Chemical Constituents from Roots of *Taraxacum formosanum*

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Two new compounds, taraxafolide (1) and (+)-taraxafolin-B (2) together with eighteen known compounds, which include one sesquiterpene, thirteen benzenoids, two indole alkaloids, one pyridine derivative and steroid mixtures were isolated and characterized from the fresh roots of *Taraxacum formosanum*. Structures of new compounds were determined by spectral analysis. (+)-Taraxafolin-B had the bioactive caffeic acid moiety, but its activity was weaker than α-tocopherol in DPPH radicals scavenging activity assay.

Key words *Taraxacum formosanum*; Compositae; sesquiterpenoid

Taraxacum formosanum is a herbaceous plant belonging to a family Compositae, distributed mainly in the littoral areas of north Taichung in Taiwan.[1)] Some species of the genus, *Taraxacum* have been used in folk medicine to treat lactation, and as diuretic, anti-mastopathy and anti-inflammatory agent. Earlier pharmacological studies on this plant revealed that, the crude extract showed an *in vitro* bactericidal effect against *Staphylococcus aureus* and inhibitory action against *Mycobacterium tuberculosis* and *Leptospira*. It is also a relatively safe herb with an LD_{50} of 59 g/kg in mice and has a record of relatively few side effects.[2)] Twenty-eight components isolated from the aerial parts of *T. formosanum* had been reported.[3)] Among them, taraxacine-A, -B and taraxafolin-A were firstly reported from *T. formosanum*. In our continuing studies, we have examined the roots of *T. formosanum* and two new compounds, taraxafolide (**1**) and (+)-taraxafolin-B (**2**) together with eighteen known compounds were isolated and characterized. Structures of new compounds were elucidated by spectral analysis. This paper deals with the structural determination of taraxafolide (**1**) and (+)-taraxafolin-B (**2**) by means of spectral analysis. (+)-Taraxafolin-B had the bio-active caffeic acid moiety, but its activity was weaker than α-tocopherol in DPPH radicals scavenging activity assay.

Results and Discussion

Compound **1** was an optically colorless syrup, $[\alpha]_D$ −9.47° (c=0.24, H_2O), and its molecular formula, $C_{21}H_{28}O_{10}$, was gained by HR-FAB-MS (high resolution fast atom bombardment mass). The UV spectrum of **1** revealed the maximum absorption at 255 nm and the signals at 3433 and 1770 cm^{-1} showed hydroxyl and carbonyl groups in its IR spectrum.

In the ^{13}C-NMR spectrum of compound **1**, 21 carbon signals were revealed and the signal at δ 6.20 (1H, s) suggested the presence of an olefinic proton in ^{1}H-NMR spectrum. Three singlet methyl signals appeared at δ 2.31, 2.22 and 1.60 and the anomeric proton of sugar revealed at δ 4.62 (1H, d, J=7.6 Hz). In COSY spectrum of **1**, the mutual coupled signals at δ 3.60 (1H, d, J=10.0 Hz), 4.01 (1H, dd, J=11.2, 10.0 Hz), 2.54 (1H, dd, J=11.2, 6.4 Hz), 3.97 (1H, m) and 2.79 (2H, d, J=6.4 Hz) revealed the –CHCHCHCHCH$_2$– moiety in this molecule. The carbons at δ 103.6, 76.2, 76.1, 73.8, 69.6 and 60.9[4)] indicated the presence of glucose moiety in ^{13}C-NMR spectrum, and the β-orientation of C-1′ was confirmed by the coupling constant (J=7.6 Hz) of the anomeric proton.

In HMBC experiment (Fig. 1), the signal at δ_C 133.0 showed the ^2J and ^3J correlations with δ_H 6.20, 4.01, 3.60, 2.79 and 2.31, respectively, and the carbon at δ_C 149.3 revealed the correlations with δ_H 2.79 and 2.31. These data suggested that the structure of compound **1** had the cycloheptane skeleton. The correlations between δ_C 199.3 and δ_H 6.20, δ_C 134.8 and δ_H 3.60, 2.22, δ_C 174.6 and δ_H 6.20, 3.60, 2.22 proved the cyclopentanone moiety. In addition, δ_C 81.8 and H-5, H-7; δ_C 60.6 and H-5, H-6, H-8, H-9; δ_C 178.8 and H-13 also showed ^2J and ^3J correlations, respectively. Above these data, the nucleus of compound **1** could be determined. The substituted position of glucose was determined at C-8 by the HMBC correlation between δ_C 75.0 (C_8) and anomeric proton (δ 4.62).

The relative stereochemistry of compound **1** could be determined by NOESY experiment and the coupling constants of H-5 and 6. The coupling constants was 10.0 Hz, and indicated the dihedral angle was 180° between H-5 and 6.[5)] Anti-

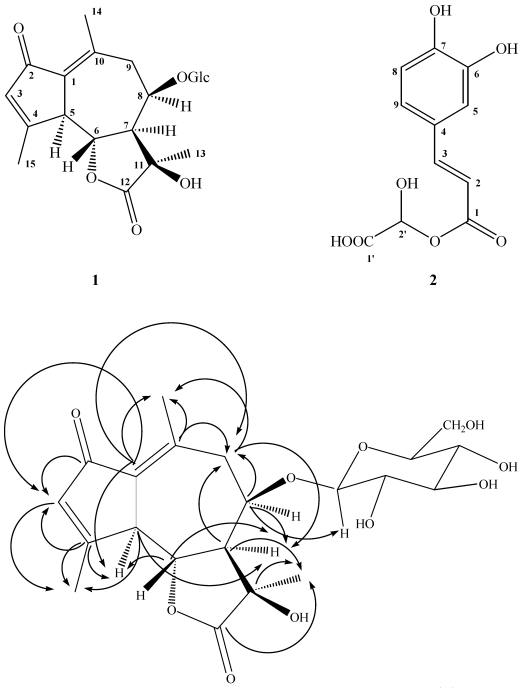

Fig. 1. HMBC Experiment for Taraxafolide (**1**)

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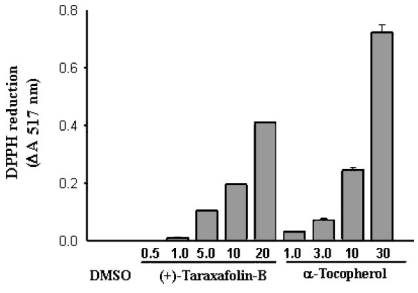

Fig. 2. Antioxidant Effects of (+)-Taraxafolin-B (**2**) and α-Tocopherol in Scavenging DPPH Radicals

After incubation of various concentrations of (+)-taraxafolin-B (0.5—20 μM) with DPPH and α-tocopherol (1—30 μM) with DPPH at 25 °C for 15 min, decrease in absorbance (ΔA) of DPPH at 517 nm was measured. For all data, value were the mean±S.E.M. of 3 separated experiments.

form between H-6 and 7 also be determined by the coupling constant (11.2 Hz). The relative stereochemical assignment could be further confirmed by the NOE signal between H-7 and 8. The strong NOE signal was also revealed between H-7 and 13-CH_3.

From the foregoing spectral analyses, the structure **1** was established as taraxafolide.

(−)-Taraxafolin B (**2**) was isolated as optically active brown powder, $[\alpha]_D$ +130° (c=0.56, H_2O), with a *pseudo*-molecular formula of $C_{11}H_{10}O_7Na$, deduced from its HR-FAB mass spectrum. The UV absorption bands at 218, 242, 300 (sh), and 326 nm indicated the presence of aromatic ring.[6] IR absorption bands at 3194 and 1696 cm^{-1} inferred the hydroxyl and carbonyl groups, respectively. The presence of trisubstituted phenyl ring was confirmed by an ABX pattern signals at δ 7.11 (1H, d, J=1.6 Hz), 7.04 (1H, dd, J=8.0, 1.6 Hz), 6.84 (1H, d, J=8.0 Hz) in the ^{1}H-NMR spectrum of **2**. The signals at δ 7.60 (1H, d, J=16.0 Hz) and 6.37 (1H, d, J=16.0 Hz) also indicated the presence of *trans*-olefinic protons. The ^{13}C-NMR spectrum, combined with HMQC and HMBC experiments, suggested a caffeic acid nucleus for compound **2**.[3] In addition, there was a proton signal appeared at δ 5.49 (s). In the HMBC spectrum, the signal at δ 5.49 showed ^2J-correlation with carbonyl carbon (δ_C 173.9) and C-1 (δ_C 169.0) revealed ^2J,^3J-correlations with H-2, H-3 and H-2′ suggested that substituted group was attached to C-1. It was further confirmed by the FAB-MS, which showed a fragment ion, [M+Na−OCH(OH)COOH−1]$^+$ at m/z 185. Consequently, the structure of (+)-taraxafolin-B was assigned as **2**.

The known compounds, taraxinic acid β-D-glucoside (**3**),[7] methyl ferulate (**4**),[8] 4-hydroxy-3-methoxy-*trans*-cinnamaldehyde (**5**),[9] syringaldehyde (**6**),[3] syringic acid (**7**),[3] methyl syringate (**8**),[9] 4-hydroxybenzaldehyde (**9**),[3] 4-hydroxybenzoic acid (**10**),[10] methylparaben (**11**),[11] 4-methoxy benzoic acid (**12**),[11] vanillin (**13**),[12] vanillic acid (**14**),[3] methyl vanillate (**15**),[13] 3-formyl indole (**16**),[3] methyl indole-3-carboxylate (**17**),[14] nicotinamide (**18**),[15] and stigmasterol and β-sitosterol mixtures (**19**+**20**),[3] were also isolated and identified by comparison of their spectral data with corresponding literature values.

The DPPH radical scavenging activity of (+)-taraxafolin-B had been examined. However, (+)-taraxafolin-B had the bioactive caffeic acid moiety, its activity was weaker than α-tocopherol in DPPH radicals scavenging activity assay (Fig. 2).

Experimental

Melting points were measured on a Yanagimoto MP-S3 micromelting point apparatus and were uncorrected. The UV spectra were recorded on a Hitachi U-3010 spectrophotometer in MeOH solution. The IR spectra were recorded on a Jasco IR Report-100 spectrophotometer as KBr discs. The ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker Avance-400 spectrometer. Chemical shifts are shown in δ values with tetramethylsilane as internal reference. The mass spectra were performed in the FAB (matrix: glycerol) mode on a VG 70-250 S spectrometer. Specific rotations were determined at 25 °C on a Jasco P-1010 polarimeter.

Plant Material *T. formosanum* was bought from a market and authenticated by Prof. C. S. Kouh. A voucher specimen (CGU-TFL-1) was deposited in the herbarium of Chang Gung University, Taoyuan, Taiwan.

Extraction and Isolation The fresh root of *T. formosanum* (1.45 kg) were extracted with MeOH (3 l×6) under reflux for 8 h and concentrated to give brown syrup (93.01 g). The syrup was suspended in H_2O and partitioned with $CHCl_3$, *n*-BuOH, successively. The $CHCl_3$ extract (8.08 g) was subjected to column chromatography over silica gel and eluted with a $CHCl_3$ and MeOH step gradients to afford six fractions. Fraction 1 was filtered to gain sterol mixtures (**19**+**20**) (256.9 mg). Repeated column chromatography of second fraction, over silica gel with *n*-hexane and EtOAc mixtures yielded **4** (1.0 mg), **8** (0.9 mg), **13** (2.1 mg) and **15** (1.3 mg). The third fraction was applied on silica gel column and eluted with a gradient of *n*-hexane and EtOAc to give **5** (3.2 mg), **11** (0.8 mg), **14** (2.3 mg) and **17** (1.5 mg), respectively. The fourth fraction was repeatedly chromatographed over silica gel with $CHCl_3$ and MeOH (14 : 1) to get **6** (0.7 mg), **10** (1.6 mg), **16** (2.3 mg) and **18** (5.1 mg), successively. The sixth fraction was purified by recrystallisation to afford **7** (2.9 mg) and **12** (0.9 mg). The *n*-BuOH layer (20.55 g) was applied on Diaion HP-20 gel and eluted with gradients of H_2O and MeOH to give eight fractions. The sixth fraction was repeatedly column chromatographed over silica gel with $CHCl_3$: $(CH_3)_2CO$ (1 : 1) gradients to give **1** (4.8 mg) and **3** (21.3 mg). Compound **2** (5.7 mg) was crystallized from eighth fraction with MeOH.

DPPH Free-Radical Scavenging Activity The scavenging activity of the DPPH radical was assayed by the modified method of Shimada *et al.*[16] The absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free-radical scavenging activity. The DPPH radical-scavenging activity (%) was calculated by the following equation:

$$\text{scavenging activity (\%)} = (1 - A_{\text{sample}}/A_{\text{control}}) \times 100\%$$

Taraxafolide (**1**): Colorless oil. $[\alpha]_D$ −9.47° (c=0.24, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ: 6.20 (1H, s, H-3), 4.62 (1H, d, J=7.6 Hz, H-1′), 4.01 (1H, dd, J=11.2, 10.0 Hz, H-6), 3.97 (1H, m, H-8), 3.87 (1H, dd, J=12.4, 2.0 Hz, H-6′a), 3.69 (1H, dd, J=12.4, 5.2 Hz, H-6′b), 3.60 (1H, d, J=10.0 Hz, H-5), 3.43 (2H, m, H-4′, H-5′), 3.35 (1H, dd, J=9.2, 9.2 Hz, H-3′), 3.29 (1H, dd, J=9.2, 8.4 Hz, H-2′), 2.79 (2H, d, J=6.4 Hz, H-9), 2.54 (1H, dd, J=11.2, 6.4 Hz, H-7), 2.31 (3H, s, H-14), 2.22 (3H, s, H-15), 1.60 (3H, s, H-13). ^{13}C-NMR (D_2O, 100 MHz) δ: 199.3 (C-2), 178.8 (C-12), 174.6 (C-4), 149.3 (C-10), 134.8 (C-3), 133.0 (C-1), 103.6 (C-1′), 81.8 (C-6), 76.2 (C-5′), 76.1 (C-4′), 75.0 (C-8), 74.7 (C-11), 73.8 (C-2′), 69.6 (C-3′), 60.9 (C-6′), 60.6 (C-7) , 51.0 (C-5) , 45.5 (C-9), 22.9 (C-13), 21.4 (C-14), 19.3 (C-15). IR ν_{max} cm^{-1}: 3433, 1770. UV λ_{max} nm (log ε): 255 (2.4). HR-FAB-MS: Calcd for $C_{21}H_{29}O_{10}$ m/z [M+1]$^+$ 441.1764, Found 441.1762. FAB-MS m/z (rel. int. %): 463 ([M+Na]$^+$, 5), 421 (5), 413 (19), 176 (21) 149 (34), 136 (18), 70 (33).

(+)-Taraxafolin B (**2**): Brown powder (MeOH). mp >280 °C. $[\alpha]_D$ +130° (c=0.56, H_2O). ^{1}H-NMR (D_2O, 400 MHz) δ: 7.60 (1H, d, J=16.0 Hz, H-2), 7.11 (1H, d, J=1.6 Hz, H-5), 7.04 (1H, dd, J=8.0, 1.6 Hz, H-9), 6.84 (1H, d, J=8.0 Hz, H-8), 6.37 (1H, d, J=16.0 Hz, H-3), 5.49 (1H, s, H-2′). ^{13}C-NMR (D_2O, 100 MHz) δ: 173.9 (C-1′), 169.0 (C-1), 147.5 (C-7), 147.1 (C-3), 144.6 (C-6), 127.4 (C-4), 123.3 (C-9), 116.6 (C-8), 115.6 (C-5), 114.5 (C-2), 75.1 (C-2′). IR ν_{max} cm^{-1}: 3194, 1696. UV λ_{max} nm (log ε): 326 (1.61), 300 (1.52, sh), 242 (1.38), 218 (1.53). HR-FAB-MS: Calcd for $C_{11}H_{10}O_7Na$ m/z [M+Na]$^+$ 277.0324, Found 277.0325. FAB-MS m/z (rel. int. %): 277 ([M+Na]$^+$, 7), 246 (39), 185 (100), 254 (64), 137 (64).

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