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Sesquiterpene Glucosides from Anti-leukotriene B₄ Release Fraction of *Taraxacum Officinale*

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SESQUITERPENE GLUCOSIDES FROM ANTI-LEUKOTRIENE B₄ RELEASE FRACTION OF *TARAXACUM OFFICINALE*

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Chemical examination of the MeOH extract of the root of *Taraxacum officinale*, which exhibited inhibitory activity on the formation of leukotriene B₄ from activated human neutrophils, has resulted in the isolation of 14-*O*-β-D-glucosyl-11,13-dihydro-taraxinic acid (**1**) and 14-*O*-β-D-glucosyl-taraxinic acid (**2**). The absolute stereostructure of **1** has been established by X-ray chrystallographic examination.

Keywords: *Taraxacum officinale*; Compositae; Anti-leukotriene B₄ formation; Germacrane; Sesquiterpene

INTRODUCTION

Various *Taraxacum* plants are widely distributed in Japan, and the roots of these plants have been used in China and Japan for anti-inflammatory and analgesic, anti-mastopathy, diuretics medication [1, 2]. In our evaluation of the anti-inflammatory activity of *Taraxacum* plants by inhibitory activity

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toward the formation of leukotriene B₄ from human neutrophils, activated with a calcium ionophore (A23187) [3], the MeOH extract of the root of *T. officinale* was found to have activity (90% inhibition at 3 µg/mL). Further activity-guided fractionation has resulted in the isolation of germacrane-type sesquiterpene lactone glucosides, 14-*O*-β-D-glucosyl-11,13-dihydro-taraxinic acid (**1**) and 14-*O*-β-D-glucosyl-taraxinic acid (**2**). The absolute stereostructure of **1** has been firstly established by spectral examinations and X-ray crystallographic analysis.

RESULTS AND DISCUSSION

The aqueous MeOH extract of the root of *T. officinale* was partitioned successively with hexane, EtOAc, and BuOH to give the hexane-, EtOAc-, BuOH-, and water-soluble fractions. Significant anti-leukotriene B₄ formation activity was found in the BuOH-soluble fraction (86% inhibition at 3 µg/mL), while EtOAc- and water-soluble fractions displayed weak inhibitory activities (32 and 21% inhibition at 3 µg/mL, respectively). Subsequent column chromatography of the EtOAc-soluble fraction, containing relatively less-polar compounds, over SiO₂ follow by preparative TLC gave an active fraction (84% inhibition at 3 µg/mL). This fraction contained mainly two compounds as revealed by the HPLC analysis, which were successively separated by semi-preparative scale HPLC to give pure samples (**1** and **2**).

Compound **1** exhibited [M+Na]⁺ peak at *m/z* 449 in the positive FABMS, and its molecular formula was established as C₂₁H₃₀O₉ by HR-FABMS. The glycosidic nature of **1** was indicated by the anomeric resonances [δ 6.38 (1H, d, *J* = 8 Hz); δ 95.7] and also confirmed by acid hydrolysis with 10% HCl to yield D-glucose. The ¹³C-NMR exhibited, along with six carbon resonances ascribable to a glucosyl moiety (Tab. I), fifteen carbon signals including two methyl groups, two trisubstituted double bonds, and two ester carbonyl groups. Examination of the ¹H-¹H COSY and ¹H-¹³C COSY spectra provided two fragment structures shown by bold lines in Figure 1. The long-range couplings in the HMBC spectrum established the connectivity of these fragment units, including methyl groups, carbonyl groups and double bonds, as shown in Figure 1, to furnish a germacrane-type sesquiterpene lactone glucoside. The NOE correlations indicated, together with the configuration of two double bonds in **1**, the relative configuration at C-11 to be α as shown in Figure 1. Furthermore, the absolute stereostructure of **1** was confirmed by X-ray crystallographic

analysis as shown in Figure 2. Compound **1** was assumed to be 14-*O*- β -D-glucosyl-11,13-dihydro-taraxinic acid, previously obtained from the same plant together with 14-*O*- β -D-glucosyl-taraxinic acid (**2**) [4]. Although the structure of **1** was elucidated based on the spectral examination of its tetraacetate, the physical and spectral data of **1** itself has not been shown. Furthermore, the stereostructure of this compound had not been established [4]. The $^1\text{H-NMR}$ spectral data for tetraacetyl derivative of **1** was identical with those reported in the literature [4].

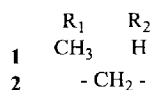
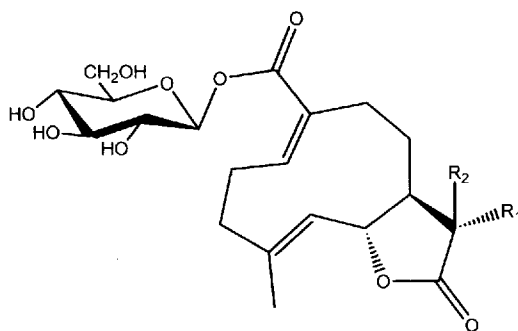


TABLE I ^1H (δ, J in Hz) and ^{13}C (δ) NMR Data for Compounds **1** and **2**

	1		2	
	<i>H</i>	<i>C</i>	<i>H</i>	<i>C</i>
1	5.67 (<i>dd</i> , 3.5, 12.5)	148.4 (<i>d</i>)	5.68 (<i>dd</i> , 4, 12.5)	148.5 (<i>d</i>)
2	ca. 2.2 3.60 (<i>m</i>)	26.9 (<i>t</i>)	ca. 2.3 (<i>m</i>) 3.66 (<i>m</i>)	26.9 (<i>t</i>)
3	ca. 2.2 (<i>m</i>)	39.4 (<i>t</i>)	ca. 2.15 (<i>m</i>)	39.3 (<i>t</i>)
4		142.0 (<i>s</i>)		141.0 (<i>s</i>)
5	4.88 (<i>d</i> , 10)	126.9 (<i>d</i>)		126.8 (<i>s</i>)
6	4.76 (<i>t</i> , 10)	81.4 (<i>d</i>)	4.78 (<i>t</i> , 10)	82.1 (<i>d</i>)
7	1.69 (<i>m</i>)	54.5 (<i>d</i>)	2.60 (<i>m</i>)	50.3 (<i>d</i>)
8	1.81 (<i>dt</i> , 6.5, 15) ca. 2.2	30.6 (<i>t</i>)	ca. 2.15 (<i>m</i>)	30.6 (<i>t</i>)
9	1.93 (<i>t</i> , 12.5) 2.91 (<i>dd</i> , 6.5, 12.5)	36.8 (<i>t</i>)	2.01 (<i>t</i> , 12.5) 2.94 (<i>dd</i> , 6.5, 13.5)	36.7 (<i>t</i>)
10		131.4 (<i>s</i>)		131.4 (<i>s</i>)
11	ca. 2.2 (<i>m</i>)	42.4 (<i>d</i>)	2.05 (<i>m</i>)	143.2 (<i>s</i>)
12		178.5 (<i>s</i>)		170.4 (<i>s</i>)
13	1.21 (<i>d</i> , 7)	13.3 (<i>q</i>)	6.27 (<i>d</i> , 3.5) 5.45 (<i>d</i> , 3.5)	119.3 (<i>t</i>)
14		166.8 (<i>s</i>)		166.8 (<i>s</i>)

TABLE I (Continued)

	1		2	
	<i>H</i>	<i>C</i>	<i>H</i>	<i>C</i>
15	1.77 (<i>s</i>)	17.0 (<i>q</i>)	1.78 (<i>s</i>)	17.1 (<i>q</i>)
Glucosyl				
1	6.38 (<i>d, 8</i>)	95.7 (<i>d</i>)	6.37 (<i>d, 8</i>)	95.7 (<i>d</i>)
2		74.2 (<i>d</i>)		74.1 (<i>d</i>)
3		78.8 (<i>d</i>)		78.8 (<i>d</i>)
4		71.2 (<i>d</i>)		71.2 (<i>d</i>)
5		79.5 (<i>d</i>)		79.5 (<i>d</i>)
6		62.3 (<i>t</i>)		62.3 (<i>t</i>)

^a Measured at 400 MHz (¹H) and 100 MHz (¹³C) in pyridine-*d*₅.

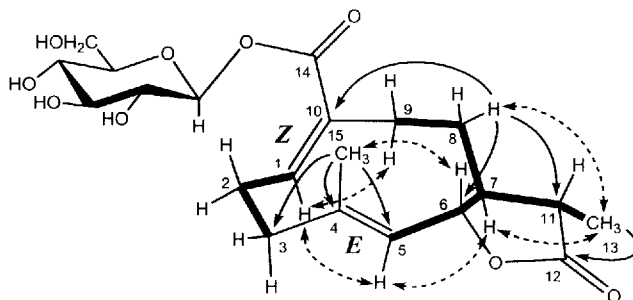


FIGURE 1 Long-range ¹H-¹³C correlations (H→C) and NOE correlations (↔) in 1.

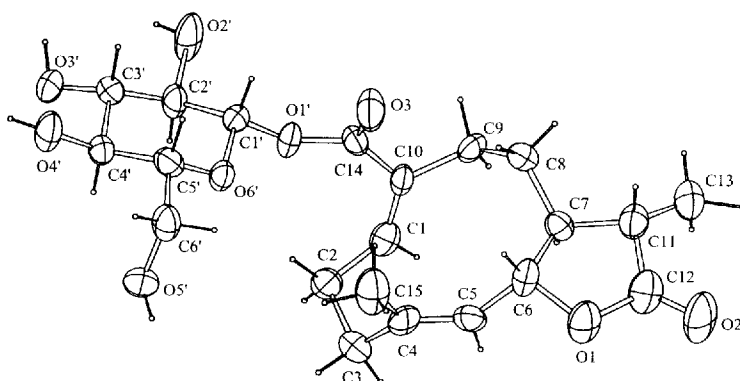


FIGURE 2 The structure of 1 showing 5.0% probability displacement ellipsoids.

The molecular formula of compound **2** was confirmed as C₂₁H₂₈O₉ by HR-FABMS. The ¹H- and ¹³C-NMR examination of **2** indicated the presence of an exomethylene group instead of a CH₃-13 secondary methyl

group. This structure is identical with that of 14-*O*- β -D-glucosyl-taraxinic acid (**2**), and the structural confirmation was established by comparing the spectral data with those reported in the literature [4].

Evaluation of the inhibitory activity for **1** and **2** on the formation of leukotriene B₄ from activated human neutrophils as well as a search for the active constituents of the BuOH- and H₂O-soluble fractions are in progress. Anti-HIV activities for **1** and **2** were also evaluated [5]. Compound **2** inhibited HIV-1 replication in acutely infected H9 cells with an EC₅₀ value of 1.68 μ g/mL, and was slightly toxic against uninfected H9 cell growth with an IC₅₀ value of 7.94 μ g/mL. In contrast, **1** showed no viral suppression up to 100 μ g/mL and no toxicity to H9 cells (IC₅₀ > 100 μ g/mL).

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were measured on a Yanaco micro melting point apparatus, and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeters. NMR spectra were recorded on JEOL A-400 spectrometer with TMS as int. standard, and chemical shifts are given in δ (ppm). FAB-MS and High resolution FAB-MS were taken with a JEOL HX-110 spectrometer. Column chromatography was performed with Kieselgel 60 PF₂₅₄ (Merck). TLC was performed on precoated Kieselgel 60 F₂₅₄ plates (0.2 mm, Merck), and spots were detected by spraying 10% H₂SO₄ with heating. The HPLC consisted of a Hitachi L-6250 solvent delivery system, an L-4200 UV-VIS spectrometer (240 nm) equipped with a Nova-PAK HR C₁₈ (Waters, Inc.) column (8 mm i.d. \times 100 mm or 25 mm i.d. \times 100 mm, for analytical and semi-preparative scale, respectively).

Plant Material

The roots of *Taraxacum officinale*, cultivated in Yamato-machi, Niigata Prefecture, Japan, were purchased and a voucher specimen has been deposited at the Herbarium of the Niigata College of Pharmacy.

Extraction and Isolation

The roots of *Taraxacum officinale* (3.57 kg) were extracted three time with MeOH (5 L) at room temperature. After removal of the solvent by evaporation, the extract (930 g) was suspended in water, which was partitioned successively with hexane, EtOAc, and BuOH to give hexane-(86 g),

EtOAc- (23 g), BuOH- (64 g), and water-soluble (738 g) fractions. The EtOAc soluble fraction was subsequently chromatographed over SiO₂ [solvent: CHCl₃-MeOH-H₂O (40:1:0→8:2:0.2)] to give seven fractions (I-IV). Fraction V was further separated by preparative TLC [CHCl₃-MeOH-H₂O (8:2:0.2)] to yield two fractions; Frs. V-1 and V-2. Fr. V-2 was separated by semi-preparative scale HPLC on Nova-PAK HR C₁₈ (25 mm i.d. × 100 mm) with 38% MeOH to yield **1** (278 mg) and **2** (249 mg).

14-O-β-D-glucosyl-11,13-dihydro-taraxinic acid (1)

Colorless needles, mp 186–186°, $[\alpha]_D^{15} - 57.6^\circ$ (MeOH; *c* 0.33), HR FAB-MS *m/z*: calcd for C₂₁H₃₀O₉Na ([M+Na]⁺) 449.1788; found 449.1797. ¹H-NMR: Table I, ¹³C-NMR: Table I.

X-ray Crystallographic Analysis of 1

C₂₁H₃₀O₉, *FW* = 425.45, monoclinic, space group *P*2₁, *a* = 11.856 (2) Å, *b* = 7.426 (5) Å, *c* = 12.283 (3) Å, β = 102.12 (1)°, *V* = 1057.3 Å³, *Z* = 2, *D*_{calcd} = 1.336 g/cm³, *T* = 22°C, μ (Mo Kα radiation, λ = 0.71073 Å) = 0.977 cm⁻¹, *F*(000) = 456, *R* = 0.051, *R*_w = 0.044; crystal dimensions: 0.3 × 0.3 × 0.2 mm. X-ray diffraction data were measured on a Rigaku AFC-5RU diffractometer. Intensity data collection was accomplished by the ω-2θ scan method with graphite-monochromated MoKα radiation up to θ = 25°; 1662 unique reflections with *F* > 3.0σ(*F*) was used for refinement. The structure was solved by the direct methods program MULTAN88. All atomic parameters, with anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms, were refined by a block-diagonal least-squares method. The final *R* value was 0.051. Crystallographic parameters have been deposited in the editorial office of JANPR.

14-O-β-D-glucosyl-taraxinic acid (2)

A white powder, mp 175–177°, $[\alpha]_D^{15} - 56.7^\circ$ (MeOH; *c* 0.3), HR FAB-MS *m/z*: calcd for C₂₁H₂₈O₉Na ([M+Na]⁺) 447.1631; found 447.1638. ¹H-NMR: Table I, ¹³C-NMR: Table I.

Acid Hydrolysis of 1 and 2

A solution of each sample (10 mg) in 10% HCl [H₂O-EtOH (1:1)] (5 mL) was refluxed for 8 hr. The reaction mixture was neutralized by Amberlite

IRA-400 (OH^- form) resin, filtered, and concentrated under reduced pressure. Silica gel chromatography of the residue with CHCl_3 -MeOH- H_2O (40:10:1) yielded D-glucose.

Biological Assay

The anti-leukotriene B_4 formation assay was carried out using the assay procedure, which has been reported [3]. The anti-HIV assay was performed by the procedure described in the literature [5].

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References

- [1] Jiangsu New Medical College (1977). *Dictionary of Traditional Chinese Materia Medica*, **2**, 2459-2462, Shanghai People's Press.
- [2] Duke, J. A., *Handbook of Medicinal Herbs*, p. 476, CRC Press, Inc., New York, 1985.
- [3] Taniguchi, K., Masuda, Y. and Takanaka, K. (1990). *Jpn. J. Pharmacol.*, **42**, 101-108.
- [4] Hänsel, R., Kartahardja, M., Huang, J. T. and Bohlmann, F. (1980). *Phytochemistry*, **19**, 857-861.
- [5] Sun, I. C., Wang, H. K., Kashiwada, Y., Shen, K. K., Cosentino, L. M., Chen, C. H., Yang, L. M. and Lee, K. H. (1998). *J. Med. Chem.*, **41**, 4648-4657.