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Glucosides from the Leaves of *Cynanchum liukiuense* (II)[1]

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Abstract

Three glucosides were isolated from an *n*-BuOH soluble fraction in a methanol extract of the leaves of *Cynanchum liukiuense* Werb. These compounds were identified as (*1R*, *6R*)-3-oxo-6-hydroxy-α-ionol 11-*O*-β-D-glucopyranoside (**9**), quercetin 3-*O*-β-D-glucopyranoside (**10**), and kaempferol 3-*O*-β-D-glucopyranoside (**11**), respectively, by spectroscopic methods and chemical evidences.

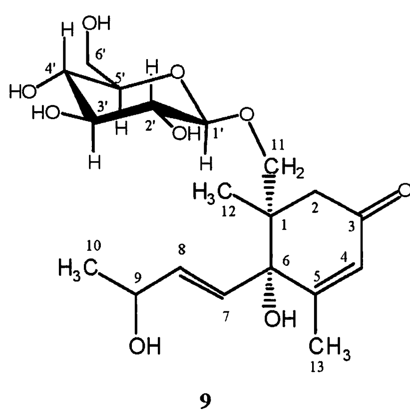
Introduction

Cynanchum liukiuense is a perennial liana and grows in the Sakishima-Islands of Japan. This plant is known as food plant of danaid buttefly, *Salatura genutia*. In connection with a study on the available constituents from food plants of some butterflies in Okinawa, we have previously reported isolation of five triterpenoids (**1-5**), methyl stearate (**6**), and phytosterols (**7a-c**) and phytosterol glycosides (**8a-c**) from the chroloform soluble fraction in the methanol extract of the leaves of *C. liukiuense*. [1)] We continuously examined constituents in the methanol extract of the leaves of *C. liukiuense* and isolated three glucosides from the 1-butanol soluble fraction in the methanol extract.

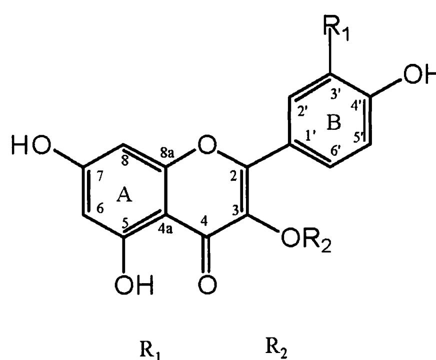
Herein, we describe the separation and identification of these constituents.

Results and Discussion

A 1-butanol soluble fraction from a methanol extract of the leaves of *C. liukiuense* was subjected to column chromatography on silica gel to give **9-11**.



9



	R_1	R_2
10	OH	β-D-glucopyranosyl
10a	OH	H
11	H	β-D-glucopyranosyl
11a	H	H

Structures

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[1] Part I: K. Ogihara et al., Bull. Fac. Sci. Univ. Ryukyu, **70**, 83 (2000).

Compound **9** was obtained as colorless oil. ^{1}H and ^{13}C NMR spectra of **9** showed 25 proton and 19 carbon signals, respectively. The SIMS showed of a quasi-molecular ion peak at *m/z* 425 due to $[M+Na]^{+}$ and a fragment ion peak at *m/z* 223 due to $[M-180+H]^{+}$, which indicated **9** to be a glycoside with a sugar moiety. The IR spectrum of **9** showed wide bands at 3700-3100 (OH) and 1200-950 cm^{-1} (C-O), which supported **9** to be a glycoside. The IR spectrum also showed a band characteristic to α, β-unsaturated carbonyl group at 1650 cm^{-1}. ^{1}H and ^{13}C NMR signals were completely assigned by means of DEPT, HMQC, and ^{1}H-^{1}H COSY spectroscopic techniques, elucidating **9** to have seven partial structures (**a-g**) shown in Fig. 1. The partial structures were mainly determined by ^{1}H and ^{13}C NMR

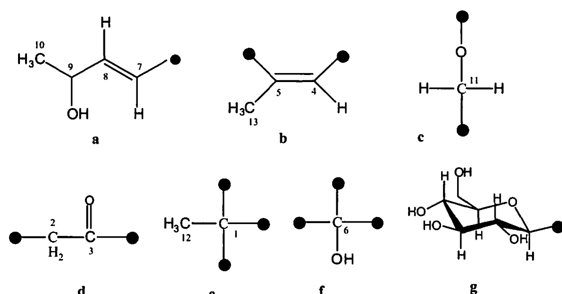

Fig. 1. Partial structures of **9**

spectra and ^{1}H-^{1}H COSY spectra as described below. In the ^{1}H NMR spectrum, each doublet of doublets at δ_H 5.73 (1H, J = 0.5,15.5 Hz) and 5.82 (1H, J = 5.5,15.5 Hz) were assigned as *trans*-olefin protons at H-7 and H-8. In the ^{1}H-^{1}H COSY spectra, a multiplet at δ_H 4.32 showed a cross peak with signal due to H-8 at δ_H 5.82, which indicated this signal to be assigned to H-9 which connected to carbon atom bonded to a oxygen atom. A doublet at δ_H 1.23 showed a cross peak with signal due to H-9 at δ_H 4.32 in the ^{1}H-^{1}H COSY spectra and was assigned to H-10. These results indicated the presence of a 3-hydroxy-1-butenyl moiety as the partial structure **a**. A signal due to H-4 olefinic proton at δ_H 5.89 showed a cross peak with methyl proton signal at δ_H1.90 in the ^{1}H-^{1}H COSY spectra and the signal at δ_H1.90 was assigned to H-13. These facts indicated the presence of a methylvinylene moiety as the partial structure **b**. An AB quartet due to a methylene group bonded to an oxygen atom at δ_H 3.76 was assigned to H-11, which indicated the presence of an oxymethylene group bonded to a quaternary carbon atom as the partial structure **c**. Another AB quartet due to a methylene group bonded to a carbonyl group at δ_H 2.49 was assigned to H-2, which indicated the presence of an oxomethylene group as the partial structure **d**. In the ^{1}H-^{1}H COSY spectra, no relation between a singlet due to a methyl group at δ_H 1.06 and any signals was observed, which indicated the presence of a methyl group bonded to a quaternary carbon atom as the partial structure **e.** In the ^{13}C NMR spectrum, signal at δ_C 79.3 was assigned to the quaternary carbon atom bonded to an oxygen atom, which indicated the presence of a hydroxyl group bonded the quaternary carbon atom as the partial structure **f.** Signals at δ_C 104.6, 75.1, 78.0, 71.5, 77.9, and 62.6 indicated the presence of a sugar moiety which was assigned to glucose by comparison of these data with those described in reference [2] as the partial structure **g**.

Connections of these partial structures were established by means of its HMBC spectral analyses shown in Fig. 2. Observation of a cross peak between

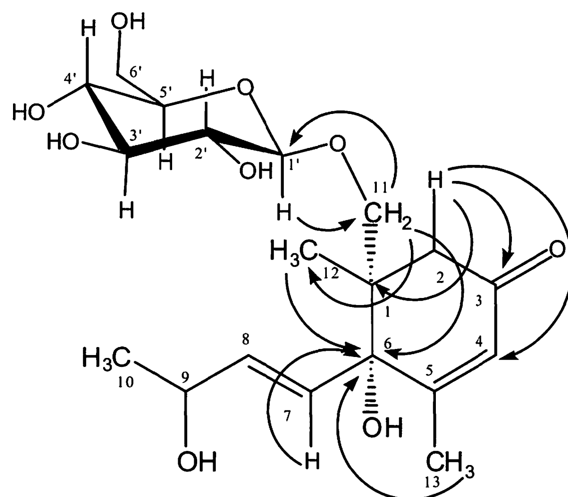

Fig. 2. H-C Interaction observed by HMBC spectra of **9**.

the signal due to H-13 at δ_H 1.90 and that due to C-6 at δ_C 79.3 indicated that C-5 of the partial structure **b** was bonded to C-6 of the partial structure **f.** Observations of each cross peak between the signal due to H-2 at δ_H 2.49 and that due to C-4 at δ_C 127.7, between the signal due to H-2 at δ_H 2.49 and that due to C-1 at δ_C 46.2, and between the signal due to H-12 at δ_H 1.06 and that due to C-6 at δ_C 79.3 indicated that C-3 of the partial structure **d** was bonded to C-4 of the

partial structure **b**, C-2 of **d** to C-1 of **e**, and C-1 of **e** to C-6 of **f**, respectively. These facts indicated that a cyclohexenone skeleton was formed from the partial structures **b**, **d**, **e**, and **f**. Moreover, observations of each cross peak between the signal due to H-7 at δ_H 5.73 and that due to C-6 at δ_C 79.3 and between the signal due to H-11 at δ_H 3.76 and that due to C-1 at δ_C 46.2 suggested that C-7 of the partial structure **a** was bonded to C-6 of the partial structure **f**, and C-11 of the partial structure **c** to C-1 of the partial structure **e**, respectively. Observation of the cross peak between the signals due to anomeric proton (H-1') of the glucose moiety at δ_H 4.13 and that due to C-11 at δ_C 74.5 indicated that C-1' of glucose was bonded to C-11 of the partial structure **c**. The coupling constant of the anomeric proton of the glucose was observed with 7.5 Hz, which indicated that the glucose was bonded to the corresponding aglycone in β-configuration. The compound **9** was hydrolyzed with 0.1M H_2SO_4 to give the D-glucose. Thus, planar structure of **9** was elucidated to be 3-oxo-6-hydroxy-α-ionol 11-*O*-β-D-glucopyranoside (**9**).

In the NOESY spectra of **9**, observation of a cross peak between a signal due to H-12 at δ_H 1.06 and that due to H-7 at δ_H 5.73 and no observation a signal due to H-11 at δ_H 3.76 and that due to H-7 at δ_H 5.73 indicated the relative configuration between 12-CH_3 group and 3-hydroxy-1-butenyl group (partial structure **a**) as *cis*. The absolute configuration at C-6 position was established by application of the CD helicity rule of a cyclohexenone derivative [3]. In the CD spectrum of **9**, curve due to each R and K band of a carbonyl group showed negative and positive Cotton effect, respectively, indicating the absolute configurations of C-1 and 6 as *R* and *R*, respectively.

Thus, compound **9** was identified as (1*R*, 6*R*)-3-oxo-6-hydroxy-α-ionol 11-*O*-β-D-gluco-pyranoside (**9**). The physical and spectral data of **9** were in agreement with those described in reference [4]. It was already reported that **9** was isolated from a water-soluble fraction of leaves of this plant [4] and that a substance having the same planar structure as **9** was isolated from *Juniperus phoenicea* [5].

Table 1. ^{1}H NMR data and coupling constnts (Hz)* of compounds **9-11**

H	9			10			11		
2	2.49	AB*q*	(17.0)						
4	5.89	*s*							
6				6.34	*d*	(1.9)	6.36	*d*	(1.0)
7	5.73	*dd*	(0.5, 15.5)						
8	5.82	*dd*	(5.5, 15.5)	6.16	*d*	(1.9)	6.18	*d*	(1.0)
9	4.32	*m*							
10	1.23	*d*	(6.0)						
11	3.76	AB*q*	(10.0)						
12	1.06	*s*							
13	1.90	*d*	(1.0)						
1'	4.13	*d*	(7.5)						
2'	3.13	*t*	(8.5)	7.69	*d*	(1.9)	8.03	*d*	(8.9)
3'	3.32	*t*	(8.5)				6.88	*d*	(8.9)
4'	3.26	*t*	(8.5)						
5'	3.20	*m*		6.85	*d*	(8.6)	6.88	*d*	(8.9)
6'	3.64	*dd*	(5.5, 11.7)	7.57	*dd*	(1.9, 8.6)	8.03	*d*	(8.9)
	3.83	*dd*	(2.0, 11.7)						
1''				5.18	*d*	(7.3)	5.19	*d*	(7.3)
2''				3.47	*t*	(8.6)	3.28		
3''				3.41	*t*	(8.6)	|	*m*	
4''				3.35	*m*		3.44		
5''				3.20	*m*		3.21	*m*	
6''				3.56	*dd*	(5.4, 12.0)	3.51	*dd*	(5.1, 11.9)
				3.70	*dd*	(2.2, 12.0)	3.68	*dd*	(2.1, 11.9)

* Coupling constants are in parentheses.

Table 2. ^{13}C NMR spectral data of compounds **9-11**

C	9	10	10a	11	11a
1	46.2				
2	45.5	159.7	148.7	158.9	148.8
3	200.9	136.3	138.0	135.4	137.9
4	127.7	180.1	178.1	179.3	178.1
4a		106.2	105.3	105.4	105.2
5	167.1	163.6	163.3	163.0	163.2
6	79.3	100.7	100.0	100.2	100.1
7	129.6	166.9	166.3	166.9	166.5
8	137.2	95.5	95.1	94.9	95.2
8a		159.1	159	158.5	159.0
9	68.6				
10	23.8				
11	74.5				
12	20.1				
13	19.5				
1'	104.6	123.9	124.9	122.7	124.5
2'	75.1	116.7	116.7	132.2	131.4
3'	78.0	146.5	146.9	116.0	117.0
4'	71.5	150.5	149.5	161.5	161.3
5'	77.9	118.3	117.0	116.0	117.0
6'	62.6	123.7	122.4	132.2	131.4
1''		105.2		104.1	
2''		76.4		75.7	
3''		79.0		78.4	
4''		71.9		71.3	
5''		78.8		78.0	
6''		63.2		62.5	

Compound **10** gave positive UV/NH_3, aluminum chloride, Mg-HCl, and Zn-HCl reactions, which suggested **10** to be a flavonol 3-*O*-glycoside derivative [6]. The compound **10** was suggested to have a molecular formula of $C_{21}H_{20}O_{12}$ by observations of 5 proton and 21 carbon signals in its ^{1}H and ^{13}C NMR spectra, respectively, and of quasi-molecular ion peaks at *m/z* 465 and 487 due to $[M+H]^+$ and $[M+Na]^+$, respectively, in its SIMS and by the reason that **10** was a glycoside derivative. The IR spectrum of **10** showed characteristic bands due to an α, β-unsaturated carbonyl group, aryl alkyl ethers, and an aromatic ring at 1650, 1200 and 1010, and 1600 and 1500 cm^{-1}, respectively. The IR spectrum also showed characteristic bands due to a sugar moiety at 3650-3000 (OH) and 1300-950 cm^{-1} (C-O), which supported **10** to be a flavonol glycoside derivative. The ^{1}H NMR spectrum of **10** showed presences of a 1,2,3,5-tetrasubstituted aromatic ring [δ_H 6.34 (*d*, J = 1.9 Hz) and 6.16 (*d*, J = 1.9 Hz)] and a 1,3,4-trisubstituted aromatic ring [δ_H 7.69 (*d*, J = 1.9 Hz), 7.57 (*dd*, J = 1.9, 8.6 Hz), and 6.85 (*d*, J = 8.6 Hz)]. The ^{13}C NMR spectrum of **10** showed presences of two carbon atoms bonded oxygen atom at δ_C 166.9, 163.6 due to A ring, two carbon atoms bonded to oxygen atom at δ_C 150.5, 146.5 due to B ring, and a carbon atom bonded to oxygen atom at δ_C136.3 due to C-3 of γ-pyrone ring. These spectral data suggested **10** to be a quercetin glycoside.

The compound **10** was hydrolyzed with dil. HCl to give D-glucose and quercetin (**10a**). In the ^{1}H NMR spectrum, the coupling constant of an anomeric proton of the glucose moiety was observed with 7.3 Hz, which indicated the D-glucose was bonded to the corresponding aglycone in β-configuration. In comparison of ^{13}C NMR spectral data of **10** with those of quercetin (**10a**) from **10** by the hydrolysis, the signals due to C-2, 3, and 4 of **10** at δ_C 159.7, 136.3,

and 180.1 were shifted from those due to C-2, 3, and 4 of **10a** at δ_C 148.7, 138.0, and 178.1, which indicated that the D-glucose was located at C-3 position of **10a**.

These spectral data coincided with those described in reference[2]. Thus, compound **10** was identified as quercetin 3-*O*-β-D-glucopyranoside (**10**).

Compound **11** also showed positive UV/NH3, aluminum chloride, Mg-HCl, and Zn-HCl reactions, which suggested **11** to be a flavonol 3-*O*-glycoside derivative. The SIMS showed quasi-molecular ion peaks at *m/z* 449 due to $[M+H]^+$ and 471 due to $[M+Na]^+$ which were an oxygen atom less than **10**. The ^1H and ^{13}C NMR spectra of **11** coincided with those of **10**, except for the signals due to B ring. In the ^1H NMR spectrum, the signals due to 1,3,4-trisubstituted aromatic ring moiety for **10** disappeared and those due to 1,4-disubstituted aromatic ring moiety [δ_H 8.03 (2H, *d*, *J* = 8.9 Hz) and 6.88 (2H, *d*, *J* = 8.9 Hz)] newly appeared. In the ^{13}C NMR spectrum, a signal due to carbon atom bonded to an oxygen atom for **10** disappeared. Thus, compound **11** was suggested to be a kaempferol glycoside which lacks oxygen atom at B in **10**. The compound **11** was hydrolyzed with dil. HCl to give D-glucose and kaempferol (**11a**). In the ^1H NMR spectrum, the coupling constant of an anomeric proton of the glucose moiety was observed with 7.3 Hz, which indicated that the D-glucose was bonded to the corresponding aglycone in β-configuration. In comparison of ^{13}C NMR spectral data of **11** with those of kaempferol (**11a**) from **11** by the hydrolysis, the signals at δ_C 158.9, 135.4, and 179.3 due to C-2, 3, and 4 of **11** were shifted from those at δ_C 148.8, 137.9, and 178.1 due to C-2, 3, and 4 of **11a**, which indicated that the D-glucose was located at C-3 position of **11a**.

These spectral data coincided with those described in reference[2]. Thus, compound **11** was identified as kaempferol 3-*O*-β-D-glucopyranoside (**11**).

Experimental

Analytical TLC was carried out on Merck 60 F_{254} silica gel plate (thickness: 0.25 mm). HPLC analyses for D-glucose were performed on a Waters HPLC 600 instrument equipped with an ORD (OR-990, Jasco Co. Ltd) and a polyamide II column (4.6 I.D. x 300 mm, YMC Co. Ltd) with a solvent system of MeCN-H_2O (3:1) at a flow rate of 2 mL/min. ^1H (500 and 270 MHz) and ^{13}C NMR (125 and 67.5 MHz) spectra were taken on JEOL α 500 and EX-270 spectrometer in CD_3OD with TMS as int. standard. SIMS spectra were obtained on a Hitachi M-2500 double focusing mass spectrometer. CD spectrum was recorded on a Jasco J-720 spectropolarimeter.

Extraction and isolation. Fresh leaves (5.9 kg) of *C. liukiuense*, collected at Hateruma Island, Okinawa-prefecture in April, were ground in a mixer and immersed in MeOH for 1 month. The MeOH soln was concd *in vacuo* and the obtained concentrate (201.6 g) was suspended with H_2O. The suspension was partitioned succesively with Hexane, $CHCl_3$, EtOAc, and *n*-BuOH. The *n*-BuOH layer was concd *in vacuo* and the BuOH-soluble fraction (2.56 g) was subjected to column chromatography on silica gel developed with a solvent system of $CHCl_3$–MeOH-H_2O (7:3:0.5) to give 13 fractions. Fractions 7 and 8 were re-chromatographed on a C-18 column developed with MeOH-H_2O (1:4) to give **9** (33 mg). Each fraction 11 and 12 was individually subjected to gel-filtration on Sephadex LH-20 with MeOH to give **10** (34 mg) from fraction 11 and **11** (24 mg) from fraction 12, respectively.

***(1S,6S)-3-Oxo-6-hydroxy-α-ionol 11-O-β-D-glucopyranoside* (9).** Colorless oil. $[\alpha]_D^{25}$ +44.2 (*c* 0.17, MeOH); CD (MeOH):λ_{ext} 400.0 ([θ]=0.0), 318.4 (-1900.8), 286.0 (0.0), 243.2 (23100), 205.0 (0.0); IR υ_{max}^{KBr} cm^{-1}: 3700-3100 (OH), 1650 (C=C-C=O), 1200-950 cm^{-1} (C-O); ^1H (500 MHz) and ^{13}C NMR (125 MHz): see Tables 1 and 2; SIMS *m/z* (rel. int.): 425 ($[M+Na]^+$, 23), 223 ($[M\text{-glc}+H]^+$, 5). The physical and spectral data coincided with those described in reference [4].

***Hydrolysis of* 9.** According to the method described in reference [1], **9** was hydrolyzed. The compound **9** (3 mg) dissolved in *n*-BuOH and 0.1M H_2SO_4 (1:1) were heated at 80°C for 2 hr. After hydrolysis, reaction mixture was shaken with $CHCl_3$ and H_2O. The H_2O layer was subjected to HPLC analysis and D-glucose was detected.

***Quercetin 3-O-β-D-glucopyranoside* (10).**

Yellow amorphous, mp 258°C (decomposed); IR υ_{max}^{KBr} cm^{-1}: 3650-3000 (OH), 1650 (C=C-CO), 1200 and 1010 (ph-o), 1600 and 1500 (aromatic ring), and 1300-950 cm^{-1} (C-O); ^{1}H (270 MHz) and ^{13}C NMR (67.5 MHz): see Tables 1 and 2; SIMS *m/z* (rel. int.): 487 ($[M+Na]^+$, 5), 465 ($[M+H]^+$, 15). The physical and spectral data coincided with those described in reference[2].

***Hydrolysis of* 10**. The compound **10** (17 mg) dissolved in 1.5 mL of 2M HCl were heated at 80°C for 1 hr. After hydrolysis, reaction mixture was shaken with $CHCl_3$ and H_2O. The $CHCl_3$ layer was washed with water and dried over anhydrous Na_2SO_4. The $CHCl_3$ layer, after concd, was subjected to a column chromatography on Si-gel to give quercetin (**10a**, 6 mg): light yellow powder, mp > 300°C ([lit. [2]: 314°C]); ^{13}C NMR (125 MHz): see Table 2. The H_2O layer was subjected to HPLC analysis and D-glucose was detected.

Kaempferol 3-O-β-D-glucopyranoside (**11**). Yellow amorphous, mp 255°C (decomposed); IR υ_{max}^{KBr} cm^{-1}: 3650-3000 (OH), 1650 (C=C-CO), 1200 and 1010 (ph-O), 1600 and 1500 (aromatic ring), and 1300-950 cm^{-1} (C-O); ^{1}H (270 MHz) and ^{13}C NMR (67.5 MHz): see Tables 1 and 2; SIMS *m/z* (rel. int.): 471 ($[M+Na]^+$, 30) and 449 ($[M+H]^+$, 10). The physical and spectral data coincided with those described in reference [2].

***Hydrolysis of* 11**. The compound **11** (12 mg) dissolved in 1.5 mL of 2M HCl were heated at 80°C for 4 hr. After hydrolysis, reaction mixture was shaken with *n*-BuOH and H_2O. The *n*-BuOH layer was washed with water and was subjected to a column chromatography on Si-gel to give kaempferol (**11a**, 4 mg): light yellow powder, mp. 259-264°C (lit. [2]: mp. 276-278°C); ^{13}C NMR (125 MHz): see Table 2. The H_2O layer was subjected to HPLC analysis and D-glucose was detected.

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