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Sesquiterpene Lactones from *Ainsliaea acerifolia* SCH. BIP. and *A. dissecta* FRANCH. *et* SAV.

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A new sesquiterpene glycoside, ainsliaside A (II) was isolated from *Ainsliaea acerifolia* SCH. BIP., together with glucozaluzanin C (I). Another new sesquiterpene glycoside, ainsliaside B (III) was isolated from *A. dissecta* FRANCH. *et* SAV. The structures of II and III were determined on the basis of chemical and spectral data.

Keywords——*Ainsliaea acerifolia*; *Ainsliaea dissecta*; sesquiterpene glycoside; guaianolide; melampolide; glucozaluzanin C; ainsliaside A; ainsliaside B

Some species of *Ainsliaea* taste bitter. However, the chemistry of *Ainsliaea* spp. had not been much investigated, though recently five guainaolide were isolated from *A. fragrans* CHAMP.[1] and four germacranolides from *A. acerifolia* SCH. BIP. var. *subapoda* NAKAI.[2]

We now report the structures of two new sesquiterpene glycosides, ainsliaside A (II) isolated (together with glucozaluzanin C) from *A. acerifolia* SCH. BIP., and ainsliaside B (III) isolated from *A. dissecta* FRANCH. *et* SAV. These compounds taste bitter.

Glucozaluzanin C (I), $C_{21}H_{28}O_8 \cdot 1/2H_2O$, mp 105—106 °C, $[\alpha]_D^{21}$ −18.4°. The infrared (IR) spectrum suggested the presence of hydroxyl groups (3400 cm^{-1}) and an unsaturated γ-lactone (1755 cm^{-1}). The proton magnetic resonance (^{1}H-NMR) spectrum exhibited doublets at δ 5.37 (1H, J=3.3 Hz) and 6.20 (1H, J=3.3 Hz), which are characteristic of exocyclic α-methylene γ-lactone. The ^{13}C- nuclear magnetic resonance (^{13}C-NMR) spectrum indicated the presence of three exomethylene groups at δ 112.4, 113.9, 119.4 (each triplet); 141.0, 148.8, 150.3 (each singlet). Acetylation of I afforded the tetraacetate (Ia), $C_{29}H_{36}O_{12}$, mp 159.5—161.0 °C, while acid hydrolysis of I afforded glucose.

From these results, I was assumed to be glucozaluzanin C (I), which had been isolated from *Pertya robusta* (MAXIM.) BEAUV.[3] as a bitter principle. This conclusion was confirmed by direct comparison [thin layer chromatography (TLC), IR, mixed mp] of I with an authentic sample.

Ainsliaside A (II), $C_{30}H_{34}O_{11} \cdot H_2O$, $[\alpha]_D^{19}$ +59.7°. The ^{1}H-NMR spectrum was similar to that of I except that it showed a pair of doublets at δ 6.37 and 7.82 (each 1H, J=15 Hz) and a multiplet at δ 6.9—7.5 due to *trans* olefinic proton and aromatic protons, respectively. Acetylation of II afforded the pentaacetate (IIa), $C_{40}H_{44}O_{16}$. Its ^{1}H-NMR spectrum showed two phenolic acetoxyl groups at δ 2.30, 2.31 and three alcoholic acetoxyl groups at δ 1.94, 2.03 and 2.10. Acid hydrolysis of II afforded glucose, while saponification of II afforded glucozaluzanin C (I) and caffeic acid.

The ^{13}C-NMR spectrum of II was similar to that of I in the aglycone moiety, but C-2 of glucose was shifted to δ 76.1 (Δ +1.0 ppm) and C-1 and C-3 of glucose were shifted to δ 98.5 (Δ −5.4 ppm) and 74.9 (Δ −3.1 ppm), respectively. Thus, the caffeoyl group was attached to C-2 of the glucose moiety.[4] This was supported by the ^{1}H-NMR spectrum, which showed a broad triplet signal at δ 5.65 (1H, J=10 Hz) due to H-2 of the glucose moiety. These results led to the structure II for ainsliaside A.

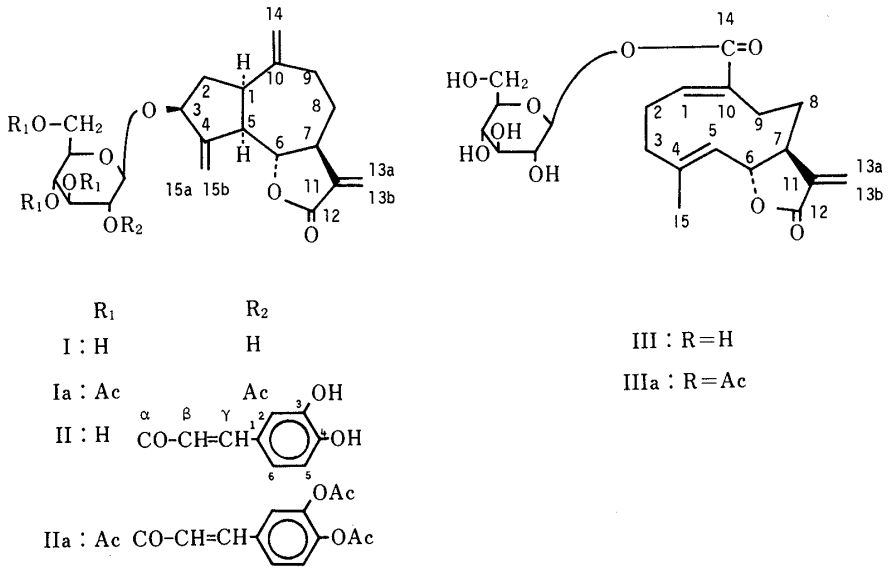

Chart 1

Ainsliaside B (III), $C_{21}H_{28}O_9 \cdot 1/2H_2O$, mp 157—161 °C, $[\alpha]_D^{19}$ +83.4°. The IR spectrum showed the presence of hydroxyl groups (3400 cm^{-1}), unsaturated γ-lactone (1750 cm^{-1}) and α,β-unsaturated ester (1700 cm^{-1}). The ^{1}H-NMR spectrum exhibited doublets at δ 5.45 (1H, d, J=3.1 Hz) and 6.11 (1H, d, J=3.3 Hz) which are characteristic of exocyclic α-methylene γ-lactone and a broad singlet at δ 1.71 (3H) due to an olefinic methyl group. Further, two olefinic protons were observed at δ 4.81 (1H, br d, J=10 Hz) and 6.72 (1H, br t, J=7 Hz); the former was coupled with a carbinol proton at δ 4.58 (1H, dd, J=10 Hz, J=9 Hz) and a methyl group at δ 1.71 (J<1 Hz) and the latter was assigned as the β proton of an α,β-unsaturated carboxylic ester. Acetylation of III afforded the tetraacetate (IIIa), $C_{29}H_{36}O_{13}$, while acid hydrolysis of III afforded glucose.

From these results, III was assumed to be a germacranolide sesquiterpene glucoside. In germacranolide-type sesquiterpenes there are four configurational isomers due to two double bonds. The β proton of the α,β-unsaturated carboxylic ester resonated at δ 6.72. If the 10,1-double bond has *Z*-configuration, the olefinic proton (H-1) should appear at higher field by *ca.* 1 ppm.[5] In fact, the H-1 (δ 6.72) signal of III was 1.08 ppm downfield from that (δ 5.64) of taraxin carboxylic acid 1′-*O*-β-D-glucopyranoside[2] having *Z*-configuration. Therefore, 10,1-double bond has *E*-configuration.

On the other hand, irradiation of the C_{15}-methyl signal resulted in a *ca.* 11% enhancement in the signal strength of H-6, demonstrating that the 4,5-double bond has *E*-configuration. Thus, III belongs to the melampolide subgroup of germacranolides. If it is assumed that the absolute configuration of C-7 side chain is β, as in all sesquiterpene lactones of authenticated stereochemistry, the configuration of H-6 must be β from the large coupling constants (J_{7-13}=3.1 and 3.3 Hz), showing that the lactone fusion is *trans*.[6]

In the ^{13}C-NMR spectrum, C-1 and C-2 of glucose appeared at δ 98.5 and 74.0, respectively, showing that the anomeric carbon is esterified.[4] Furthermore, in the ^{1}H-NMR spectrum, the anomeric proton appeared at δ 6.26 (1H, d, J=8 Hz), showing that the glucose linkage is β. These results led us to assign the structure III to ainsliaside B.

Experimental

Melting poins were determined on a Yanaco MP-500 micromelting point apparatus and are uncorrected. Optical

TABLE I. ^{13}C Chemical Shifts of I, II (in Pyridine-d_5) and III (in Methanol-d_4)

Carbon No.		I	II	III
Aglycone moiety	1	44.8[a]	45.9	143.0
	2	38.0	37.3	26.2[c]
	3	80.5	79.5	38.0
	4	150.3	150.2	141.3
	5	50.2	51.6	127.1
	6	83.5	83.1	82.8
	7	45.3[a]	45.9	46.7
	8	30.6	30.4	24.2[c]
	9	34.1	33.8	26.4[c]
	10	148.8	149.0	135.1
	11	141.0	140.5	139.0
	12	169.7	169.9	172.4
	13	119.2	119.5	119.4
	14	113.9	114.9	167.6
	15	112.4	114.9	17.2
Glucose moiety	1	103.9	98.5	95.7
	2	75.1	76.1	73.9
	3	78.4[b]	74.9	78.6[d]
	4	71.7	71.8	71.0
	5	78.0[b]	78.2	77.9[d]
	6	62.9	62.6	62.4
Caffeic acid moiety	1		126.7	
	2		114.9	
	3		148.2	
	4		147.3	
	5		116.3	
	6		122.1	
	α		166.4	
	β		115.6	
	γ		145.9	

a—d) Assignments may be interchanged in each column.

rotations were determined with a JASCO DIP-140 digital polarimeter. IR spectra were run on a JASCO A-202 infrared spectrophotometer and ultraviolet (UV) spectra on a Shimadzu UV-360 recording spectrophotometer. ^1H-NMR and ^{13}C-NMR spectra were recorded on a JEOL FX-90Q apparatus (89.55 and 22.5 MHz, respectively). Chemical shifts are given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; br, broad). Gas chromatography (GC) was run on a Hitachi K 53 gas chromatography. High performance liquid chromatography (HPLC) was run on a Kyowa Seimitsu model K 880 instrument.

Isolation——Air-dried aerial parts (350 g) of *A. acerifolia* were extracted twice with methanol under reflux. The extract was concentrated under reduced pressure and the residue was suspended in water. This suspension was extracted with ether and *n*-butanol, successively. The *n*-butanol soluble fraction (3 g) was chromatographed on a polyamide (30 g) column with water–methanol (3 : 1) as the eluent to give Fr. 1 (1.4 g) and Fr. 2 (170 mg). Fr. 1 was rechromatographed on a silica gel column with chloroform–methanol (93 : 7) as the eluent to give glucozaluzanin C. Fr. 2 was rechromatographed on a silica gel column with chloroform–methanol (92 : 8) to give ainsliaside A. Air-dried whole plants (100 g) of *A. dissecta* were treated in the same manner as *A. acerifolia*. The *n*-butanol-soluble fraction (2.3 g) was chromatographed on a silica gel column with chloroform–methanol (91 : 9) to give ainsliaside B.

Glucozaluzanin C (I)——Recrystallization from water gave colorless needles, mp 105—106 °C. $[\alpha]_D^{21}$ −18.4° (c = 0.93, methanol). *Anal.* Calcd for $C_{21}H_{28}O_8 \cdot 1/2H_2O$: C, 60.42; H, 7.00. Found: C, 60.72; H, 7.06. IR ν_{max}^{KBr} cm^{-1}: 3400, 1755. ^1H-NMR (pyridine-d_5) δ: 5.37 (1H, d, J = 3.3 Hz, H-13a), 5.51 (1H, br s, H-15b), 5.81 (1H, br s, H-15a), 6.20 (1H, d, J = 3.3 Hz, H-13b). ^{13}C-NMR (pyridine-d_5) δ: Table I. This was identified by direct comparison (mixed mp, IR, TLC) with an authentic sample.[3]

Ainsliaside A (II)——Amorphous powder. (52 mg), $[\alpha]_D^{19}$ +59.7° (c = 0.50, methanol). *Anal.* Calcd for $C_{30}H_{34}O_{11} \cdot H_2O$: C, 61.22; H, 6.16. Found: C, 61.14; H, 6.11. IR ν_{max}^{KBr} cm^{-1}: 3400, 1740—1700, 1515, 1440, 1260, 1150. UV λ_{max}^{MeOH} (log ε): 332 (4.24), 302 (sh 4.10), 245 (sh 4.02). ^1H-NMR (pyridine-d_5) δ: 5.13 (2H, br s, H_2-14), 5.45 (2H,

br s, H_2-15), 5.65 (1H, br t, $J=10$ Hz, H-2 of glucose), 6.37 (1H, d, $J=15$ Hz, CO–CH=C), 6.9—7.5 (3H, m, aromatic H), 7.82 (1H, d, $J=15$ Hz, CO–C=CH). ^{13}C-NMR (pyridine-d_5) δ: Table I.

Ainsliaside B (III)——Recrystallization from methanol gave colorless needles (525 mg). mp 157—161 °C. $[\alpha]_D^{19}$ +83.4° ($c=0.51$, methanol). *Anal.* Calcd for $C_{21}H_{28}O_9 \cdot 1/2H_2O$: C, 58.19; H, 6.74. Found: C, 58.01; H, 6.80. IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1700, 1620, 1270, 1205, 1130, 1065, 1045, 970. ^{1}H-NMR (pyridine-d_5) δ: 1.71 (3H, br s, CH_3), 4.58 (1H, dd, $J=10$ Hz, $J=9$ Hz, H-6), 4.81 (1H, d, $J=10$ Hz, H-5), 5.45 (1H, d, $J=3.1$ Hz, H-13a), 6.11 (1H, d, $J=3.3$ Hz, H-13b), 6.26 (1H, d, $J=8$ Hz, H-1 of glucose), 6.72 (1H, br t, $J=7$ Hz, H-1). ^{13}C-NMR (methanol-d_4) δ: Table I.

Acid Hydrolysis of Glucozaluzanin C (I), Ainsliaside A (II), Ainsliaside B (III)——A solution of each glycoside (*ca.* 1 mg) in 10% sulfuric acid (1 ml) was heated in a boiling water bath for 20 min. The solution was passed through an Amberlite IRA-45 column and the eluate was concentrated to give a residue, which was reduced with sodium borohydride (*ca.* 2 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120 column and the eluate was concentrated to dryness. Boric acid was removed by codistillation with methanol and the residue was acetylated with acetic anhydride and pyridine (each 1 drop) at 100 °C for 1 h. The reagents were evaporated off *in vacuo*. Glucitol acetate was detected by GC from each glycoside. Conditions: column, 1.5% OV-17, 3 mm × 1 m; column temperature, 230 °C; carrier gas, N_2; t_R 3.5 min.

Acetylation of Glucozaluzanin C (I)——Glucozaluzanin C (I) (80 mg) was dissolved in pyridine and acetic anhydride (each 0.5 ml) and left at room temperature overnight. The reagents were evaporated off *in vacuo* and the residue was recrystallized from methanol to give the tetraacetate (Ia) (70 mg), mp 159.5—161 °C. *Anal.* Calcd for $C_{29}H_{36}O_{12}$: C, 60.41; H, 6.29. Found: C, 60.52; H, 6.20. IR ν_{max}^{KBr} cm^{-1}: 1770, 1750, 1670, 1635, 1380, 1368, 1245, 1225, 1210, 1165, 1140, 1060, 1035. ^{1}H-NMR ($CDCl_3$) δ: 1.96, 2.00, 2.04, 2.09 (each 3H, s, $OCOCH_3$). This was identified by direct comparison (mixed mp, IR, TLC) with an authentic sample.[3)]

Acetylation of Ainsliaside A (II)——Ainsliaside A (II) (20 mg) was dissolved in pyridine and acetic anhydride (each 0.3 ml) and the mixture was left at room temperature overnight. The reagents were evaporated off *in vacuo* and the residue was chromatographed on a silica gel column with hexane–ethyl acetate (2 : 1) to give the pentaacetate (IIa) (15 mg), as a colorless powder. *Anal.* Calcd for $C_{40}H_{44}O_{16}$: C, 61.53; H, 5.68. Found: C, 61.48; H, 5.72. IR ν_{max}^{KBr} cm^{-1}: 1755—1725, 1635, 1505, 1370, 1260, 1225, 1205, 1175, 1125, 1108, 1030. ^{1}H-NMR ($CDCl_3$) δ: 1.94, 2.03, 2.10, 2.30, 2.31 (each 3H, s, $OCOCH_3$), 6.16 (1H, d, $J=16$ Hz, CO–CH=C), 7.50 (1H, d, $J=16$ Hz, CO–C=CH).

Saponification of Ainsliaside B (II)——Ainsliaside B (II) (*ca.* 1 mg) was treated with 2% sodium hydroxide (0.5 ml) under a nitrogen atmosphere for 1 h at room temperature. The reaction mixture was acidified with hydrochloric acid and extracted with *n*-butanol. the *n*-butanol extract was washed with water and concentrated. Caffeic acid and glucozaluzanin C (I) were identified in the residue by HPLC. Conditions: column, Lichrosorb RP-8, 4 mm × 25 cm; solvent, acetonitrile–water (25 : 75); flow, 1.5 ml/min; detector, UV 220 nm; t_R 2.3 min (caffeic acid), 6.5 min (glucozaluzanin C).

Acetylation of Ainsliaside B (III)——Ainsliaside B (III) (30 mg) was acetylated in the same manner as described for glucozaluzanin C to give the tetraacetate (IIIa) (20 mg) as a colorless powder. *Anal.* Calcd for $C_{29}H_{36}O_{13}$: C, 58.78; H, 6.12. Found: C, 58.54; H, 6.10. IR ν_{max}^{KBr} cm^{-1}: 1755, 1665, 1630, 1370, 1220, 1130, 1265, 1235, 975. ^{1}H-NMR ($CDCl_3$) δ: 1.87 (3H, br s, CH_3), 1.96, 2.02, 2.04, 2.07 (each, 3H, s, $OCOCH_3$), 4.50 (1H, t, $J=10$ Hz, H-6), 4.61 (1H, d, $J=10$ Hz, H-5), 5.46 (1H, d, $J=3.5$ Hz, H-13a), 5.79 (1H, d, $J=8$ Hz, H-1 of glucose), 6.14 (1H, d, $J=3.5$ Hz, H-13b), 6.80 (1H, br t, $J=7$ Hz, H-1).

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