

A New Phytoecdysteroid from *Ajuga taiwanensis*

Yu-Yi CHAN,*^a Tian-Shung WU,^{b,c} Chang Sheng KUOH,^d and Amooru G. DAMU^b

^aDepartment of Biotechnology, Southern Taiwan University of Technology; Tainan, Taiwan 710, R.O.C.; ^bDepartment of Chemistry, National Cheng Kung University; Tainan, Taiwan 701, R.O.C.; ^cNational Research Institute of Chinese Medicine, Taipei; Taiwan 112, R.O.C.; and ^dDepartment of Life Sciences, National Cheng Kung University; Tainan, Taiwan 701, R.O.C. Received January 19, 2005; accepted March 26, 2005

A new phytoecdysteroid, namely ajugalide-E (1), together with twenty five known compounds were isolated from *Ajuga taiwanensis* collected from Tainan, Taiwan. Their structures were determined by spectral analysis including high resolution one- and two-dimensional NMR spectroscopy.

Key words *Ajuga taiwanensis*; Labiatae; phytoecdysteroid; ajugalide-E

Many plants contain ecdysteroids, polyhydroxysteroids with a *cis*-fused A/B ring junction and 14 α -hydroxy-7-ene-6-one system, structurally related with ecdysone, the biosynthetic precursor of the insect moulting hormone.¹⁾ These compounds exhibit interesting physiological activities such as regulation of moulting in insects, hormonal functions involve regeneration, metamorphosis, reproduction and differentiation in all arthropods, and antiulcer, antirheumatic, insulin regulation and diuretic or tonic effects in mammals.²⁾

Among the numerous plants containing phytoecdysteroids, those pertaining to *Ajuga* genus are unique for the great variety of such compounds produced.³⁾ Species belonging to genus *Ajuga* have been used as folk medicinal plants as anthelmintic against intestinal disorders, antifungal, hypoglycaemic, antitumor and antimicrobial agents.^{4,5)} Furthermore, it has also been reported that some species of this genus were especially used as diuretic agents, as a remedy for fever, toothache, dysentery and hypertension.⁶⁾ Earlier chemical investigations have led to the isolation of clerodane diterpenoids,^{7–9)} ecdysteroids,^{3,5,10,11)} flavonoids,¹²⁾ iridoids^{13–15)} and triglycerides.¹⁶⁾ The compounds isolated from the genus *Ajuga*, exert a broad spectrum of biological and pharmacological actions such as cytotoxic, anti-mitotic, anti-bacterial, cardiogenic, vasorelaxing, antifungal and antifeedant and insect growth-inhibitions. These facts encouraged us to investigate the chemical constituents of an *Ajuga* species *A. taiwanensis* NAKAI ex MURATA (Labiatae) mainly distributed in the Taiwan, Philippines and Ryukyus which has been often used for the treatment of hepatitis and hepatoma in Taiwanese folk medicine.¹⁷⁾ The isolation of ajugalide A to D from title plant has recently been reported from our laboratories. In this paper, we now describe the isolation and structural elucidation of one new phytoecdysteroid, ajugalide-E (1) and twenty five known compounds.

Results and Discussion

Ajugalide-E (1) was isolated as an optically active, white amorphous solid, mp 210–212 °C. The high-resolution fast-atom bombardment mass spectrum (HR-FAB-MS) of 1 showed a *pseudo* molecular ion peak ($[M+H]^+$) at m/z 563.3221. This data is compatible with the molecular formula C₃₁H₄₇O₉, which was also in good agreement with the 31 carbon atom resonances observed in the ¹³C-NMR spectrum. The ¹H-NMR spectra revealed the presence of five methyl groups, two of them doublets with chemical shifts and coupling constants similar to those characteristic ones of

phytoecdysteroids with γ -lactone side chain.¹⁸⁾ The occurrence of a γ -lactone ring in the side chain was evidenced by the carbonyl absorptions at 1730 cm⁻¹ in the IR and δ 178.6 in the ¹³C-NMR spectrum as well as the signal at δ 79.2 assigned to C-28, carbon enclosed in the lactone ring. A low field signal at δ 5.43 (d, $J=10.0$ Hz) of one deshielded CH was assigned to H-22 and the adjacent signals of side chain were assigned by the corresponding COSY correlation to H-23 (2.0–1.6, m), H-24 (2.0–1.6, m), H-25 (2.25, qd, $J=6.8, 6.8$ Hz), Me-27 (1.41, d, $J=6.8$ Hz), H-28 (3.95, qd, $J=6.8, 6.8$ Hz) and Me-29 (1.29, d, $J=6.8$ Hz). These data were consistent with the occurrence of characteristic side chain with γ -lactone by similitude with 22-acetylcaesterone signals. Additional functionalities included the signal of an olefinic proton at δ 6.21 (br s) as well as nine methine protons and seven methylene groups and an acetyl methyl group also supported the basic skeleton of phytoecdysteroid. As shown in Table 1 the ¹H- and ¹³C-NMR spectra for compound 1 showed a high analogy with those of 22-acetylcaesterone with a *cis* fused A/B ring junction and 14 α -hydroxy-7-ene-6-one skeleton.¹⁹⁾ Results of two-dimensional correlation spectroscopy (2D-COSY) and the long-range ¹³C–¹H correlation in heteronuclear multiple bond correlation (HMBC) experiments also confirmed that the planar structure of 1 was the same as 22-acetylcaesterone (2). But the differences in the chemical shifts and the multiplicities of A-ring protons suggesting that the new compound might be one epimer at C-2 and/or C-3. The signals of H-2 and H-3 appeared in ¹H-NMR spectrum as ddd with three coupling constants, 7.6, 7.6, 4.0 Hz characteristic for two diquasi-axial and a quasiaxial-quasiequatorial interactions, thereby H-2 and H-3 are in a quasiaxial relative position. These findings also showed the *trans* relationship of the hydroxyl group at C-2 and C-3. The upfield shifts of H-2, H-1_{ax}, and H-5 revealing the inversion of configuration at C-2, and thus compound 1 was a C-2 epimer of 2. The final assignment was achieved by NOESY experiment. The presence of NOEs of H-5 with H-19 and H-3 with H-9 (Fig. 1) in compounds 1 and 2 indicated that the A and B rings adopted boat-*cis*-boat conformation. The NOEs between H-2 and H-5 and between H-3 and H-9 showed the hydroxyl groups at C-2 and C-3 to be quasiequatorial, respectively, with *trans* geometry. On the basis of these results, the structure of ajugalide-E was elucidated as 1.

The known compounds, 22-acetylcaesterone (2),¹⁹⁾ ajugalactone (3),²⁰⁾ cyasterone (4),²¹⁾ isocyasterone (5),²¹⁾ ajugamacrin B (6),²²⁾ ajugapantins A (7),²³⁾ ajugamarin C1 (8),¹⁵⁾

* To whom correspondence should be addressed. e-mail: yuyichan@mail.stut.edu.tw

Table 1. ¹H- and ¹³C-NMR Spectral Data of Compounds **1** and **2**

	1		2	
	¹ H-NMR ^{a)}	¹³ C-NMR ^{b)}	¹ H-NMR ^{a)}	¹³ C-NMR ^{b)}
1	2.20—2.00 m 2.00—1.60 m	33.7	2.32—2.12 m 2.04—1.60 m	38.0
2	3.81 ddd (7.6, 7.6, 4.0)	75.2	4.23 br s	68.1
3	4.18 ddd (7.6, 7.6, 4.0)	71.8	4.16 br d (9.2)	68.1
4	2.43—2.38 m 1.45—1.38 m 2.43—2.38 m	43.1	2.04—1.60 m	32.5
5	2.43—2.38 m	57.1	3.02 dd (12.8, 3.2)	51.4
6		201.6		203.4
7	6.21 br s	121.7	6.27 d (2.0)	122.0
8		165.6		165.6
9	3.67 t (9.0)	35.2	3.60 t (8.4)	34.4
10		39.0		38.8
11	2.00—1.60 m	21.0	2.04—1.60 m	21.1
12	2.59—2.53 m 2.00—1.60 m	31.9	2.68—2.57 m 2.04—1.60 m	32.1
13		48.1		48.1
14		84.0		84.2
15	2.20—2.00 m 2.00—1.60 m	31.8	2.32—2.12 m 2.04—1.60 m	31.9
16	2.59—2.53 m 2.20—2.00 m	21.5	2.68—2.57 m 2.32—2.12 m	21.6
17	2.79 t (8.8)	50.2	2.84 t (9.2)	50.3
18	1.14 s	17.8	1.19 s	17.9
19	1.01 s	23.9	1.07 s	24.5
20		76.2		76.2
21	1.53 s	21.7	1.58 s	21.8
22	5.43 d (10.0)	76.9	5.47 d (10.4)	76.9
23	2.00—1.60 m	33.1	2.04—1.60 m	33.3
24	2.00—1.60 m	48.2	2.04—1.60 m	48.2
25	2.25 qd (6.8, 6.8)	42.7	2.32—2.12 m	42.8
26		178.6		178.7
27	1.41 d (6.8)	15.6	1.45 d (7.2)	15.7
28	3.95 qd (6.8, 6.8)	79.2	4.00 qd (6.4, 6.4)	79.3
29	1.29 d (6.8)	19.0	1.33 d (6.4)	19.0
OH	6.53 s		6.53 s	
OH	5.53 s		6.15 br s	
OH			5.96 br s	
OH			5.64 s	
OAc	2.01 s	170.9	2.06 s	170.9
		21.2		21.3

a) Spectral data were obtained in pyridine-*d*₅ at 400 MHz. Chemical shift are given in δ values. b) Spectral data were obtained in pyridine-*d*₅ at 100 MHz. Chemical shift are given in δ values.

ajugalide-A (**9**),²⁴ -B (**10**),²⁴ -C (**11**),²⁴ -D (**12**),²⁴ pheophytin-a (**13**),²⁵ pheophytin-b (**14**),²⁵ 13²-hydroxy(13²-*R*)pheophytin-a (**15**),²⁵ 13²-hydroxy(13²-*S*)pheophytin-a (**16**),²⁵ 8-*O*-acetylharpagide (**17**),¹⁴ harpagide (**18**),²⁶ galiridoside (**19**),¹⁴ teuhiroside (**20**),²⁷ β -sitosterol (**21**),²⁸ stigmasterol (**22**),²⁸ β -sitosteryl-D-glucoside (**23**),²⁸ vanillic acid (**24**),²⁸ kaempferol (**25**)²⁹ and nicotinic acid (**26**)³⁰ were also isolated from *Ajuga taiwanensis*. Their structures were identified by comparison of their spectroscopic data (UV, IR, NMR and mass spectrometry) with values in the literature.

The chemistry of secondary metabolites of the members of *Ajuga* including *A. taiwanensis* appears to be very similar in that all are rich sources of phytoecdysteroids and clerodane diterpenoids, since our phytochemical studies on *A. taiwanensis*, collected in Taiwan afforded five phytoecdysteroids and seven clerodane diterpenoids. Particularly, the widespread occurrence of various phytoecdysteroids in *Ajuga* was very significant and their presence might be used as chemotaxonomic marker for the genus.

Experimental

General Melting points were determined on a Yanagimoto MP-S3 apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-370 polarimeter. IR and UV spectra were recorded on Shimadzu FTIR-8501 and Hitachi UV-3210 spectrophotometers, respectively. EI-MS was obtained on a VG-70-250S mass spectrometer. The ¹H- and ¹³C-NMR, DEPT, COSY, HMQC, NOESY, and HMBC experiments were recorded on a Bruker AMX-400 spectrometer.

Plant Material The whole plant of *A. taiwanensis* was collected from Tainan, Taiwan, in August 2001 and verified by Prof. C.-S. Kuoh. A voucher specimen (TSWu 90003) has been deposited in the Herbarium of National Cheng Kung University, Tainan, Taiwan.

Extraction and Isolation The dried and powdered whole plants of *A. taiwanensis* (68 g) was extracted with MeOH ($\times 10$) at room temperature and concentrated under reduced pressure to give a dark brown syrup (14.7 g). The syrup was partitioned successively between H₂O and CHCl₃. The CHCl₃ layer (3.4 g) was chromatographed directly on silica gel and eluted with a gradient of *n*-hexane and EtOAc to afford 13 fractions. Fraction 2 was rechromatographed on silica gel and eluted with *n*-hexane-acetone (3:1, v/v) to give **13** (5 mg), **14** (1 mg), **15** (0.8 mg), **16** (0.7 mg), **21** and **22** (3 mg). Fractions 6, 7 and 8 were combined and rechromatographed on silica gel and eluted with CHCl₃-MeOH (30:1, v/v) to give **6** (56 mg). Fraction 10 underwent series of chromatographic separations on silica gel using

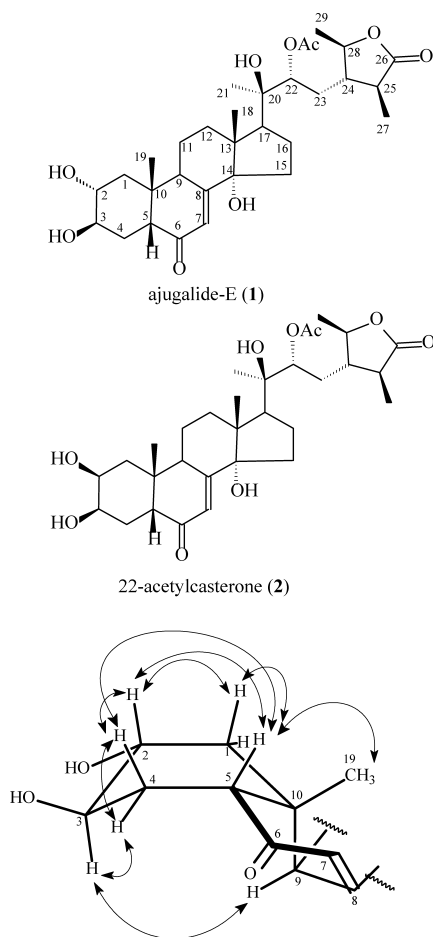


Fig. 1

CHCl_3 -MeOH (15:1, v/v) as an eluent to afford **7** (8 mg), **8** (11 mg), **9** (2 mg), **10** (5 mg), **11** (1 mg), **12** (0.8 mg) and **23** (1 mg), successively. Fraction 12 was underwent chromatography on silica gel using CHCl_3 -MeOH (12:1, v/v) as eluent to afford **1** (2 mg), **2** (2 mg), **3** (1 mg), **4** and **5** (1 mg) and **26** (2 mg), respectively. Fraction 13 was also separated by chromatography on silica gel using CHCl_3 -MeOH (9:1, v/v) to give **24** (0.7 mg). The H_2O layer subjected to column chromatography on Diaion HP-20 and eluted with gradient of H_2O -MeOH gave 8 fractions. Fraction 4 was separated by chromatography on silica gel using CHCl_3 -MeOH (3:1, v/v) as eluent to give **25** (1 mg). Fraction 5 was chromatographed on silica gel using a gradient of CHCl_3 -MeOH (3:1, v/v) to afford **18** (13 mg). Fraction 6 was also purified by chromatography on silica gel and eluted with a gradient of CHCl_3 -MeOH to give **20** (1 mg). Fraction 7 was separated by chromatography on silica gel using CHCl_3 -MeOH (4:1, v/v) as eluent to yield **19** (2 mg). Fraction 8 was also separated by chromatography on silica gel using CHCl_3 -MeOH (5:1, v/v) as eluent to obtain **17** (96 mg).

Ajugalide-E (1): White amorphous solid, mp 210–212 °C. $[\alpha]_{\text{D}}^{25} +133.6^\circ$ ($c=0.007$, CHCl_3). UV λ_{max} (MeOH) nm (log ϵ): 240 (4.10). IR (KBr) cm^{-1} : 3375, 1730, 1654, 1248, 1197, 1063, 1038. ^1H - and ^{13}C -NMR data, see Table 1. FAB-MS m/z : 563 ($[\text{M}+\text{H}]^+$, 1), 562 (1), 544 (1), 526 (1), 516 (1), 508 (3), 498 (2), 484 (1), 466 (1), 456 (1), 451 (1), 448 (2), 433 (2), 377 (2), 371 (13), 363 (3), 360 (2), 353 (3), 345 (2), 327 (3), 301 (4), 300 (3), 299 (3), 250 (3), 249 (5), 183 (8), 149 (100), 139 (20). HR-FAB-MS m/z : 563.3221 (Calcd for $\text{C}_{31}\text{H}_{47}\text{O}_9$; 563.3220).

22-Acetylcasteron (2): White amorphous solid, mp 212–214 °C. $[\alpha]_{\text{D}}^{25} +111.7^\circ$ ($c=0.01$, CHCl_3). UV λ_{max} (MeOH) nm (log ϵ): 241 (4.02). IR (KBr) cm^{-1} : 3382, 1769, 1735, 1655, 1259, 1188, 1042. ^1H - and ^{13}C -NMR data, see Table 1. FAB-MS m/z : 563 ($[\text{M}+\text{H}]^+$, 5), 545 (6), 544 (2), 526 (3),

517 (2), 508 (3), 498 (2), 484 (2), 466 (2), 456 (2), 451 (3), 448 (4), 433 (3), 377 (4), 371 (26), 363 (7), 360 (3), 353 (5), 345 (5), 327 (5), 301 (7), 300 (5), 299 (6), 250 (7), 249 (11), 183 (15), 139 (33), 119 (100). HR-FAB-MS m/z : 563.3218 (Calcd for $\text{C}_{31}\text{H}_{47}\text{O}_9$; 563.3220).

Acknowledgments We thank National Science Council, R.O.C. (NSC 90-2113-M-273-001) for financial support of this research.

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