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KAURANE GLYCOSIDES FROM *INULA BRITANNICA*

YU SHAO,* NAI-SHEN BAI and BING-NAN ZHOU

Department of Phytochemistry, Shanghai Institute of Materia Medica, Academia Sinica, Shanghai 200031, China

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Key Word Index—*Inula britannica*; Compositae; kaurane glycosides; diterpene glycosides.

Abstract—Two new diterpene glycosides have been isolated from *Inula britannica* and their structures established as 17-*O*-β-D-glucopyranosyl-16-β-H-ent-kauran-19-oic acid and 17-*O*-β-D-glucopyranosyl-16-β-H-ent-kauran-19-oic acid-19-*O*-β-D-glucopyranoside, respectively, from their spectral data, and chemical and enzymatic evidence. In addition, six known compounds were also identified.

INTRODUCTION

Inula britannica L. var *chinensis* has been used in Chinese folk medicine for the treatment of bronchitis and inflammation [1]. In a previous paper, we reported on the isolation, structural elucidation and cytoxic activities of three new sesquiterpene lactones from the flowers of this plant [2]. In a continuation of our investigation on this plant, we have now studied the polar fraction. In this paper, we deal with the isolation and structure elucidation of two new kaurane glycosides (**1** and **2**).

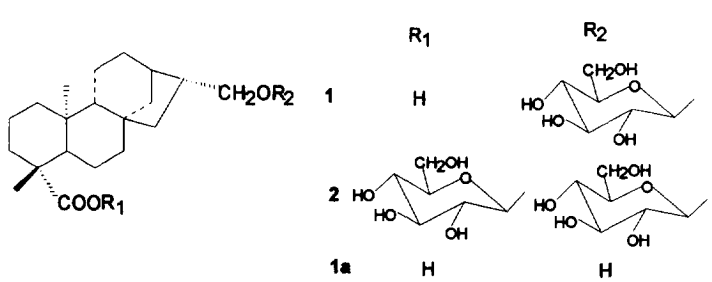
RESULTS AND DISCUSSION

Repeated chromatography of the *n*-butanol-soluble fraction obtained from the ethanol extract of *I. britannica* led to the isolation of compounds **1–8**.

Compound **1**, was assigned the molecular formula $C_{26}H_{42}O_8$ by means of the FAB-mass data (*m/z* 505 $[M+Na]^+$ and 489 $[M+Li]^+$) and the ^{13}C NMR (Table 1) and DEPT spectral data. The IR spectrum indicated the presence of a hydroxyl group (3400 cm^{-1}) and a carboxylic group (1685 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of two methyl groups (δ 1.09 and 1.32) and one anomeric proton [δ 4.84 (*d*, $J = 7.7$ Hz)] (Table 2). The ^{13}C NMR spectrum displayed the signals of 26 carbons, including two methyl groups (δ 16.10 and 29.47), one anomeric carbon (δ 104.96) and one free carboxyl group (δ 180.06). These data suggested **1** was a diterpene glycoside.

Acid hydrolysis of **1** yielded the sugar part which was identified as glucose by PC and TLC in direct comparison with an authentic sample. The evidence for the aglycone (**1a**) was obtained by means of enzymatic hydrolysis of compound **1**. **1a** gave the molecular ion at *m/z* 320 in the El-mass spectrum, corresponding to the molecular formular $C_{20}H_{32}O_3$ which was confirmed by the ^{13}C NMR data. Comparison of the ^{13}C NMR spectral data with those of methyl 17-hydroxy-16-β-H-ent-kauran-19-oate [3] indicated that they had almost identical structures except that the latter had an additional methyl ester group. The mass spectral fragments at *m/z* 302 $[M-H_2O]^+$, 284 $[M-2H_2O]^+$, 290 $[M-CH_2O]^+$ and 274 $[M-HCOOH]^+$ supported the above conclusion [4]. Thus, compound **1a** was determined as 17-hydroxy-16-β-H-ent-kauran-19-oic acid.

When the ^{13}C NMR spectrum of **1** was compared with that of **1a**, the signal of C-17 in **1** was shifted downfield by +7.28 ppm, which indicated that the glycosylation took place at this position. The anomeric centre of the glucosyl group was confirmed as β based on $J_{1,2} = 7.8$ Hz. Consequently, the structure of **1** was



*Present address: Dept of Plant Science, Rutgers, The State University of New Jersey, New Brunswick, NJ 08904-0231, U.S.A.

Table 1. ^{13}C NMR data of compounds **1**, **2** and **1a** (pyridine-d_5)

C	1	2	1a	DEPT
1	41.2	40.9	41.4	CH_2
2	20.0	19.5	20.0	CH_2
3	38.9	38.4	38.9	CH_2
4	45.1	44.9	45.2	C
5	57.3	57.4	57.4	CH
6	23.3	22.7	23.4	CH_2
7	42.1	41.9	42.3	CH_2
8	44.0	44.0	44.1	C
9	55.8	55.4	55.9	CH
10	40.1	39.9	40.2	C
11	19.2	19.1	19.4	CH_2
12	31.7	31.3	32.1	CH_2
13	38.9	38.5	38.9	CH
14	37.5	37.0	37.6	CH_2
15	46.0	45.4	46.0	CH_2
16	41.3	41.0	41.4	CH
17	74.8	74.6	67.2	CH_2
18	29.5	28.6	29.5	CH_3
19	180.1	176.9	180.1	C
20	16.1	15.8	16.2	CH_3
17-Glc-1′	105.0	105.0		CH
2′	75.3	75.1		CH
3′	78.7	78.6		CH
4′	72.0	71.7		CH
5′	78.4	78.5		CH
6′	63.1	62.8		CH_2
19-Glc-1″		95.7		CH
2″		74.1		CH
3″		79.3		CH
4″		71.0		CH
5″		79.1		CH
6″		62.1		CH_2

established as that of 17-*O*-*β*-D-glucopyranosyl-16-*β*-H-ent-kauran-19-oic acid.

Compound **2** gave rise to IR absorptions for hydroxy groups (3400 cm^{-1}), an ester group (1725 cm^{-1}) and a glycosidic linkage (1000–1100 cm^{-1}). The molecular formula calculated as $C_{32}H_{52}O_{13}.H_2O$ by elemental analysis (Analyt. Calc.: C, 58.01, H, 8.16. Found: C, 58.25; H, 8.24) was in agreement with the data from the FAB-MS spectrum (*m/z* 667 $[M+Na]^+$ and 651 $[M+Li]^+$). A comparison of the ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) with those of **1** indicated the same aglycone as in **1**. On acid hydrolysis, **2** afforded glucose as the sugar component. The ^1H NMR spectrum showed the presence of two *β*-glucosyl moieties [*δ* 6.22 (*d*, $J = 7.7$ Hz) and 4.80 (*d*, $J = 7.9$ Hz)]. The ^{13}C NMR spectrum showed the signals of two glucosyl anomeric carbons (*δ* 95.72 and 104.95). The former was the signal of the esterified anomeric carbon of glucose, which was further supported by hydrolysis with alkali. Alkaline hydrolysis of compound **2** provided computer **1**, i.e. **2** was the glucosyl ester of **1** at the C-19 position. Therefore, the structure of **2** was determined as 17-*O*-*β*-D-glucopyranosyl-16-*β*-H-ent-kauran-19-oic acid-19-*O*-*β*-D-glucopyranoside.

Compound **3** was identified as 2-*O*-*β*-D-glucopyranosylatractyligenin by comparison of its spectral data with those reported in the literature [5]. This compound has been isolated from coffee for the first time. Four flavonoids were found to be identical to 4′,5,7-trihydroxy-3, 6-dimethoxy flavone-7-*O*-*β*-D-glucopyranoside (**4**) [6], isorhamnetin-3-*O*-*β*-D-glucopyranoside (**5**) [7], rhamnetin-3-*O*-*β*-D-glucopyranoside (**6**) [8] and kaemferol-3-*O*-*β*-D-glucopyranoside (**7**) [9], respectively, by comparison of various diagnostic data with reported values and authentic samples. Compound **8** was determined as sitosterol 3-*O*-*β*-D-glucopyranoside by direct comparison with an authentic sample.

EXPERIMENTAL

Mp: uncorr; $[\alpha]_D$: 28°; FAB-MS: direct inlet, glycerol as matrix; NMR: 400 MHz for δ_H and 100 MHz for δ_C; PC: Whatman No. 1 using the solvent systems *n*-BuOH–pyridine–H_2O (6:4:3) and *n*-BuOH–AcOH–H_2O (4:1:5, upper layer), respectively, and detected with aniline phthalate.

Plant materials. The flowers of *I. britannica* L. var *chinensis* were collected in 1990 from the suburb of Yanan city, Shanxi Province, northwestern China, and identified by professor Tian-Niang Pan (Yanan Institute of Medicine Inspection, Shanxi Province, China).

Extraction and separation. 60 kg of flowers of *I. britannica* were percolated with 95% EtOH (×3) at

Table 2. ^{1}H NMR data of compounds **1**, **2** and **1a** (pyridine-d_5)

H	1	2	1a
1a	0.78 *dt* (12.5, 3.2)	0.72 *dt* (12.5, 3.0)	0.83 *dt* (12.5, 3.3)
1*b*	1.81 *br d* (12.5)	1.74 *br d* (12.5)	1.89 *br d* (12.5)
2a	1.43 *m*	1.36 *m*	1.50 *m*
2b	2.21 *m*	2.14 *m*	2.35 *m*
3a	1.00 *m*	0.99 *m*	1.10 *m*
3b	2.44 *br d* (12.7)	2.36 *br d* (12.8)	2.47 *br d* (12.7)
5	0.95 *dd* (13.0, 3.0)	0.98 *dd* (13.0, 3.0)	1.07 *dd* (13.0, 3.1)
6a	2.15 *m*	2.33 *m*	2.18 *m*
6b	2.02 *br d* (13.0)	1.94 *m*	2.04 *br d* (13.0)
7	1.41 *m*	1.29 *m*	1.46 *m*
9	0.93 *dd* (6.8, 4.4)	0.90 *dd* (6.8, 4.4)	1.01 *dd* (6.8, 4.4)
11	1.49 *m*	1.45 *m*	1.57 *m*
12a	1.31 *m*	1.23 *m*	1.33 *m*
12b	1.38 *m*	1.37 *m*	1.42 *m*
13	2.18 *m*	2.19 *m*	2.22 *m*
14a	0.96 *m*	0.89 *m*	1.09 *m*
14b	1.80 *br d* (11.6)	1.92 *d* (11.7)	1.86 *d* (10.7)
15a	0.97 *m*	0.91 *m*	1.10 *m*
15b	1.53 *dd* (13.2, 10.8)	1.48 *dd* (13.2, 10.7)	1.63 *dd* (13.2, 10.6)
16	2.176 *m*	2.17 *m*	2.19 *m*
17a	3.44 *dd* (8.9, 8.8)	3.35 *dd* (9.0, 9.0)	3.67 *m*
17b	3.94 *dd* (8.8, 7.1)	3.90 *dd* (6.6, 9.0)	
18	1.32 *s*	1.23 *s*	1.35 *s*
20	1.09 *s*	1.22 *s*	1.16 *s*
17-Glc-1′	4.84 *d* (7.8)	4.80 *d* (7.9)	
2′	4.04 *dd* (7.8, 8.2)	4.02 *dd* (7.9, 8.2)	
3′	4.26 *dd* (8.2, 9.0)	4.23 *dd* (8.2, 9.0)	
4′	4.24 *dd* (9.0, 9.0)	4.21 *dd* (9.0, 9.0)	
5′	3.91 *dd* (9.0, 5.3, 1.8)	3.98 *ddd* 9.0, 5.0, 2.2)	
6′a	4.58 *dd* (11.7, 1.8)	4.59 *dd* (11.6, 2.2)	
6′b	4.40 *dd* (11.7, 5.3)	4.43 *dd* (11.6, 5.0)	
19-Glc-1″		6.22 *d* (7.7)	
2″		4.19 *dd* (7.7, 8.8)	
3″		4.24 *dd* (8.8, 9.2)	
4″		4.33 *dd* (9.2, 8.8)	
5″		4.33 *dd* (9.2, 8.8)	
6″a		4.00 *m* overlap	
6″b		4.42 *m* overlap	

room temp. After evapd of EtOH *in vacuo*, the extract was suspended in H_2O and then extracted exhaustively with petrol, $CHCl_3$, EtOAc and *n*-BuOH. The BuOH-soluble was chromatographed on a silica gel column (1.5 kg) with $CHCl_3$–MeOH (10:1–1:1) gradient to give five crude frs (1–5). Fr. 1 yielded **8** (60 mg) by recrystallization from MeOH. Fr. 2 was rechromatographed on a silica gel column ($CHCl_3$–MeOH–H_2O, 7:1:0.1) to give **4** (500 mg). Fr. 3 was further chromatographed on a silica gel column ($CHCl_3$–MeOH–H_2O, 60:10:1) to yield **5** (150 mg) and **6** (70 mg). Fr. was rechromatographed on a Sephadex LH-20 column (MeOH as solvent) to give fr. A (flavonoids) and fr. B (nonflavonids). 240 mg of **7** and 35 mg of **1** were obtained by recrystallization from fr. A and fr. B, respectively. The flavonoids in fr. 5 were removed by chromatography on Sephadex LH-20, and the remaining nonflavonoids were chromatographed on a RP-8 column with MeOH–H_2O (2:3) as eluent to afford **2** (54 mg) and **3** (456 mg).

Compound **1**. Needles, mp 230–232°. $[\alpha]_D$ −81.07° (MeOH, *c* 0.18), $C_{26}H_{42}O_8$. FAB-MS *m/z*: 505 $[M+Na]^+$ and 489 $[M+Na]^+$; IR ν_{KBr} cm^{-1}: 3400, 2920, 1685, 1450, 1000–1100.

Acid hydrolysis of **1**. A soln of compound **1** (8 mg) in 2 M HCl–MeOH (4 ml) was heated at 100° for 4 hr. After cooling to room temp., the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evapd *in vacuo*. The residue was dissolved in H_2O and extracted with Et_2O. From the aq. layer, glucose was identified by PC and TLC in direct comparison with a standard sugar.

Enzymatic hydrolysis of **1**. Compound **1** (12 mg) was incubated in 0.2 M NaOAc–HOAc (pH 5.4, 5 ml) with cellulase (80 mg) for 7 days at 37°. The hydrolysate was extracted with Et_2O. After removal of Et_2O, the residue was recrystallized from MeOH to afford the aglycone **1a** (9 mg): colourless needles, mp 182–184°, $C_{20}H_{32}O_3$, EI-MS *m/z*: 320 $[M]^+$, 302 $[M^+ - H_2O]$, 284 $[M^+ - 2H_2O]$, 274 $[M^+ - HCOOH]$, 305 $[M^+ -$

CH_3], 290 [$M^+ - CH_2O$], 123, 109, 107, 94.

Compound **2**. Needless, mp 224–226°, $[\alpha]_D$ −70.85° (MeOH, *c* 0.09). Analyt. Calcd for $C_{32}H_{52}O_{13}.H_2O$: C, 58.01; H, 8.16. Found: C, 58.25; H, 8.24. FAB-MS *m/z*: 667 $[M + Na]^+$ and 651 $[M + Li]^+$.

Conversion of **2** *to* **1**. A soln of **2** (15 mg) in 5% KOH (4 ml) was heated at 100° for 4 hr. The reaction mixture was cooled to room temp. and neutralized with dil HCl. After removal of MeOH, the remaining mixt. was passed through a column of highly porous resin eluted with H_2O and then MeOH. The H_2O eluent was followed by acid hydrolysis which showed the presence of glucose (identified by PC and TLC in direct comparison with a standard sugar. The MeOH eluate was subjected to CC over silica gel using $CHCl_3$–MeOH (5:1) as eluent to afford the prosapogenin which was identical with **1** (co-TLC and ^1H NMR).

Acid hydrolysis of **2**. A soln of **2** (3 mg) in 2 M HCl–MeOH (2 ml) was allowed to stand at 100° for 4 hr, and the precipitates formed were collected. The filtrate was evaporated repeatedly at 40° until the soln showed a neutral reaction. The residue was identified as glucose by PC and TLC in direct comparison with standard sugars.

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