccaase of the fungus *Trametes versicolor* to its oxidative coupling compound or dimer.[25)]

Spiraformin A (**1**), a colorless syrup was shown to have a molecular formula of $C_{20}H_{20}O_6$ by high resolution electron impact mass spectrum (HR-EI-MS), ($[M]^+$, *m*/*z* 356.1259) which was consistent with ^{13}C-NMR and DEPT experiments. The UV spectrum of **1** in MeOH showed absorption maxima at 223 and 313 nm characteristic of cinnamoyl chromophore.[26)] The IR absorption bands at 3426 and 1710 cm^{-1} indicated the presence of hydroxyl and carbonyl functions, respectively. A 1,3,4-trisubstituted phenyl group was observed in the ^{1}H- and ^{13}C-NMR spectra [ABX system at δ 6.98 (1H, d, J=2.0 Hz, H-2), 7.03 (1H, d, J=8.0 Hz, H-5), and 7.20 (1H, dd, J=8.0, 2.0 Hz, H-6), and δ 116.0 (C-2), 116.5 (C-6), 125.2 (C-5), 127.6 (C-1), 149.4 (C-3) and 154.5 (C-4)] and the attachment of a –CH=CH–$COOCH_3$ group to the aromatic nucleus was evidenced by signals at δ 6.17 (1H, d, J=16.0 Hz, H-8), 7.52 (1H, d, J=16.0 Hz, H-7) and 3.67 (3H, s, OMe-10); δ 144.3 (C-7), 115.8 (C-8), 173.3 (C-9) and 51.8 (OCH_3-10), and ^3J-HMBC connectivities from H-7 to C-2 and from OCH_3-10 to C-9, thus confirming the presence of a phenyl propenoyl derivative. The ^{1}H- and ^{13}C-NMR spectra of **1** also revealed the presence of a *para* substituted phenyl group [A_2B_2 system at δ 6.96 (2H, d, J=8.4 Hz, H-3′, -5′) and 7.21 (2H, d, J=8.4 Hz, H-2′, -6′), δ 118.4 (C-3′, -5′), 130.1 (C-2′, -6′), 136.8 (C-1′) and 144.6 (C-4′)] to which is attached a –$CH_2CH_2COOCH_3$ group [δ 2.65 (2H, t, J=8.0 Hz, H-8′), 2.96 (2H, t, J=8.0 Hz, H-7′) and 3.75 (3H, s, OCH_3-10′); δ 29.9 (C-7′), 35.9 (C-8′), 51.9 (OCH_3-10′) and 173.5 (C-9′)] as evidenced by HMBCs from H-7′ to C-2′, C-1′, and C-9′. These data indicated the presence of a second phenylpropanoyl moiety leading to **1** being a neolignan. These two moieties were connected with ether linkage was suggested by the molecular formula and the two oxygenated quaternary aromatic carbons at δ 144.6 (C-4′) and 149.4 (C-3). The negative Gibb's test for compound **1** indicated that the *para*-position of free hydroxyl group was substituted with the olefinic fragment, so that C-3 should be involved in ether linkage. To further confirm the position of free hydroxyl group and thus the site of ether linkage, compound **1** was methylated with iodomethane in acetone solution. The resulting compound exhibited one more methoxy group at δ 3.67 (3H, s). This methoxy signal displayed NOESY correlation with the proton signal at δ 7.03 (H-5)

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Table 1. ^{1}H- and ^{13}C-NMR Data of Compounds **1**—**4**[a)]

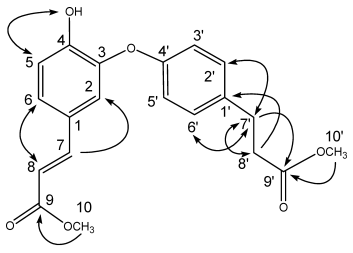
	1		**2**		**3**		**4**[b)]	
	H	C	H	C	H	C	H	C
1		127.6		126.3		127.3		109.5
2	6.98 (1H, d, 2.0)	116.0	6.98 (1H, d, 2.0)	116.3	6.98 (1H, d, 2.0)	116.3	7.16 (1H, d, 2.0)	119.6
3		149.4		144.4		146.5		146.7
4		154.5		149.3		150.2		150.8
5	7.03 (1H, d, 8.0)	125.2	7.03 (1H, d, 8.0)	123.1	7.03 (1H, d, 8.0)	123.1	7.28 (1H, d, 8.0)	117.2
6	7.20 (1H, dd, 8.0, 2.0)	116.5	7.20 (1H, dd, 8.0, 2.0)	118.8	7.20 (1H, dd, 8.0, 2.0)	118.9	7.36 (1H, dd, 8.0, 2.0)	124.8
7	7.52 (1H, d, 16.0)	144.3	7.52 (1H, d, 16.0)	138.2	7.52 (1H, d, 16.0)	139.2	7.55 (1H, d, 16.0)	144.0
8	6.17 (1H, d, 16.0)	115.8	6.17 (1H, d, 16.0)	114.5	6.17 (1H, d, 16.0)	112.1	6.30 (1H, d, 16.0)	116.4
9		167.4		167.7		170.3		167.9
10	3.67 (3H, s)	51.8	3.75 (3H, s)	51.3	3.75 (3H, s)	51.9		
1′		136.8		134.3		136.2		137.4
2′, 6′	7.21 (2H, d, 8.4)	130.1	7.21 (2H, d, 8.4)	130.1	7.21 (2H, d, 8.4)	130.2	7.21 (2H, d, 8.4)	129.4
3′, 5′	6.96 (2H, d, 8.4)	118.4	6.96 (2H, d, 8.4)	119.9	6.96 (2H, d, 8.4)	119.9	6.87 (2H, d, 8.4)	117.7
4′		144.6		142.5		144.3		155.7
7′	2.96 (2H, t, 8.0)	29.9	2.96 (2H, t, 8.0)	30.4	2.96 (2H, t, 8.0)	30.6	2.87 (2H, t, 8.0)	31.7
8′	2.65 (2H, t, 8.0)	35.9	2.65 (2H, t, 8.0)	35.2	2.65 (2H, t, 8.0)	35.9	2.44 (2H, t, 8.0)	39.6
9′		173.5		173.5		175.4		172.3
10′	3.75 (3H, s)	51.9	4.13 (2H, q, 7.2)	51.3	4.09 (2H, t, 6.8)	59.5		
11′			1.25 (3H, t, 7.2)	27.0	1.61 (2H, m)	26.8		
12′					1.55 (2H, m)	22.7		
13′					0.92 (3H, t, 7.4)	19.1		
1″							5.02 (1H, d, 7.6)	100.8
2″							3.55—3.86 (5H, m)	73.6
3″								76.7
4″								70.0
5″								77.3
6″								61.2

a) Chemical shifts are shown as δ values recorded by 400 MHz NMR in $CDCl_3$ with reference to tetramethylsilane (TMS), and coupling constants (*J*) are expressed in Hertz (Hz). Signal multiplicities are represented by s (singlet), br (broad), d (doublet), t (triplet), q (quartet), dd (doublets of doublet), and m (multiplet). *b*) In CD_3OD.

confirmed that the free hydroxyl group was located at C-4 and thus carbons C-3 and C-4′ were involved in the ether linkage between two phenylpropanoid moieties. Accordingly **1** was confirmed to be a new neolignan 3-*p*-methyldihydrocoumaroyloxy methyl-*p*-coumarate and assigned the trivial name spiraformin A.

The second lignan, compound **2** was isolated as a colorless syrup. It has the molecular formula of $C_{21}H_{22}O_6$ based on HR-EI-MS analysis ($[M]^+$, *m*/*z* 370.1414) which was 14 mass units more than that of **1**. The UV absorption maxima and IR absorption bands of **2** were similar to those in **1** at 230 and 285 nm, and 3374 and 1723 cm^{-1}, respectively, indicated that **2** has similar basic skeleton and functionalities. Analysis of the ^{1}H-NMR data showed that **2** had spectral data very similar to those of **1**, indicating a close structural relationship. In the ^{1}H-NMR spectrum of **1** and **2** the evident difference between them the signal of one methoxy group was changed to an ethoxy group [δ 1.25 (3H, t, J=7.2 Hz, CH_3-11′) and 4.13 (2H, q, J=7.2 Hz, CH_2-10′)]. The observation of ^{3}J-correlation between CH_2-10′ (δ 4.13) and C-9′ (δ 173.5) in the HMBC spectrum of **2** confirmed the connection of the ethoxy group with C-9′ of dihydrocoumaroyl moiety. Thus, **2** was determined to be 3-*p*-ethyldihydrocoumaroyloxy methyl-*p*-coumarate and was assigned the trivial name spiraformin B.

Spiraformin C (**3**) a colorless syrup had a molecular formula of $C_{23}H_{26}O_6$ as determined by HR-EI-MS ($[M]^+$, *m*/*z* 398.1731). The UV and IR spectra of **3** were also similar with those of **1**. Its NMR spectral data was very similar to those of **1** indicating the same basic skeleton for **3**. However,



1

Fig. 1. HMBC (→) and NOESY (↔) Correlations of Compound **1**

in compound **3**, a set of signals for *n*-butyloxy group was found at δ 0.92 (3H, t, J=7.4 Hz, CH_3-13′), 1.55 (2H, m, CH_2-12′), 1.61 (2H, m, CH_2-11′), and 4.09 (2H, t, J=6.8 Hz, CH_2-10′) instead of a methoxy signal. The evident difference in the molecular formula and the NMR signals between **3** and **1** concluded that one methyl ester group was changed to *n*-butyl ester group in **3**. The HMBC correlations of methylene protons at δ 4.09 (CH_2-10′) with C-9′ (δ 175.4) and CH_3-10 (δ 3.75) with C-9 (δ 170.3) confirmed that the *n*-butyl ester was connected with C-9′. Thus the structure of **3** was assumed to be 3-*p*-*n*-butyldihydrocoumaroyloxy methyl-*p*-coumarate and named as spiraformin C.

Compound **4** obtained as optically active colorless syrup, $[\alpha]_D^{25}$ −2.1°, was shown to have the molecular formula $C_{24}H_{26}O_{11}$ as deduced by the HR-FAB-MS (*m*/*z* 491.1554). The UV absorption maxima of **4** at 222 and 289 nm was typical of a cinnamoyl derivative.[26)] The IR absorption bands at 3464 and 1695 cm^{-1} suggested the existence of hydroxyl and

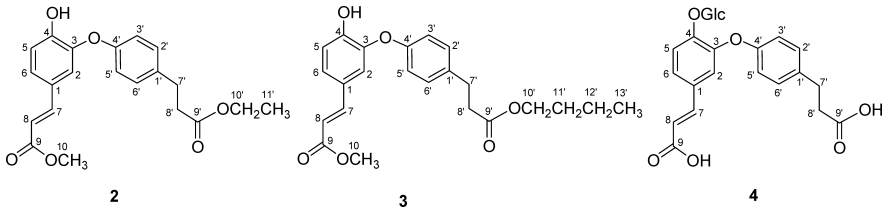


Fig. 2. Structures of **2**—**4**

carboxylic acid functionalities, respectively. The ^{1}H-NMR spectrum of **4** displayed an ABX system resonated at δ 7.16 (1H, d, J=2.0 Hz, H-2), 7.28 (1H, d, J=8.0 Hz, H-5), and 7.36 (1H, dd, J=8.0, 2.0 Hz, H-6); an A_2B_2 system at δ 6.87 (2H, d, J=8.4 Hz, H-3′, -5′) and 7.21 (2H, d, J=8.4 Hz, H-2′, -6′); two *trans*-coupled doublets at δ 6.30 (1H, d, J=16.0 Hz, H-8) and 7.55 (1H, d, J=16.0 Hz, H-7); and two mutually coupled triplets at δ 2.44 (2H, t, J=8.0 Hz, H-8′) and 2.87 (2H, t, J=8.0 Hz, H-7′) as in **1** indicating that the basic neolignan skeleton is same with that of **1**. Compound **4** differs from **1** by an anomeric proton signal at δ 5.02 (1H, d, J=7.6 Hz, H-1″), a five proton multiplet at δ 3.86—3.55 for β-D-glucopyranosyl unit and by lacking of two methoxy singlets. Accordingly, the ^{13}C-NMR spectrum of **4** revealed the signals for a glucose unit together with 18 carbon signals for the aglycone as expected. In the 2D analyses, the anomeric proton correlated to the aromatic doublet at δ 7.28 (H-5) in the NOESY spectrum, and exhibited ^3J HMBC correlation to C-4 (δ 150.8), confirmed the attachment of glucose moiety to the carbon C-4. Thus, **4** was concluded to be 3-dihydrocoumaroyloxy-4-*O*-β-D-glucopyranosyl-*p*-coumaric acid and trivially named as spiraformin D.

In addition, spiraeaine A (**5**),[27] β-sitosterol (**6**),[27] bakuchiol (**7**),[27] glutinol (**8**),[27] β-amyrin (**9**),[23] β-sitosterone (**10**),[28] mixture of β-sitosterol (**6**) and stigmasterol (**11**),[27] methyl vanillate (**12**),[29] methyl ferulate (**13**),[30] ethyl *p*-hydroxy-*trans*-cinnamate (**14**),[31] ethyl ferulate (**15**),[32] agrimonolide (**16**),[33] aurantiamide acetate (**17**),[34] β-sitosteryl glucoside (**18**),[27] nonadecyl ferulate (**19**),[27] nonadecyl-3-(4-hydroxyphenyl)propionate (**20**),[35] uridine (**21**),[36] 3-*O*-β-D-glucoside-*p*-vanillic acid (**22**),[37] vanillic acid (**23**),[26] *p*-hydroxybenzoic acid (**24**),[38] veratric acid (**25**),[38] syringic acid (**26**),[39] 3-*O*-β-D-glucoside-4′,5-dihydroxystilbene (**27**),[40] *p*-hydroxybenzaldehyde (**28**),[27] (−)-isolariciresinol-3a-*O*-β-D-glucopyranoside (**29**),[26] methyl quinate (**30**),[41] quercetin-3-*O*-β-D-glucopyranoside (**31**),[42] quercetin-3-*O*-β-D-galactopyranoside (**32**),[42] kaempferol-3-*O*-β-D-glucopyranoside (**33**),[42] *p*-coumaric acid (**34**),[42] 3,4′,5-trihydroxystilbene (**35**),[26] *p*-hydroxycinnamaldehyde (**36**),[43] quercetin (**37**),[42] (+)-5,7-dihydroxy-2-(3′,4′-methylenedioxyphenyl)chroman-4-one (**38**),[44] and *N*,*N*-dimethyladenine (**39**)[45] were also identified by comparison of their physical and spectral data with those reported in the literature.

Experimentaøl

General Experimental Methods Melting points were measured on Yanaco MP-S3 micro-melting point apparatus and uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained on the Bruker Avance-300 and AMX-400 NMR spectrometers, with tetramethylsilane (TMS) as internal standard. IR spectra were determined as KBr discs on a Shimadzu FTIR-8501 spectrophotometer, and UV spectra were recorded in MeOH on a Hitachi UV-3210 spectrophotometer. EI- and HR-EI-MS were measured with a 70 eV direct inlet system on a VG 70-250S spectrometer, and the FAB- and HR-FAB-MS were obtained on a Jeol JMS-700 spectrometer. Optical rotations were determined on a Jasco DIP-370 digital polarimeter.

Plant Material Stems of *S. formosana* was collected in Ilan Hsien, Taiwan, Republic of China, in 1991. A voucher specimen (Wu 1991010) is deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation The fresh stem (8.6 kg) of *S. formosana* was powdered and extracted with hot EtOH (10 L×7). The combined extracts were concentrated to give dark brown syrup (1.01 kg) and it was partitioned with $CHCl_3$ and *n*-BuOH, successively, to afford four individual portions: $CHCl_3$ layer (320 g), *n*-BuOH layer (220 g), H_2O layer (290 g), and residue (180 g). The condensed $CHCl_3$ solubles were subjected to alkaloids extraction using 3% HOAc and 5% NH_4OH to yield a crude alkaloid extract (9.2 g). It was chromatographed over silica gel and with a gradient of $CHCl_3$ and MeOH to afford 6 fractions. Fraction 5 was further purified by HPLC [Cosmosil 5C-18-AR-II Waters (5 μm)] with MeOH–H_2O (60 : 40) to give **5** (5.7 mg). The non-alkaloidal fraction was chromatographed on silica gel by eluting with gradient of *n*-hexane and EtOAc, to give 10 fractions. Fraction 2 was subjected to chromatography on a silica gel column with *n*-hexane and C_6H_6 (19 : 1) eluent followed by recrystallization of a subfraction obtained to give **6** (7.2 g). Fraction 3 was repeatedly column chromatographed over silica gel with *n*-hexane and $CHCl_3$ (9 : 1) to yield **7** (16.2 mg) and **8** (3.8 g). Recrystallization of fourth fraction with C_6H_6 afforded **9** (5.8 g). Fraction 5 on a silica gel column chromatography with a gradient of *n*-hexane and $(CH_3)_2CO$ afforded **10** (23.7 mg) and a mixture of **6** and **11** (4.8 g). Silica gel column chromatography of fraction 6 and followed by PTLC purification with the mixture of *n*-hexane and $(CH_3)_2CO$ (5 : 1) resulted in **3** (1.3 mg), **12** (13.6 mg), **13** (17.4 mg), **14** (47.6 mg), and **15** (67.8 mg). Fraction 7 on a silica gel column chromatography with C_6H_6 and $(CH_3)_2CO$ (19 : 1) followed by purification of subfractions 2 and 3 by PTLC with *n*-hexane and $(CH_3)_2CO$ (19 : 1) afforded **1** (9.8 mg) and **2** (1.1 mg). Purification of fraction 8 and 9 by repeated silica gel column chromatography using C_6H_6 and EtOAc (15 : 1), and $CHCl_3$ and MeOH (20 : 1) gave **16** (5.6 mg), **17** (20.5 mg), and **18** (36.3 mg), **19** (37.5 mg) and **20** (18.9 mg), respectively.

The *n*-BuOH layer (220 g) was subjected to column chromatography on Diaion HP-20, and eluted with a step gradient of H_2O and MeOH to give 12 fractions. Recrystallization of third fraction with MeOH afforded **21** (9.6 mg). Fraction 4 was separated by column chromatography on a silica gel column with a mixture of EtOAc and MeOH (8 : 1) saturated with H_2O to afford **22** (15.3 mg) and **23** (12.3 mg). Fraction 5 on a silica gel column chromatography with EtOAc and MeOH (10 : 1) saturated with H_2O followed by recrystallization of subfraction 3 in acetone gave **24** (17.5 mg). Purification of fraction 6 on silica gel column chromatography with EtOAc–MeOH–H_2O (15 : 1 : sat) eluent resulted in the isolation of **25** (18.3 mg) and **26** (21.6 mg). Fraction 7 was recrystallized to afford **27** (27.3 mg). A series of silica gel column chromatography of fraction 8 by $CHCl_3$–MeOH–H_2O (5 : 1 : sat) with a step gradient of MeOH yielded **28** (15.3 mg), **29** (208.5 mg), **30** (21.4 mg), **31** (98.3 mg), **32** (132.8 mg), and **33** (78.8 mg), successively. Compound **34** (26.1 mg) was obtained from fraction 9 on silica gel column chromatography using $CHCl_3$ and MeOH (10 : 1) saturated with H_2O. Fraction 10 was subjected to chromatography on a silica gel column repeatedly with EtOAc–MeOH–H_2O (15 : 1 : sat) to give **35** (17.8 mg) and **36** (7.8 mg). Recrystallization of fraction 11 with EtOAc–MeOH solvent system resulted **37** (13.2 mg). Repeated column chromatography of fraction 12 on silica gel by $CHCl_3$–MeOH–H_2O (8 : 1 : sat) yielded **4** (9.3 mg) and **38** (26.7 mg).

The water layer (290 g) was directly chromatographed on Diaion HP-20 column and eluted with a gradient of H_2O and MeOH to give 10 fractions. Among them, fraction 5 was further purified by HPLC [Cosmosil 5C-18-AR-II Waters (5 μm)] with MeOH–H_2O (40 : 60) to give **39** (17.2 mg).

Spiraformin A (**1**): Colorless syrup. ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3426, 2927, 1710, 1635, 1509, 1439, 1216. UV λ_{max} (MeOH) nm (log ε): 313, 223. HR-EI-MS m/z: 356.1259 (Calcd for $C_{20}H_{20}O_6$:

356.1260). EI-MS *m/z*: 356 (M^+), 325, 296, 284, 283, 281.

Spiraformin B (**2**): Colorless syrup. ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3375, 2923, 1723, 1604, 1510, 1443, 1220. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 286. HR-EI-MS *m/z*: 370.1414 (Calcd for $C_{21}H_{22}O_6$: 370.1416). EI-MS *m/z*: 370 (M^+), 363, 349, 329, 315, 283, 241, 226, 121.

Spiraformin C (**3**): Colorless syrup. ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3374, 2920, 1724, 1603, 1509, 1428, 1223. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 312, 222. HR-EI-MS *m/z*: 398.1713 (Calcd for $C_{23}H_{26}O_6$: 398.1729). EI-MS *m/z*: 398 (M^+), 365, 342, 316, 283, 121.

Spiraformin D (**4**): Colorless syrup. $[\alpha]_D^{25}$: $-2.1°$ (c=0.9, MeOH). ^{1}H- and ^{13}C-NMR: see Table 1. IR (KBr) cm^{-1}: 3464, 2366, 1695, 1626, 1424, 1211. UV λ_{max} (MeOH) nm ($\log \varepsilon$): 289, 222. HR-FAB-MS *m/z*: 491.1554 (Calcd for $C_{24}H_{27}O_{11}$: 491.1553). FAB-MS *m/z*: 491 ($[M+H]^+$), 490.

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