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 spiriformin-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 CHCl₃, *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 laccase of

the fungus *Trametes versicolor* to its oxidative coupling compound or dimer.²⁵⁾

Spiriformin A (1), a colorless syrup was shown to have a molecular formula of C₂₀H₂₀O₆ by high resolution electron impact mass spectrum (HR-EI-MS), ([M]⁺, *m/z* 356.1259) which was consistent with ¹³C-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 ¹H- and ¹³C-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–COOCH₃ 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 (OCH₃-10), and ³*J*-HMBC connectivities from H-7 to C-2 and from OCH₃-10 to C-9, thus confirming the presence of a phenyl propenoyl derivative. The ¹H- and ¹³C-NMR spectra of 1 also revealed the presence of a *para* substituted phenyl group [A₂B₂ 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 –CH₂CH₂COOCH₃ 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, OCH₃-10'); δ 29.9 (C-7'), 35.9 (C-8'), 51.9 (OCH₃-10') 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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Fig. 2. Structures of 2–4

carboxylic acid functionalities, respectively. The ^1H -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 spiraeformin 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.

Experimental

General Experimental Methods Melting points were measured on Yanaco MP-S3 micro-melting point apparatus and uncorrected. ^1H - 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 $\times$ 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 $(\text{CH}_3)_2\text{CO}$ 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 $(\text{CH}_3)_2\text{CO}$ (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 $(\text{CH}_3)_2\text{CO}$ (19:1) followed by purification of subfractions 2 and 3 by PTLC with *n*-hexane and $(\text{CH}_3)_2\text{CO}$ (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 (15:1:1 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 (5:1:1 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 (15:1:1 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 (8:1:1 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).

Spiraeformin A (1): Colorless syrup. ^1H - 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 $\text{C}_{20}\text{H}_{20}\text{O}_6$:

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

Spiraformin B (2): Colorless syrup. 1H - and ^{13}C -NMR: see Table 1. IR (KBr) cm^{-1} : 3375, 2923, 1723, 1604, 1510, 1443, 1220. UV λ_{max} (MeOH) nm (log ϵ): 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. 1H - and ^{13}C -NMR: see Table 1. IR (KBr) cm^{-1} : 3374, 2920, 1724, 1603, 1509, 1428, 1223. UV λ_{max} (MeOH) nm (log ϵ): 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). 1H - and ^{13}C -NMR: see Table 1. IR (KBr) cm^{-1} : 3464, 2366, 1695, 1626, 1424, 1211. UV λ_{max} (MeOH) nm (log ϵ): 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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