Chemical Constituents of *Taraxacum formosanum*

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Three new compounds, taraxacine-A (1), taraxacine-B (2) and taraxafolin (3) together with twenty-five known compounds, which include two β-carboline alkaloids, two indole alkaloids, two chlorophylls, two flavonoids, one coumarin, two triterpenoids, one monoterpenoid, one ionone, four steroids and eight benzenoids, were isolated and characterized from the fresh aerial parts of *Taraxacum formosanum*. Structures of new compounds were determined by spectral analysis.

Key words *Taraxacum formosanum*; Compositae; β-carboline alkaloid

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, antimastopathy 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)] However, *T. formosanum* was hitherto uninvestigated for its chemical constituents. As a part of our ongoing phytochemical work on Chinese medicinal plants, we have examined the aerial parts of *T. formosanum*, which resulted in the isolation of three new and twenty-five known compounds. This paper deals with the structural determination of two new β-carbolines, taraxacine-A (**1**) and taraxacine-B (**2**), and one phenylpropanoid, taraxafolin (**3**) by means of spectral analysis. This is the first report of the isolation of β-carboline alkaloids from the genus of *Taraxacum*.

Results and Discussion

Taraxacine-A (**1**) was obtained as pale yellow syrup, which gave a positive reaction with Dragendroff's reagent, indicating it to be an alkaloid. The molecular formula was established as $C_{12}H_{10}N_2O$ by high resolution electron impact (HR-EI)-MS. The UV absorptions at 212, 246, 257, 273, 300 and 367 nm were typical of a β-carboline alkaloid.[3)] Its IR spectrum showed bands at 3300 and 1460 cm^{-1}, accounted for amine and aromatic groups, respectively. A broad singlet at δ 10.29 (1H, D_2O exchangeable) in ^{1}H-NMR spectrum was assigned for NH. Presence of four mutually coupled aromatic proton system at δ 8.31 (1H, d, J=8.0 Hz), 7.85 (1H, t, J=8.0 Hz), 7.72 (1H, d, J=8.0 Hz), and 7.56 (1H, t, J=8.0 Hz), in ^{1}H-NMR spectrum indicated that aromatic ring of β-carboline was unsubstituted. Protons H-3 and H-4 constituted an isolated vicinal pair, which could be assigned α and β pyridine protons from their homonuclear coupling constant (J=5.6 Hz), from their proton chemical shifts at δ 8.90 (H-3) and 8.54 (H-4). A methoxyl singlet appeared at δ 4.30 was thus, located at C-1. On the basis of the above analysis, structure **1** was assigned to taraxacine-A. This data was coincided well with that of synthetic sample.[4)]

Taraxacine-B (**2**) was obtained as pale yellow syrup, which also gave a positive reaction with Dragendroff's reagent, indicating it to be an alkaloid. The HR-EI-MS of **2** revealed the molecular formula as $C_{13}H_{10}N_2O_3$. The UV absorption maxima at 216, 232, 270, 287 (sh), 303, 333 and 346 nm characteristic of a β-carboline alkaloid.[3)] Its IR absorption bands suggested the presence of amine (3200 cm^{-1}), carboxyl (1721 cm^{-1}), and aromatic (1437 cm^{-1}) groups. Presence of carboxylic acid group was also supported by a prominent fragment ion $[M-CO_2H]^+$ peak at m/z 199 in EI-MS spectrum. The ^{1}H-NMR spectrum showed four adjacent aromatic proton system at δ 8.32 (1H, d, J=8.0 Hz, H-5), 7.97 (1H, d, J=8.0 Hz, H-8), 7.85 (1H, t, J=8.0 Hz, H-7), and 7.54 (1H, t, J=8.0 Hz, H-6), and a singlet at δ 9.07 (H-4) corresponding to heteroaromatic proton and a methoxyl signal at δ 4.24 (3H, s, 1-OCH_3). Two broad singlets at δ 13.46, and 10.14 (each 1H, br s, D_2O exchangeable) were attributed to –OH, and NH, respectively. The downfield shift of H-4 signal to δ 9.07 suggested that a carboxyl group was attached to C-3. It was confirmed by the nOe signal between H-4 and H-5. From the foregoing spectral analyses, the structure **2** was assigned to taraxacine-B.

Taraxafolin (**3**) was isolated as optically active colorless syrup with a molecular formula of $C_{11}H_{14}O_5$, deduced from its HR-EI mass spectrum. Its UV absorption bands at 234 (sh), 249 (sh), 291, and 326 nm indicated the presence of aromatic ring. IR absorption bands at 3331, and 1713 cm^{-1} inferred the hydroxyl, and carbonyl groups, respectively. Accordingly, a D_2O exchangeable broad singlet integrated for two protons was observed at δ 7.85 in ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum of **3** also showed the presence of two methoxyl groups at δ 3.61 (3H, s), and 3.12 (3H, s), a methylene group at δ 2.69 (1H, dd, J=15.2, 9.2 Hz), and 2.50 (1H, dd, J=15.2, 4.8 Hz), and an oxymethine group at δ 4.45 (1H, dd, J=9.2, 4.8 Hz), corresponding to a –$CH(OCH_3)$-CH_2COOCH_3 moiety. The presence of trisubstituted phenyl ring was confirmed by an ABX pattern signals at δ 6.82 (1H, d, J=2.0 Hz), 6.80 (1H, d, J=8.0 Hz), and 6.68 (1H, dd, J=8.0, 2.0 Hz), attributable to H-2′, 5′, and 6′, respectively. The ^{13}C-NMR spectrum, combined with ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments, suggested a methyl 3-(3′,4′-dihydroxyphenyl)-3-methoxy propionate nucleus for compound **3**. In the HMBC spectrum, the signals at δ 4.45 showed ^2J, ^3J-correlations with methoxyl carbon (δ_C 55.9) and with C-6′ (δ_C 118.7) and C-2 (δ_C 43.4)

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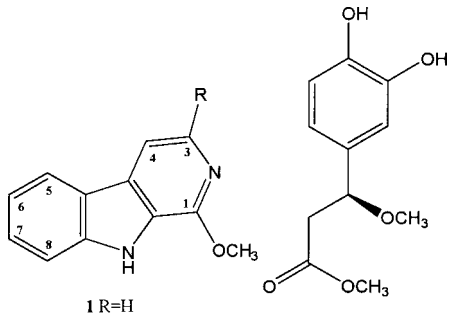

Fig. 1

suggested that methoxyl group was attached to C-3. It was further confirmed by the EI-MS, which showed a fragment ion, $[M-CH_2COOCH_3]^+$ at *m/z* 153. The negative optical rotation of **3** inferred the *S* stereochemistry for C-3.[5] Consequently, the structure of (*S*)-taraxafolin was assigned as **3**.

The known compounds, 3-carboxy-1,2,3,4-tetrahydro-β-carboline,[6] 1,2,3,4-tetrahydro-1,3,4-trioxo-β-carboline,[7] indole-3-carboxaldehyde,[8] indole-3-carboxylic acid,[9] 13^2-hydroxy-(13^2-*R*)-pheophytin-b,[10] methyl pheophorbide-b,[11] ψ-taraxasteryl acetate,[12] β-amyrin acetate,[12] luteolin-7-*O*-glucoside,[13] isoetin-7-*O*-glucosyl-2′-*O*-xyloside,[14] aesculetin,[15] dihydrosyringin,[16] *p*-hydroxy-cinnamic acid,[9] caffeic acid,[9] ferulic acid,[17] phenylalanine,[18] benzoic acid,[9] methyl paraben,[17] *p*-hydroxyphenyl acetic acid methyl ester,[19] (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigma-dien-9-one,[20] 2,6,6-trimethyl-4-hydroxy-1-cyclohexene-1-carboxaldehyde,[21] β-sitosterol,[9] stigmasterol,[9] β-sitosteryl-3-*O*-glucoside,[9] and stigmasteryl-3-*O*-glucoside,[22] were also isolated and identified by comparison of their spectral data with corresponding literature values.

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 EI or FAB (matrix: glycerol) mode on a VG 70-250 S spectrometer. Specific rotations were determined 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 aerial parts of *T. formosanum* (4.68 kg) were extracted with MeOH (10 l×6) under reflux for 8 h and concentrated to give brown syrup (230.2 g). The syrup was suspended in H_2O and partitioned with $CHCl_3$, *n*-BuOH successively. The $CHCl_3$ extract (72.3 g) was subjected to column chromatography over silica gel and eluted with a $CHCl_3$ and MeOH step gradients to afford ten fractions. Repeated column chromatography of combined third and fourth fractions, over silica gel with *n*-hexane and EtOAc mixtures yielded ψ-taraxasteryl acetate (909.1 mg) and β-amyrin acetate (2.3 mg). The fifth fraction was purified by recrystallisation to afford β-sitosterol (2.9 g) and stigmasterol (2.0 g). The sixth fraction was applied on silica gel column and eluted with a gradient of *n*-hexane and EtOAc to give 13^2-hydroxy-(13^2-*R*)-pheophytin-b (8.2 mg). The seventh fraction was repeatedly chromatographed over silica gel with $CHCl_3$ and $(CH_3)_2CO$ (14 : 1) to get taraxacine-A (**1**) (1.5 mg), indole-3-carboxaldehyde (7.3 mg), methyl pheophorbide-b (250.0 mg), (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigma-dien-9-one (2.5 mg) and 2,6,6-trimethyl-4-hydroxy-1-cyclohexene-1-carboxaldehyde (2.1 mg), successively. The tenth fraction was purified by recrystallisation to afford β-sitosteryl-3-*O*-glucoside (1.2 g) and stigmasteryl-3-*O*-glucoside (2.0 g). The *n*-BuOH layer (36.0 g) was applied on Diaion HP-20 gel and eluted with gradients of H_2O and MeOH to give six fractions. Phenylalanine (315.1 mg) was crystallized from second fraction with MeOH. The third fraction was chromatographed on Sephadex LH-20 column and eluted with gradients of H_2O and MeOH to afford 3-carboxy-1,2,3,4-tetrahydro-β-carboline (54.6 mg), luteolin-7-*O*-glucoside (72.1 mg), dihydrosyringin (72.0 mg), caffeic acid (1.17 g), and ferulic acid (5.0 mg), successively. The fifth fraction was repeatedly column chromatographed over silica gel with $CHCl_3$: MeOH gradients to give taraxacine-B (**2**) (3.0 mg), (*S*)-taraxafolin (**3**) (2.3 mg), 1,2,3,4-tetrahydro-1,3,4-trioxo-β-carboline (2.0 mg), indole-3-carboxylic acid (10.0 mg), isoetin-7-*O*-glucosyl-2′-*O*-xyloside (39.4 mg), aesculetin (1.4 mg), *p*-hydroxy-cinnamic acid (1.5 mg), benzoic acid (2.0 mg), and *p*-hydroxyphenyl acetic acid methyl ester (1.5 mg). The sixth fraction was also rechromatographed as above to obtain methyl paraben (1.0 mg).

Taraxacine-A (**1**): Pale yellow syrup. ^{1}H-NMR ($CDCl_3$) δ: 10.29 (1H, s, NH), 8.90 (1H, d, J=5.6 Hz, H-3), 8.54 (1H, d, J=5.6 Hz, H-4), 8.31 (1H, d, J=8.0 Hz, H-5), 7.85 (1H, t, J=8.0 Hz, H-7), 7.72 (1H, d, J=8.0 Hz, H-8), 7.56 (1H, d, J=8.0 Hz, H-6), 4.30 (3H, s, OCH_3). IR ν (KBr) cm^{-1}: 3300, 2924, 2853, 1460. UV λ_{max} (MeOH) nm (log ε): 212 (3.9), 246 (3.6), 257 (3.6), 273 (3.5), 300 (3.4), 367 (3.2). EI-MS *m/z*: 198.0794 (Calcd for $C_{12}H_{10}N_2O$: 198.0793). MS *m/z*: 198 (M^+), 166, 140, 71, 57.

Taraxacine-B (**2**): Pale yellow syrup. ^{1}H-NMR ($CDCl_3$) δ: 13.46 (1H, br s, OH), 10.14 (1H, s, NH), 9.07 (1H, s, H-4), 8.32 (1H, d, J=8.0 Hz, H-5), 7.97 (1H, d, J=8.0 Hz, H-8), 7.85 (1H, t, J=8.0 Hz, H-7), 7.54 (1H, t, J=8.0 Hz, H-6), 4.24 (3H, s, OCH_3). IR ν (KBr) cm^{-1}: 3200, 1721, 1437, 1341, 1255, 1105, 756. UV λ_{max} (MeOH) nm (log ε): 216 (4.3), 232 (4.3), 270 (4.4), 287 (4.0, sh), 303 (3.8), 333 (3.5), 346 (3.5). EI-MS *m/z*: 242.0693 (Calcd. for $C_{13}H_{10}N_2O_3$: 242.0691). MS *m/z*: 242 (M^+), 227, 199, 167, 149, 121, 95, 81, 67, 55.

(*S*)-Taraxafolin (**3**): Colorless syrup. ^{1}H-NMR ($CDCl_3$) δ: 7.85 (1H, br s, OH), 6.82 (1H, d, J=2.0 Hz, H-2′), 6.80 (1H, d, J=8.0 Hz, H-5′), 6.68 (1H, dd, J=8.0, 2.0 Hz, H-6′), 4.45 (1H, dd, J=9.2, 4.8 Hz, H-3), 3.61 (3H, s, OCH_3), 3.12 (3H, s, OCH_3), 2.69 (1H, dd, J=15.2, 9.2 Hz, H-2), 2.50 (1H, dd, J=15.2, 4.8 Hz, H-2). ^{13}C-NMR ($CDCl_3$) δ: 171.0 (C-1), 145.5 (C-3′), 145.2 (C-4′), 132.9 (C-1′), 118.7 (C-6′), 115.2 (C-5′), 113.9 (C-2′), 80.1 (C-3), 55.9 (CH–O$\underline{C}H_3$), 51.0 ($CO_2\underline{C}H_3$), 43.4 (C-2). IR ν (KBr) cm^{-1}: 3331, 1713, 1440, 1281, 1060, 757. UV λ_{max} (MeOH) nm (log ε): 234 (4.4, sh), 249 (4.3, sh), 291 (4.4), 326 (4.4). EI-MS *m/z*: 226.0842 (Calcd for $C_{11}H_{14}O_5$: 226.0841). MS *m/z*: 226 (M^+), 211, 194, 153, 134, 77. $[\alpha]_D$ −32.0° (c=0.1, $CHCl_3$).

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