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Terpenoid constituents of *Aster subspicatus* and *A. ageratoides*

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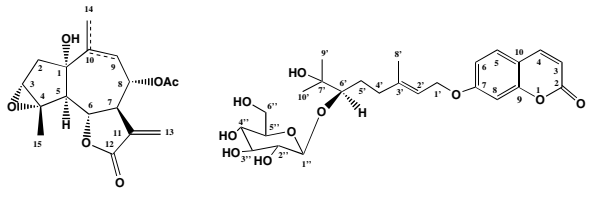
Chemical investigation of the aerial parts of *Aster subspicatus* afforded a new sesquiterpene lactone, 8α-acetoxy-1α-hydroxy-3α,4α-epoxy-5α,7αH-9,11(13)-guaiadien-12,6α-olide (**1**), in addition to the known compounds arteglasin-B (**2**), diversoside (**3**), and 2-phenylethyl β-D-glucopyranoside. Re-investigation of *Aster ageratoides* afforded the known compounds crotocorylifuran, and oplopanone. The structure of the new compound was determined by spectroscopic methods particularly high resolution ^{1}H, ^{13}C NMR, DEPT, 2D ^{1}H-^{1}H and ^{1}H-^{13}C COSY NMR and HMBC analysis.

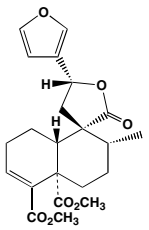
1. Introduction

Aster is a large genus of the family Asteraceae, comprising more than 250 species distributed around the world. The plants of this genus are widely distributed in China, especially in the west district of China [1], and contain more than 100 species, among them, 15 species have been used as drugs [2]. Several members of this species have been used in traditional systems of medicine for the treatment of fevers, colds, tonsillitis, snake bites, bee stings [3, 4], and some species demonstrated some biological activities including anti-inflammatory, insecticidal and antitumour [5]. The biological importance of members of this genus prompted us to investigate the aerial parts of *Aster subspicatus* Nees, which has not been previously investigated chemically, and to re-investigate the aerial parts of *Aster ageratoides* Turcz. Earlier work on the chemistry of this genus showed that triterpene glycosides are the main constituents [1–4, 6–10], however some diterpenes and sesquiterpenes were also isolated [5, 11–15]. In this work we report the isolation and structure elucidation of a new sesquiterpene lactone, 8α-acetoxy-1α-hydroxy-3α,4α-epoxy-5α,7αH-9,11(13)-guaiadien-12,6α-olide (**1**) from *A. subspicatus*, in addition to the known compounds arteglasin-B (**2**) [16], diversoside (**3**) [17,18], and 2-phenylethyl β-D-glucopyranoside [19]. *A. ageratoides* afforded the known compounds crotocorylifuran (**4**) [20] and oplopanone (**5**) [21–22].



1 : Δ^9
2 : $\Delta^{10(14)}$

3



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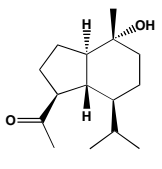

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2. Investigation, results and discussion

The aerial parts of *Aster subspicatus* contained a new sesquiterpene lactone **1**. High resolution positive ion CIMS showed a molecular ion peak at *m/z* 321.1331 in accordance with a molecular formula $C_{17}H_{21}O_6$ (confirmed by ^{13}C NMR and DEPT analysis). Two fragments at *m/z* 303 and 261, resulting from loss of a water and an acetic acid molecule, respectively, indicated the presence of hydroxyl and acetoxy groups. The ^{1}H and ^{13}C NMR spectral data of **1** established the presence of a guaianolide type sesquiterpene with an acetoxy group. The spectra also contained signals typical of an acetate group at δ 2.18 (s, 3H) in the ^{1}H NMR and δ 21.0 (q), 170.0 (s) in the ^{13}C NMR. The α-methylene-γ-lactone grouping with two doublets at δ 5.79 and 6.38 (each 1 H, J = 3.0 Hz) correlated with an olefinic methylene carbon signal at δ 124.6 in the HMQC spectrum (C-13), a one-proton doublet of doublets at δ 3.84 (J = 10.5, 11.5 Hz) corresponding to the lactonic proton at C-6 (d, 75.3), and the carbonyl signal at δ 168.5 (s), which is characteristic for C-12. The presence of an epoxy group at C-3/C-4 was inferred from the sharp singlet at δ 1.72 (3 H, H-15), together with a broadend-singlet at δ 3.55 (H-3), which correlated with a methine carbon signal at δ 62.9, in the HMQC spectrum. The structure of **1** was followed from the ^{1}HNMR spectrum and was very similar to that of arteglasin-B (**2**) [16]. The difference between the two structures being the exomethylene protons H-14 and H-14′ of **2** were replaced by a broad singlet at δ 5.29 and a new olefinic methyl signal in the ring was observed at δ 1.92. Accordingly, compound **1** was the Δ^9-isomer of **2**. This was supported by the ^{13}CNMR spectrum, which showed a trisubstituted double bond signals at δ 122.5 (d, C-9) and 139.0 (s, C-10), instead of the exomethylene signals in **2**. The absence of a proton at C-1 suggested the presence of a hydroxyl group at this position. The stereochemistry of **1** was followed from the coupling constants and the NOE's. The relative configuration and stereochemistry at C-5, C-6 and C-7 were derived from the coupling constants ($J_{5,6} = 10.5$ and $J_{6,7} = 11.5$ Hz), which were in agreement with the *trans*-diaxial disposition of the protons at C-5 (α), C-6 (β) and C-7 (α). The appearance of H-3 as a broad singlet deduced the α-configuration of the epoxide at C-3/C-4 (16, 22). The presence of H-5 at lower field (δ 2.58) suggested that the 1-hydroxyl group was in the α-orientation [23–25]. These results were supported by the NOE effects, irradiation of the signal at δ 3.30 (H-7) and 4.21 (1-OH) enhanced the signal at δ 2.58 (H-5). Irradiation of the signal at δ 3.84 (H-6) enhanced the signal at δ 5.28 (H-8) Furthermore,

Table 1: ^{1}H, ^{13}C, and HMBC spectral data ($CDCl_3$, 500 MHz) of compounds 1 and 2

	1*			2*		
Position	δ_H (mult., J in Hz)	δ_C (mult.)	HMBC	δ_H (mult., J in Hz)	δ_C (mult.)	HMBC
1	–	80.4 (s)	–	–	81.7 (s)	
2a	2.47 (1 H, d, J =15.5)	42.0 (t)	C-1, C-3, C-4, C-5	2.22 (1 H, d, J = 15.5)	40.4 (d)	C-1
2b	1.93 (1 H, d, J = 15.5)		C-1, C-5	1.90 (1 H, d, J = 15.5)		C-1, C-3, C-4
3	3.55 (1 H, s)	62.9 (d)	C-1, C-2	3.57 (1 H, s)	64.3 (d)	C-1, C-2, C-5
4	–	67.1 (s)	–	–	67.2 (s)	–
5	2.58 (1 H, d, J = 10.5)	60.1 (d)	C-1, C-2, C-3, C-6, C-7	2.35 (1 H, d, J = 12.0)	60.9 (d)	C-1, C-2, C-3, C-6, C-7
6	3.84 (1 H, dd, J = 10.5, 11.5)	75.3 (d)	C-8	3.91 (1 H, t, J = 12.0)	75.4 (d)	C-4, C-8, C-11
7	3.30 (1 H, m#)	47.8 (d)	C-6, C-11	3.23 (1 H, m)	46.5 (d)	C-6, C-11, C-13
8	5.28 (1 H, m#)	72.0 (d)	C-7, C-9, CO (AcO)	4.93 (1 H, m)	73.1 (d)	C-11, CO (AcO)
9a	5.29 (1 H, brs)	122.5 (d)	C-1, C-8, C-7, C-14	2.57 (1 H, dd, J = 15.0, 2.5)	35.0 (t)	C-1, C-7, C-8, C-10, C-14
9b				2.25 (1 H, brd, J = 15.0)		C-8, C-14
10	–	139.0 (s)	–	–	140.4 (s)	–
11	–	135.5 (s)	–	–	136.3 (s)	–
12	–	168.5 (s)	–	–	168.5 (s)	–
13a	6.38 (1 H, d, J = 3.0)	124.6 (t)	C-7, C-11, C-12	5.62 (1 H, d, J = 3.0)	122.6 (t)	C-7, C-11, C-12
13b	5.79 (1 H, d, J = 3.0)		C-7, C-11, C-12	6.22 (1 H, d, J = 3.0)		C-7, C-11, C-12
14a	1.92 (3 H, s)	24.7 (q)	C-1, C-9, C-10	5.53 (1 H, brs)	118.0 (t)	C-1, C-9, C-10
14b				5.00 (1 H, brs)		C-1, C-9
15	1.72 (3 H, s)	19.5 (q)	C-3, C-4, C-5	1.64 (3 H, s)	18.5 (q)	C-3, C-4
OAc	2.18 (3 H, s)	21.0 (q)	–	2.14 (3 H, s)	170.0 (s)	–
		170.0 (s)	–		21.1 (q)	–
OH	4.21 (1 H, brs)					

* Assignments by ^{1}H-^{1}H COSY, HMQC and HMBC experiments. Carbon multiplicities were determined by DEPT experiments; s = quaternary, d = methine, t = methylene, q = methyl carbons.
# Overlapping signals

irradiation of the signal at δ 1.72 (H-15) showed effect on the signal at 3.55 (H-3) and no effect was observed on H-5. This suggested the β-configuration of H-3 and H-15. The multiplicities of the carbon signals were deduced by DEPT experiments, whereas the assignments of all proton signals and their connectivity to adjacent protons and carbon signals were established from the results of the 2D ^{1}H-^{1}H COSY, HMQC and the long-range coupling HMBC experiments. Therefore, **1** was identified to be 8α-acetoxy-1α-hydroxy-3α,4α-epoxy-5α,7αH-9,11(13)-guaiadien-12,6α-olide. The non-acetylated skeleton of **1** and **2** reported from *Achillea clypeolata* [26]. The previously unreported NMR data of arteglasin B (**2**) are given in the Table 1 and those of diversoside (**3**) in the Experimental section.

3. Experimental

3.1. Equipment

^{1}HNMR (500 MHz, $CDCl_3$), ^{13}CNMR (125 MHz, $CDCl_3$) and the 2D spectra were recorded on a JEOL 500 MHz, Lambda spectrometer, with TMS as internal standard. Optical rotation was determined with a JASCO-20C automatic recording spectropolarimeter. TLC: precoated silica gel type 60 (Merck); CC: silica gel type 60 (Merck). HPLC was performed in the reverse phase on knauer pump 64 and different refractometer (column: RP-8, 250 × 25 mm, flow = 17 mL/min, elution with MeOH−H_2O, mixtures, refractive index).

3.2. Plant material

A. subspicatus was collected during the flowering stage in August 1997 on Doeswallips river tide flats on the Olympic Peninsula of Washington State, USA. The plant material was identified by Dr. J. Karchesy, Department of Forest Products, Oregon State University, USA. A voucher specimen has been deposited in the Department of Forest Products, USA. *A. ageratoides* was collected during the flowering stage in August 1994 on Gifu, around Gifu Pharmaceutical University, Japan. A vaucher specimen has been deposited in the Department of Chemistry, Faculty of Science, El-Minia Univeristy, El-Minia, Egypt.

3.3. Extraction and separation

For each plant, the following standard procedure was adopted. The air-dried plant materials (500 g for *A. subspicatus* and 150 g for *A. ageratoides*) were ground and extracted at room temperature with CH_2Cl_2−MeOH (1 : 1). The extracts were concentrated in vacuo to obtain residues of 12 g from *A. subspicatus* and 5.5 g *A. ageratoides*. The extract of *A. subspicatus* was pre-fractionated by cc (6 × 120 cm) on silica gel eluting with *n*-hexane (3l) followed by a gradient of *n*-hexane-CH_2Cl_2 up to 100% CH_2Cl_2 and CH_2Cl_2−MeOH up to 15% MeOH (2l each of solvent mixture). The 100% CH_2Cl_2 fraction was further purified by cc (2 × 40 cm) on Sephadex LH-20 eluted with *n*-hexane−CH_2Cl_2 (6 : 3) to obtain 8α-acetoxy-1α-hydroxy-3α,4α-epoxy-5α,7αH-9,11(13)-guaiadien-12,6α-olide (9 mg) and arteglasin-B (15 mg). The CH_2Cl_2−MeOH (9 : 1) fraction was subjected to silica gel column (3 × 50) eluted with CH_2Cl_2–MeOH (9.5 : 0.5, 9 : 1). The first fraction, was further purified by cc (2 × 30) on Sephadex LH-20 eluted with *n*-hexane−CH_2Cl_2−MeOH (7 : 4 : 0.5) to obtain diversoside (21 mg). The second fraction was purified by HPLC (MeOH-H_2O, 65 : 35, R_t = 5.6 min) to give 2-phenylethyl β-D-glucopyranoside (6 mg). The extract of *A. ageratoides* was separated by flash column, silica gel, using *n*-hexane, increasing the degree of polarity by addition of CH_2Cl_2. The 75% CH_2Cl_2 was further purified by column chromatography (2.5 × 50) on Sephadex LH-20 eluted with *n*-hexane−CH_2Cl_2 (7 : 3) to obtain oplopanone (9 mg). The CH_2Cl_2−MeOH (9 : 1) was purified by HPLC (MeOH−H_2O, 1 : 1, Rt. = 13.8 min) to give 2-phenylethyl β-D-glucopyranoside (5 mg).

3.4. 8α-Acetoxy-1α-hydroxy-3α,4α-epoxy-5α,7αH-9,11(13)-guaiadien-12,6α-olide (1)

Colorless oil, $[\alpha]^{25}$ D : +8.8 ($CHCl_3$, *c* = 0.34); IR = 3466 (OH), 2924, 1770 (unsaturated γ-lactone C=O), 1742 cm$^{-1;}$ Positive ion HR-CIMS: 321.1331 (calc. for $C_{17}H_{21}O_6$, 321.1338); CI-MS *m/z* (rel. int.): 321 $[M + H]^+$ (30), 303 $[M + H-H_2O]^+$ (18), 261 $[M + H-AcOH]^+$ (100), 243 (40).

3.5. Arteglasin-B (*2*)

Positive ion HR-CIMS: 321.1343 (calc. for $C_{17}H_{21}O_6$, 321.1333); CI-MS *m/z* (rel. int.): 321 $[M + H]^+$ (95), 303 $[M + H-H_2O]^+$ (20), 261 $[M + H-AcOH]^+$ (100).

3.6. Diversoside (*3*)

Gummy material; ^{1}H NMR ($CDCl_3$, 500 MHz): $\delta = 1.35$ (3 H, s, H-10′), 1.49 (3 H,s, H-9′), 1.73 (2 H, m, H-5′), 1.73 (3 H, br s, H-8′), 2.58 (1 H, m, H-4a′), 2.66 (1 H, m, H-4b′), 3.76 (1 H, br d, J = 9.5 Hz, H-6′), 3.87 (1 H, ddd , J = 9, 5, 3 Hz, H-5″), 4.05 (1 H, dd, J = 10, 8 Hz, H-2″), 4.20 (1 H, dd, J = 10, 9 Hz, H-3″), 4.23 (1 H, dd, J = 9, 9 Hz, H-4″), 4.42 (1 H, dd, J = 16, 5 Hz; H-6a″), 4.56 (1 H, dd, J = 16, 3 Hz, H-6b″), 4.66 (1 H, d, J = 6.5 Hz, H-1), 5.09 (1 H, d, J = 8 Hz, H-1″), 5.81 (1 H, br t, J = H-2′), 6.27 (1 H, d, J = 9.5 Hz, H-3), 6.93 (1 H, dd, J = 8.5, 2 Hz, H-6), 6.98 (1 H, d, J = 2 Hz, H-8), 7.37 (1 H, d, J = 8.5 Hz, H-5), 7.62 (1 H, d, J = 9.5 Hz, H-4); ^{13}CNMR ($CDCl_3$, 500 MHz): $\delta = 16.7$ (q, C-8′), 24.2 (q, C-9′), 26.5 (q, C-10′), 29.9 (t, C-5′), 36.2 (t, C-4′), 62.7 (t, C-6″), 65.6 (t, C-1′), 71.6 (d, C-4″), 73.3 (s, C-7′), 76.1 (d, C-2″), 78.3 (d, C-5″), 78.8 (d, C-3″), 90.2 (d, C-6′), 101.9 (d, C-8), 106.6 (d, C-1″), 112.8 (s, C-10), 113.1 (d, C-6), 113.3 (d, C-3), 119.7 (d, C-2′), 129.4 (s, C-5), 142.4 (s, C-3′), 143.8 (d, C-4), 156.4 (s, C-9), 160.9 (C-2), 162.5 (s, C-7).

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References

1 Shao, Y.; Zhou, B. N.; Ma, K.; Wu, H.; Lin, L.; Cordell, G. A.: Phytochemistry **39**, 875 (1995)
2 Shao, Y.; Li, Y. L.; Zhou, B. N.: Phytochemistry **41**, 1593 (1996)
3 Shao, Y.; Zhou, B. N.; Ma, K.; Wu, H-M.: Planta Med. **61**, 246 (1995)
4 Shao, Y.; Ho, C. T.; Chin, C. K.: J. Nat. Prod. **60**, 743 : (1997)
5 Changzeng, W.; Dequan, Y.; Phytochemistry **45**, 1483 (1997)
6 Shao, Y.; Zhou, B. N.: J. Nat. Prod. **58**, 837 (1995)
7 Schöpke, T.; Al-Tawaha, C.; Wray, V.; Nimtz, M.; Hiller, K.: Phytochemistry **45**, 125 (1997)
8 Shao, Y.; Zhou, B. N.; Lin, L. Z.; Cordell, G. A.: Phytochemistry **38**, 92 (1995)
9 Shao, Y.; Zhou, B. N.; Gao, J. H.; Lin, L. Z.; Cordell, G. A.: Phytochemistry **38**, 675 (1995)
10 Sakai, K.; Nagao, T.; Okabe, H.: Phytochemistry **51**, 309 (1999)
11 Shao, Y.; Wang, M. F.; Ho, C. T.; Chin, C. K.; Yang, S. W; Cordell, G. A.; Lotter, H.; Wagner, H.: Phytochemistry **49**, 609 (1998)
12 Bohlmann, F.; Jakupovic, J.; Hashemi-Nejad, M.; Huneck, S.: Phytochemistry **24**, 608 (1985)
13 Cheng, D.L.; Cao, X. P.; Wei, H. X.; He, L.: Phytochemistry **33,** 1181 (1993)
14 Guo, S. J.; Katalinic, J. P.; He, L.; Cheng, D.L.: Pharmazie **53**, 481 (1998)
15 Bohlmann, F.; Dutta, L. N.; Knauf, W.; Robinson, H.; King, R. M.: Phytochemistry **19,** 433 (1980)
16 Lee, K. H.; Matsueda, S.; Geissman, T. A.: Phytochemistry **10**, 405 (1971)
17 Kamilov, Kh. M.; Kiseleva, V. V.; Nikonov, G. K.: Khim. Prir. Soedin. **6**, 781 (1974)
18 Kiseleva, V. V.: Khim. Prir. Soedin. **10,** 801 (1978)
19 Umehara, K.; Hattori, I., Miyase; T., Ueno, A.; Hara, S.; Kageyama, C.: Chem. Pharm. Bull. **36**, 5004 (1988)
20 Tchissambou, L.; Chiaroni, A.; Riche, C.; Khuong-Huu, F.: Tetrahedron **46**, 5199 (1990)
21 Takeda, K.; Minato, H.; Ishikawa, M.: Tetrahedron Supplement No. **7**, 219 (1966)
22 Appendino, G.; Jakupovic, J.: Phytochemistry **46**, 1039 (1997)
23 Bohlmann, F.; Ang, W.; Trinks, C.; Jakupovic, J.; Huneck, S.: Phytochemistry **24,** 1003 (1985)
24 Al-Easa, H. S.; Rizk, A. M.; Ahmed, A. A.: Phytochemistry **43**, 423 (1996)
25 Bohlmann, F.; Hartono, L.; Jakupovic, J.;Huneck, S.: Phytochemistry **24**, 1003 (1985)
26 Todorova, M. N., Krasteva, M. L., Markova, M. M., Tsankova, E. T., Taskova, R. M., Peev, D. R.: Phytochemistry **49**, 2371 (1998)

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