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NEW DITERPENOID ALKALOID FROM *SPIRAEA FORMOSANA*

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Abstract — Chemical investigation on the ethanol extract of stem of *Spiraea formosana* has resulted in the isolation of a new diterpenoid alkaloid, spiraeaine A (**1**), together with seven known compounds, bakuchiol (**2**), *p*-hydroxybenzaldehyde (**3**) , nonadecyl ferulate (**4**), glutinol (**5**), β-sitosteryl glucoside (**6**), mixture of β-sitosterol (**7**) and stigmasterol (**8**). Their structures were determined by the spectral analyses and by comparison with the literature values.

INTRODUCTION

Spiraea formosana Hayata (Rosaceae) is an endemic shrub distributed in high altitudes of forests in central Taiwan.[1] Many species of *Spiraea* genus have been extensively investigated for their chemical constituents, and showed to contain atisine type alkaloids, monoterpenoids, triterpenoids, and flavonoids.[2-5] However, only triterpenoids were reported from *S. formosana*.[6] Some of *Spiraea* species reported to have potent inhibition of platelet aggregation induced by PAF and AA[7] and also inhibition of

generation of nitric oxide and superoxide in RAW 264.7 cells.[8-9] In the course of our study on the constituents of medicinal plants, we have collected the stem of this species for phytochemical investigation. This paper deals with the isolation and structural elucidation of a new atisine type alkaloid.

RESULTS AND DISCUSSION

Spiraeaine A (**1**) gave a positive test with Dragendorff reagent, characteristic test of alkaloid. The molecular formula $C_{22}H_{33}NO_3$ was established by HR-EIMS (molecular ion peak at m/z 359.2462), and was further corroborated by its ^{13}C-NMR, which showed signals for all the 22 carbons, including one methyl, eleven methylenes, six methines, and four quartenary carbons. The IR absorption band at 3358 and 1651cm^{-1} indicated the presence of hydroxyl group and C-C double bond, respectively. Comparison of the ^{1}H and ^{13}C-NMR spectral data of **1** with those of spiramine X (**9**)[10] showed that these two compounds possess similar skeleton with different functionalities. The presence of a slightly downfield methylene protons (δ 2.35, and 2.69) and carbon (δ_C 54.5) and the absence of carbonyl carbon suggested that the carbonyl group of spiramine X was replaced by a methylene group at C-19 position. It was further confirmed by the ^{3}J correlation of H-5 (δ 1.25) with C-19 (δ_C 54.5), and of H-19 (δ 2.35, and 2.69) with C-20 (δ_C 87.1) and C-5 (δ_C 56.7) in HMBC spectrum. The two broad singlets for hydroxyl groups at δ 6.47 and 5.47 (exchangeable by D_2O) in ^{1}H-NMR spectrum correlated with the carbon signals at δ_C 69.4 and 59.8, indicated that they were secondary and primary hydroxyl groups, respectively. This fact suggested that compound (**1**) has hydroxyl groups instead of acetoxyl groups in spiramine X. The HMQC and COSY correlations of the mutually coupled methylene protons at δ 3.90, 3.85, 3.16, 2.91 and hydroxyl signal at δ 5.47 established the -NCH_2CH_2OH fragment. This confirmed the location of primary hydroxyl group on C-22. Similarly, a vicinal dioxygen fragment -CH(O)CH(O)- was derived from the HMQC and COSY correlations of two methine protons at δ 4.40, and 3.75. The HMBC correlation of proton at δ 3.75 (H-7) with δ_C 87.1 (C-20) confirmed that fragment -CH(O)CH(O)- was involved in the ether linkage with C-20. Thus the secondary hydroxyl group should be placed on C-6. The stereochemistry of **1** was determined to be the same as that of spiramine X, since NOE correlations were

observed between H-5 / H-18, H-6 / H-7 and H-18, H-14 / H-20, and H-12 / H-17. But we have not found any NOE between H-5 and H-7. The NOEs of H-6 with H-7, H-18 and H-19 also indicated that the 6-OH should be in β-orientation. All these assignments were further supported by molecular modeling (Figure 1) also, in which H-5 and H-7 were out of the scope to show NOE correlation, as B and C rings of spiraeaine A were in boat forms. Based on the above analyses, the structure of **1** was assigned for spiraeaine A.

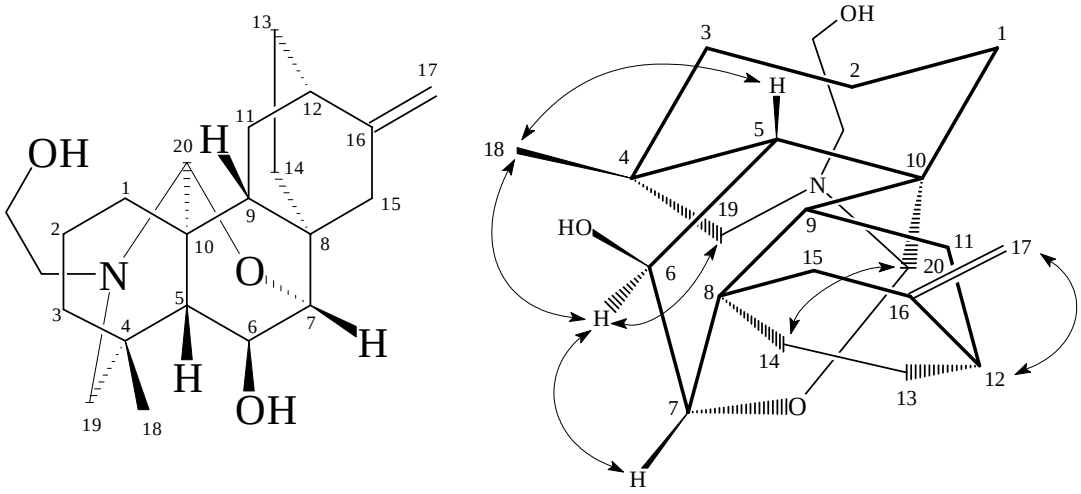
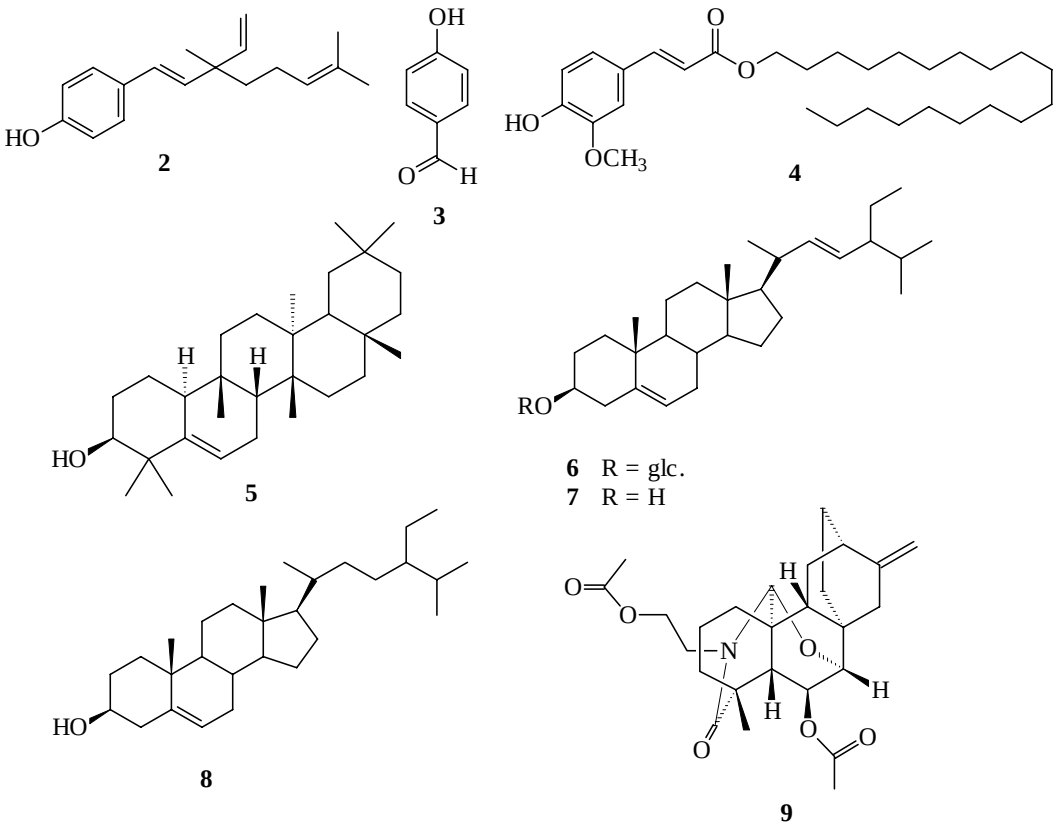


Figure 1 Structure and Key NOE correlations of Spiraeaine A (**1**)



2 **3** **4** **5** **6** R = glc. **7** R = H **8** **9**

Figure 2 Structures of isolated compounds (**2**)-(**8**) and Spiramine X (**9**)

The seven known compounds were identified as bakuchiol (**2**),[11] *p*-hydroxybenzenaldehyde (**3**),[12] nonadecyl ferulate (**4**),[9] glutinol (**5**),[9] β-sitosteryl glucoside (**6**),[12] mixture of β-sitosterol (**7**) and stigmasterol (**8**)[12] (Figure 2) by comparison of their spectroscopic data (UV, IR, NMR, MS spectrometry) with the values in literature.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. IR spectra were recorded on a Shimadzu FT-IR DR-8011 spectrophotometer. ^{1}H and ^{13}C NMR spectra were determined on Varian Unity plus 400 spectrometers. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as internal standard. EI and HR-EI MS spectra were measured on a VG-70-250S spectrometer by a direct inlet system.

Plant Material. *S. formosana* was collected in Ilan Hsien, Taiwan, Republic of China, in 1991. A voucher specimen (Wu 20011) 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 refluxed with ethanol on boiling water bath for 8 h (10 L × 7) and filtered. The filtrate was concentrated to give dark brown syrup (1.02 Kg) and it was partitioned with chloroform. The condensed chloroform extract (310 g) was dissolved in 3% acetic acid solution and filtered. The acidic solution was basified with 5% NH_4OH, and then extracted by $CHCl_3$. Evaporated off the solvent yielded a crude alkaloid extract (9.2 g). It was chromatographed over silica gel and eluted with a gradient of chloroform and methanol to afford 6 fractions. Fraction 6 was further purified by HPLC [Cosmosil 5C-18-AR-II Waters (5 μm)] with methanol-water (60:40) to give spiraeaine A (**1**) (2 mg). The non-alkaloidal fraction was chromatographed on silica gel by eluting with gradient of *n*-hexane and EtOAc, and gave 14 fractions. Further purification of these fractions by thin layer chromatography and recrystallization afforded bakuchiol (**2**) (5 mg), *p*-hydroxybenzenaldehyde (**3**) (7 mg), nanodecyl ferulate (**4**) (7 mg), glutinol (**5**) (4 mg), β-sitosteryl glucoside (**6**) (15 mg), the mixture of β-sitosterol (**7**) and stigmasterol (**8**) (55 mg), successively.

Spiraeaine A（**1**）$C_{22}H_{33}NO_3$: Colorless oil; $[\alpha]_D$-7.83° (*c* 0.7,$CHCl_3$); IR ν_{max} cm^{-1}：3358, 1651, 922.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.47(1H, br s, D_2O exchangeable, 6-OH), 5.47(1H, br s, D_2O exchangeable, 22-OH), 4.88(1H, s, H-17), 4.74(1H, s, H-17), 4.71(1H, s, H-20), 4.40(1H, br s, H-6), 3.90 (1H, m, H-22), 3.85(1H, m, H-22), 3.75(1H, d, *J* = 4.8 Hz, H-7), 3.46(1H, d, *J* = 15.6 Hz , H-15), 3.16(1H, dt, *J* = 14.0, 5.6 Hz, H-21), 2.91(1H, dt, *J* = 14.0, 5.6 Hz, H-21), 2.69(1H, d, *J* = 11.6 Hz, H-19), 2.61(1H, m, H-13), 2.35(1H, d, *J* = 11.6 Hz, H-19), 2.27(1H, m, H-12), 2.15(2H, m, H-15 and 14), 1.66(1H, m, H-1), 1.64(1H, d, *J* = 8.4 Hz, H-9), 1.59~1.38(5H, m, H-11, 14, 2, 3, and 1), 1.38~1.25(6H, m, H-11, 14, 13, 2, 3, and 1), 1.25(1H, m, H-5), 1.07(1H, ddd, *J* = 13.6, 13.6, 7.2 Hz, H-11).^{13}C-NMR (100 MHz, $CDCl_3$): δ 152.5(C-16), 107.4(C-17), 87.1(C-20), 74.4(C-7), 69.4(C-6), 59.8(C-22), 58.4(C-21), 56.7 (C-5), 54.5 (C-19), 46.1(C-9), 41.8(C-3), 40.3(C-15), 37.4(C-12), 37.0(C-8), 36.2(C-10), 33.3(C-4), 29.2 (C-11), 28.0(C-14), 26.7(C-2), 26.2(C-1), 25.2(C-18), 21.3(C-13). EI-MS *m/z* (*rel. int.* %): 359(M^+, 21), 328(83), 300(21), 180(21), 116(100). HREI-MS *m/z* 359.2462 $[M]^+$ (Calcd for $C_{22}H_{33}NO_3$: 359.2460).

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