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TRITERPENOID SAPONINS FROM *ASTER LINGULATUS*

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Key Word Index—*Aster lingulatus*; Compositae; triterpenoid saponins; asterlingulatosides A and B; DNA synthesis inhibition.

Abstract—Two new triterpenoid saponins named asterlingulatosides A and B were isolated from the whole plants of *Aster lingulatus*. Their structures were determined by spectroscopic data and chemical transformations to be 3-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid-28-*O*-α-L-*O*-arabinopyranoside and 3-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid-28-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside. They showed inhibitory activity on DNA synthesis in human leukaemia HL-60 cells. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Several species of the genus *Aster* have been studied by Shao and co-workers [1–9], Nagao and co-workers [10, 11] and Schöpke *et al.* [12] and shown to contain triterpenoid saponins. As a part of our continuing efforts to investigate saponins of plants belonging to this genus, we have now undertaken a phytochemical examination of *Aster lingulatus*, which has not been chemically studied before. As a result of this study, two new oleane-type triterpenoid saponins were isolated. This paper deals with their structural elucidation and their inhibitory activity on [^{3}H]thymidine incorporation into DNA of human leukaemia HL-60 cells, which was used as preliminary screening test for identifying new antitumour compounds [13, 14].

RESULTS AND DISCUSSION

The *n*-butanol-soluble fraction of a 70% ethanol extract from the whole plants of *A. lingulatus* was chromatographed over Diaion HP-20, silica gel, Sephadex LH-20 and C_8 reversed-phase silica gel to yield compounds **1** and **2**.

Compound **1** was obtained as an amorphous powder, mp 199–201°, $[\alpha]_D$ + 7.6° (methanol, *c* 1.0). It responded positively to the Liebermann–Burchard and Molish colour tests and showed characteristic IR absorption bands at 3420 (OH), 1730 (CO_2R), 1634 (C=C) and 1077 (glycosidic linkage) cm^{-1}. The molecular formula $C_{41}H_{66}O_{13}$ was deduced from the ^{13}C NMR spectrum and the negative-ion APCI mass spectrum showing an $[M-H]^-$ ion at *m/z* 765 and an $[M+Cl]^-$ ion at *m/z* 802 which resulted from the reaction of the molecular ion with HCl. The ^{1}H NMR spectrum of **1** displayed signals for typical pentacylic triterpenoid methyl groups at δ 0.89, 1.00, 1.03, 1.10, 1.16, 1.29 and 1.84 (each 3H, each *s*, *tert*-methyl ×7), one trisubstituted olefinic proton at δ 5.30, and two sugar anomeric protons at δ 4.97 (1H, *d*, *J* = 7.9 Hz) and 6.30 (1H, *d*, *J* = 5.9 Hz). The noise-decoupled ^{13}C NMR and DEPT spectra of **1** showed resonances indicative of six C–C bonded saturated quaternary carbons at δ 30.9, 36.9, 39.4, 39.9, 42.0 and 49.5, and two anomeric carbons at δ 122.8 and 144.4, an ester carbonyl carbon at δ 175.9, and two anomeric carbons at δ 95.9 and 106.7. The number and chemical shifts of the tertiary methyl groups and quaternary carbons suggested that the aglycone moiety was of the olean-12-ene type with an ester carbonyl at the C-28 position. The presence of an ester-linked sugar moiety was inferred from the chemical shift of the anomeric proton at δ 6.30 and the chemical shift of the anomeric carbon at δ 95.9 [11].

Alkaline hydrolysis of **1** yielded a prosapogenin and arabinose, detected by paper chromatography and TLC in direct comparison with an authentic sample. Upon treatment with diazomethane, the prosapogenin was converted into its methyl ester (**1a**). Acid hydrolysis of **1a** afforded glucose and an aglycone which was also converted into its methyl ester (**1b**). Compounds **1b** and **1a** were identified as 3β,16α-dihydroxyolean-12-en-28-oic acid methyl ester and 3-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid methyl ester, respectively, by comparison of their NMR and mass

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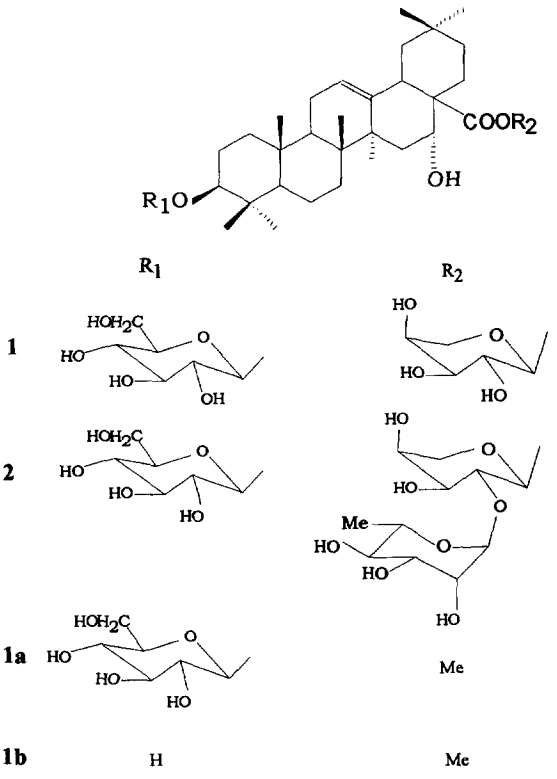
spectral data with those reported in the literature [8, 15] as well as by direct comparison with an authentic sample obtained in our laboratory [8]. These results indicated that a β-D-glucopyranosyl unit was bound to the C-3 position and an arabinose unit to the C-28 position by a glycosidic linkage. The α-configuration at the centre of the arabinose was suggested by the large $J_{1,2}$ coupling constant (5.9 Hz) and the ^{13}C NMR chemical shifts. On the basis of these findings, the structure of **1** was concluded to be 3-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid-28-*O*-α-L-arabinopyranoside and this compound has been named asterlingulatoside A.

Compound **2** was obtained as an amorphous powder, mp 218–220°, $[\alpha]_D$ −32.1° (methanol, *c* 0.8). The negative ion APCI mass spectrum showed two quasimolecular ion peaks at *m/z* 912 $[M-H]^-$ and 947 $[M+Cl]^-$ which, together with the ^{13}C NMR data, suggested the molecular formula as $C_{47}H_{76}O_{17}$. The molecular weight of **2** was 146 mu larger than that of **1**, indicating that **2** might be a deoxyhexosyl glycoside of **1**. A comparison of the NMR data for **2** with those for **1** revealed that the signals due to the aglycone moiety of **2** were identical to those of **1**. This observation suggested that **2** was also an echinocystic acid 3,28-bisdesmoside. Acid hydrolysis of **2** provided echinocystic acid, glucose, arabinose and rhamnose. Alkaline hydrolysis with 5% KOH followed by reaction with diazomethane gave a prosapogenin methyl ester which was proved to have the same structure as the prosapogenin methyl ester (**1b**) of asterlingulatoside A by comparison of their NMR data. Thus, the sugar residue at the C-3 position of **2** was established to be β-D-glucopyranosyl.

The ^1H NMR spectrum of **2** showed the presence of three anomeric signals at δ 5.84 (1H, *br* s), 4.96 (1H, *d*, $J=7.6$ Hz) and 6.54 (1H, *d*, $J=3$ Hz). Its ^{13}C NMR spectrum showed three anomeric carbon signals at δ 93.5, 101.4 and 106.9. These data indicated that the 28-*O*-sugar moiety consisted of one rhamnose unit and of one arabinose unit. Comparison of the ^{13}C NMR data of 28-*O*-sugar moieties in **2** with those for the corresponding individual methyl glycopyranoside indicated the rhamnosyl unit was located in the terminal position, because its ^{13}C NMR data were identical to those for methyl α-L-rhamnopyranoside [16]. In addition, a glycosylation shift was observed at the C-2 signal of the arabinose residue by +3.3 ppm, i.e. the rhamnose unit was linked to the C-2 position of an arabinose residue unit. The anomeric proton and carbon signals of the arabinose unit at δ 6.54 and 93.5, respectively, confirmed that the arabinose unit was directly linked to the C-28 position. The configuration of the rhamnopyranosyl group was determined as α on the basis of its ^{13}C NMR data, especially the C-5 chemical shift at δ 70.4 [16, 17]. The arabinosyl unit was presumed to be in the α-configuration and in the $^1C_4$ conformation based on the $J_{1,2}=3$ Hz coupling constant and the NMR data, which were identical to those of desacyl-lobatoside B (3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-bayogenin-28-α-L-



rhamnopyranosyl-(1→2)-α-L-arabinopyranoside) [18]. Therefore, compound **2** was deduced to be 3-*O*-β-D-glucopyranosyl-3β,16α-dihydroxyolean-12-en-28-oic acid-28-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside and named as asterlingulatoside B.

Compounds **1** and **2** were evaluated for *in vitro* antitumour activity by means of their inhibitory effect on [^3H]thymidine incorporation into DNA in HL-60 cells [13, 14]. They inhibited the rate of DNA synthesis by 20.1 and 18.2% at concentrations of 5 μM, 54.4 and 43.3% at 25 μM, and 87.2 and 81.9% at 100 μM, respectively.

EXPERIMENTAL

General. Mps (uncorr.) were determined on a Kofler apparatus; $[\alpha]_D$ values were measured at 28° on a Jasco DIP-181 polarimeter. IR spectra were obtained on a Mattson Cygnus 100 Fourier transform IR spectrometer. EIMS and APCI MS were obtained on MAT-95 and Fisons/VG Platform II mass spectrometers, respectively. No column was used for APCI mass analysis. The mobile phase was MeOH/H_2O (1:1), the H_2O contained 0.05% HCl. NMR spectra were run on a Nicolet NT-360 (360 MHz for δ_H, 90 MHz for δ_C) spectrometer. PC and TLC of sugars were developed on Whatman No. 1 paper using the solvent systems *n*-BuOH–pyridine–H_2O (6:4:3) and *n*-BuOH–HOAc–H_2O (4:1:5, upper layer), respectively, and detected with aniline phthalate. The human leukaemia cell line HL-60 was obtained from American Type Culture Collection (Rockville, MD). Radioactivity was mea-

sured in a Beckman LS 11701 scintillation counter.

Plant material. The whole plants of *A. lingulatus* were collected in August 1992 from Li-Jiang county, Yunnan Province, southwestern China. A voucher specimen was identified by Prof. Z. W. Lu and deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica, Yunnan Province.

Extraction and isolation. Dried whole plants of *A. lingulatus* (7 kg) were extracted 5× with 70% EtOH (each 1 week) at room temp. The combined extracts were evapd to dryness *in vacuo* to afford 1 kg residue. This residue was suspended in H_2O and then extracted successively with petrol, EtOAc and H_2O-satd *n*-BuOH to yield 3 corresponding frs (145, 280 and 470 g, respectively). The *n*-BuOH extract was subjected to CC over Diaion HP-20, eluted with H_2O and then MeOH. The MeOH eluate (425 g) was subjected to CC on silica gel (1.8 kg, 200–300 mesh), eluted with $CHCl_3$–MeOH–H_2O (8:1:0.1–1:1:0.1) gradient to separate into 5 crude frs (1–5). Fr.1 was repeatedly subjected to CC over silica gel using $CHCl_3$–MeOH (6:1) as solvent, and further purified over Lichroprep RP-8 eluted with MeOH–H_2O (7:4) to give 195 mg **1**. Fr.2 was initially subjected to CC over Sephadex LH-20 using MeOH as solvent to be sepd into 2 frs. The fr. mainly containing saponins was purified by Lichroprep RP-8 CC, eluted with MeOH–H_2O (3:2), to yield 346 mg **2**.

Asterlingulatoside A (**1**). Amorphous powder. Mp 199–201°. $[\alpha]_D$ + 7.6° (MeOH, *c* 1.0). $C_{41}H_{66}O_{13}$. Negative ion APCI MS *m/z*: 802 $[M+Cl]^-$, 765 $[M-H]^-$, 633 [M-Ara-H]$^-$, 603 [M-Glc-H]$^-$, 471 [M-Ara-Glc-H]$^-$. IR ν_{KBr} cm^{-1}: 3420, 1730, 1634, 1077. ^1H NMR (pyridine-d_5): aglycone moiety: δ 0.89, 1.00, 1.03, 1.10, 1.16, 1.29 1.84 (each 3H, each *s*, *tert*-Me × 7), 3.42 (1H, *dd*, *J* = 10 and 5 Hz, H-3), 5.30 (1H, *br s*, H-16), 5.64 (1H, *br s*, H-12); sugar moiety: δ 4.97 (1H, *d*, *J* = 7.9 Hz, H-1 of Glc unit), and 6.30 (1H, *d*, *J* = 5.9 Hz, H-1 of Ara unit). ^{13}C NMR data: Table 1.

Asterlingulatoside B (**2**). Amorphous powder. Mp 218–220°. $[\alpha]_D$ − 32.1° (MeOH, *c* 0.8). $C_{47}H_{76}O_{17}$. Negative ion APCI MS *m/z*: 911 $[M-H]^-$, 847 $[M+Cl]^-$, 755 [M-Rha-H]$^-$, 749 [M-Glc-H]$^-$, 633 [M-Ara-Rha-H]$^-$, 603 [M-Glc-Rha-H]$^-$, 471 [M-Glc-Ara-Rha-H]$^-$. IR $\nu_{KBr}$$cm^{-1}$: 3415, 1727, 11634, 1077, 1049. ^1H NMR (pyridine-d_5, 360 MHz): δ 0.88, 0.99, 1.03, 1.10, 1.16, 1.29, 1.83 (each 3H, each *s*, *tert*-Me × 7), 3.41 (1H, *dd*, *J* = 10, 5 Hz, H-3), 5.30 (1H, *br s*, H-16), 5.63 (1H, *br s*, H-12), 1.71 (3H, *d*, *J* = 6 Hz, Me of Rha), 5.84 (1H, *br s*, H-1 of Rha), 4.96 (1H, *d*, *J* = 7.6 Hz, H-1 of Glc), 6.54 (1H, *d*, *J* = 3 Hz, H-1 of Ara). ^{13}C NMR: Table 1.

Alkaline hydrolysis of **1** *and* **2**. A soln of each compound (50 mg **1**, or 10 mg **2**) in 5% KOH–MeOH (8 or 3 ml) was heated at 100° for 4 hr. Each reaction mixt. was cooled to room temp and neutralized with aq. HCl. After removal of MeOH, the remaining mixt. was passed through a column of Diaion HP-20 and eluted with H_2O and then MeOH. The H_2O eluate was evapd to dryness *in vacuo* and followed by acidic hydrolysis with 10% HCl to give sugar components which were identified by PC and TLC in direct comparison with authentic samples. To the MeOH eluate, ethereal CH_2N_2 was added, and the reaction mixt. kept at room temp overnight. The solvent was then evapd to dryness. Each residue was subjected to CC over silica gel using $CHCl_3$–MeOH (10:1) as solvent to afford **1a** (38 mg) as needles. Its physical properties and spectral data were identical to those reported in the lit. [8].

Table 1. ^{13}C NMR spectral data for compounds **1** and **2**

C	1	2	DEPT
Aglycone part			
1	38.8	38.8	CH_2
2	26.6	26.6	CH_2
3	88.6	88.7	CH
4	39.4	39.4	C
5	55.8	55.8	CH
6	18.6	18.5	CH_2
7	33.4	33.4	CH_2
8	39.9	40.0	C
9	47.1	47.1	CH
10	36.9	36.9	C
11	23.8	23.8	CH_2
12	122.7	122.7	CH
13	144.4	144.4	C
14	42.0	42.0	C
15	36.1	36.1	CH_2
16	73.9	74.0	CH
17	49.5	49.5	C
18	41.2	41.2	CH
19	47.1	47.1	CH_2
20	30.9	30.9	C
21	35.9	35.9	CH_2
22	32.1	32.1	CH_2
23	28.2	28.2	Me
24	17.0	17.0	Me
25	15.6	15.6	Me
26	17.5	17.5	Me
27	27.1	27.1	Me
28	175.9	175.8	C
29	33.2	33.3	Me
30	24.7	24.7	Me
3-*O*-Sugar*			
Glc-1	106.7	106.9	CH
2	75.8	75.8	CH
3	78.7	78.7	CH
4	71.8	71.7	CH
5	78.3	78.3	CH
6	62.8	62.9	CH_2
28-*O*-Sugar*			
Ara-1	95.9	93.5	CH
2	71.4	75.2	CH
3	73.6	70.0	CH
4	67.6	66.1	CH
5	65.8	63.1	CH_2
Rham-1		101.4	CH
2		72.3	CH
3		72.6	CH
4		73.8	CH
5		70.4	CH
6		18.5	Me

*Glc = β-D-glucopyranosyl; Ara = α-L-arabinopyranosyl; Rham = α-L-rhamnopyranosyl.

Acid hydrolysis of **1a** *and* **2**. A soln of each compound (20 mg **1a** or 10 mg **2**) in 2 N HCl–MeOH (4 or 3 ml) was refluxed for 3 hr. After cooling to room temp. the reaction mixt. was extracted with Et_2O. The Et_2O extracts were dried over Na_2SO_4 and evapd to dryness under red. pres. and treated with ethereal CH_2N_2, and then recrystallized from MeOH to yield the Me ester of **1b**. Each aq. soln was repeatedly evapd with addition of H_2O to give a residue which was examined by PC and TLC in direct comparison with an authentic sample to show the presence of Glu for **1a**, or Ara, Glc and Rha for **2**. The physical properties and spectral data of **1b** were identical to those reported in the lit. [8, 15].

Cell culture and assay of [^{3}H]*thymidine incorporation into DNA of the cultured cells*. HL-60 cells were cultured in RPMI-1640 medium supplemented with 10% foetal calf serum and 1% penicillin–streptomycin in a humidified atmosphere containing 5% CO_2 at 37°. To each 1 ml of HL-60 cells (5×10^5 cells ml^{-1}) suspended in RPMI-1640 medium without foetal calf serum and 3 μl [^{3}H]thymidine (50 μCi μmol^{-1}), the test sample (5, 25 or 100 μM) was added and then incubated for 2 hr at 37°. The reaction was terminated by addition of 1 ml cold phosphate buffer saline soln, and the rate of DNA synthesis was determined as previously described [13, 14]. The percentage incorporation shown is expressed relative to the control incubation and represents an average of 3 experiments.

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