Two Acetophenone Glucosides, Cynanonesides A and B, from *Cynanchum taiwanianum* and Revision of the Structure for Cynandione A

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β-Sitosterol, β-amyrin, 4-hydroxyacetophenone, 3,4-dihydroxyacetophenone, sitosteryl 3-*O*-β-D-glucopyranoside, cynandione A, and cynanonesides A (**3**) and B (**4**) were isolated from the roots of *Cynanchum taiwanianum*. The latter two compounds are new acetophenone glucosides, and their structures were elucidated on the basis of spectral and chemical evidence. Meanwhile, the structure of cynandione A, previously designated as 3′,4-diacetyl-2,2′,3,6′-tetrahydroxybiphenyl, has been revised as 2,3′-diacetyl-2′,3,6,6′-tetrahydroxybiphenyl (**9**).

Species of the genus *Cynanchum* (Asclepiadaceae) have been used in folk medicine for the treatment of chronic tracheitis and as antifebrile, diuretic, antitussive, expectorant, anodyne, and tonic drugs.[1−7] Chemical studies of *Cynanchum* species have shown the presence of disecopregnanes, 2,6-dideoxy-3-*O*-methyl sugars, and C/D-*cis*-polyoxypregnanes and their glycosides.[9−14] Previous investigation on *C. formosanum* and *C. taiwanianum* led to the isolation of pregnane glycosides and flavonoids.[15−18]

In the present study, we reinvestigated the root extract of *C. taiwanianum* and the known β-sitosterol,[19] sitosteryl 3-*O*-β-D-glucopyranoside,[19] β-amyrin,[20] 4-hydroxyacetophenone,[21] 3,4-dihydroxyacetophenone (**1**),[22] cynandione A (**2**),[23] and two new compounds, cynanonesides A (**3**) and B (**4**). This paper also deals with the structural elucidation of the compounds **3** and **4**, and the revision of the cynandione A (**2**) structure.[23]

Cynanoneside A (**3**), an amorphous solid was determined to have a molecular formula of $C_{14}H_{18}O_8$ on the basis of its molecular ion peak in the FABMS spectrum (negative) at m/z 313 ($M^+ - 1$) and elemental analysis. UV spectrum showed similar maxima to that of 3,4-dihydroxyacetophenone (**1**).[24] The IR spectrum indicated the presence of a hydroxyl group (3400 cm^{-1}), a conjugated ketone (1660 cm^{-1}), and an aromatic ring (1600 and 1500 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) of **3** showed the presence of an acetyl group at δ 2.57 (3H, s), an ABX system of phenyl protons at δ 6.88 (1H, dd, J = 8.8, 3.0 Hz), 6.95 (1H, d, J = 3.0 Hz), and 7.10 (1H, d, J = 8.8 Hz), a phenolic proton at δ 9.29 (1H, s), which disappeared on D_2O exchange, and a sugar moiety at δ 3.44 (1H, dd, J = 10.9, 2.7 Hz), 3.69 (1H, dd, J = 10.9, 4.7 Hz), and 4.81 (1H, d, J = 7.2 Hz). Among the 14 ^{13}C-NMR signals (Table 1) of **3**, two acetyl, six phenyl, and six sugar signals were observed. The signals at δ 149.3 and 151.7 suggested two *ortho* oxygenated phenyl carbons, while the six sugar ^{13}C signals indicated that the sugar moiety was a glucose. Acetylation of **3** with Ac_2O and pyridine afforded a pentaacetate product **5** [amorphous solid; 1755 and 1740 cm^{-1}; δ 2.01, 2.03, 2.05, 2.06, and 2.26 (each 3H, s)].

Table 1. ^{1}H- and ^{13}C-NMR Data for **3** and **4** (300 MHz, 75 MHz in DMSO-d_6)

H	**3**	**4**	C	**3**	**4**
2	6.95d (3.0)		1	129.3	119.4
3		6.59d (2.0)	2	117.6	162.9
5	7.10d (8.8)	6.47 dd	3	149.4	102.0
		(8.7, 2.0)	4	151.8	159.2
6	6.88 dd	7.57d (8.7)	5	114.4	109.2
	(8.8, 3.0)		6	120.2	131.5
8	2.57s	2.52s	7	198.9	196.3
1′	4.81d (7.2)	4.91d(7.1)	8	31.7	32.0
6′	3.44 dd	3.51dd	1′	101.7	100.6
	(10.9, 2.7)	(11.5, 4.2)	2′	73.3	73.2
	3.69 dd	3.70 br d	3′	76.7	76.7
	(10.9, 4.7)	(11.5)	4′	69.7	69.4
			5′	77.0	77.1
			6′	60.7	60.5

The comparison of ^{1}H- and ^{13}C-NMR data of **3** with those of **1** indicated that the former was the glucoside of the latter. Acidic hydrolysis of compound **3** yielded 3,4-dihydroxyacetophenone (**1**) and glucose. The anomeric proton of **3** at δ 4.81 showed a NOESY correlation with the phenyl proton at δ 7.10 (d, J = 8.8 Hz), indicating that compound **3** was 3,4-dihydroxyacetophenone 4-*O*-β-D-glucopyranoside.

Cynanoneside B (**4**) was an isomer of cynanoneside A (**3**) (the molecular formula $C_{14}H_{18}O_8$ was derived from its negative ion FABMS spectrum and elemental analysis). It had hydroxyl, aromatic, and conjugated ketone absorption bands in its IR spectrum, and its UV spectrum showed maxima similar to that of 2,4-dihydroxyacetophenone. Compound **4** contained an acetyl group, an ABX system of phenyl protons, and a sugar moiety as revealed from its ^{1}H-NMR spectrum (Table 1). Two signals of phenyl carbons at δ 159.2 and 162.9 showed that the two oxygenated groups were not ortho-related. The signal of the phenyl carbon at δ 102.0 indicated that this carbon was linked to two oxygenated carbons. The ^{13}C-NMR chemical shifts of the sugar moiety of **4** suggested that the sugar moiety was a glucose. The acetylation of **4** yielded a tetraacetate product **6** [amorphous solid; 3350 and 1735 cm^{-1}; δ 2.01, 2.02, 2.03, and 2.05 (each 3H, s)] instead of a pentaacetate product. Two products, glucose and 2,4-dihydroxyacetophenone (**7**),[25] were isolated from the acidic hydrolysis of **4**. Two substituents, consisting of a

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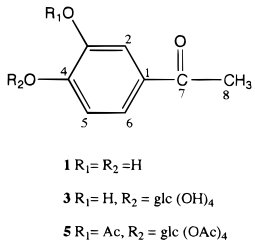
hydroxy and a glycosyl groups, were located at C-4 and C-2 or reversed positions from the above evidence. The signal at δ 4.91 (anomeric proton) showed a NOESY correlation with the phenyl proton at δ 6.59 (d, $J = 2.0$ Hz); therefore, the correct structure of **4** is 2,4-dihydroxyacetophenone 2-*O*-β-D-glucopyranoside.

Recently, Huang *et al*. isolated three new acetophenones, cynandiones A, B, and C,[23] from the same source, and the structure of cynandione A was designated as 3′,4-diacetyl 2,2′,3,6′-tetrahydroxybiphenyl (**2**). This designated structure appears to be incorrect for the following reasons. Two acetyl groups are involved in a similar chemical environment, but their chemical shifts are quite different (δ 2.18 and 2.56 as in structure **2** and δ 2.07 and 2.59 as in structure **8**).[23] The great difference in the chemical shift for H-4′ and H-5′ of compounds **2** (δ 7.79 and 6.94) and **8** (δ 7.59 and 7.37) was deduced from the effect of an acetyl group, but this difference was not observed between H-5 and H-6 (δ 6.94 and 6.77 in **2,** and 7.37 and 7.25 in **8**). The NOESY correlation between H-4′ and H-8′, but not between H-5 and H-8, is unexpected. We also isolated cynandione A from the same source and revised its structure to be **9** (2,3′-diacetyl-2′,3,6,6′-tetrahydroxybiphenyl) based on the following evidence.

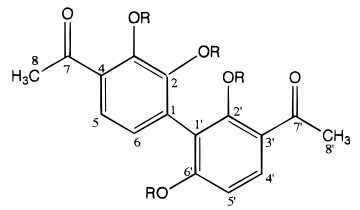
Cynandione A revealed only one intramolecular hydrogen bonding signal at δ 12.80 as it was dissolved in DMSO-d_6 solvent. This finding ruled out the designated structure **2** for cynandione A. The acetyl group situated on the B-ring was conjugated to an aryl ring and gave strong intramolecular hydrogen bonding with C-2′ hydroxy group. Due to the steric effect, the acetyl group located at ring A was not coplanar with ring A, therefore it failed to form hydrogen bonding and resonated at higher field (δ 2.18). The IR absorption bands at 1705 (isolated ketone) and 1670 cm^{-1} (conjugated ketone) in **10** and the NOE experiment of its tetrametyl product **10** provide further evidence, so that the structure of cynandione A must be revised as structure **9**.



1 $R_1 = R_2 = H$

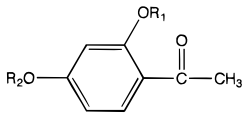
3 $R_1 = H$, R_2 = glc $(OH)_4$

5 R_1 = Ac, R_2 = glc $(OAc)_4$



2 R=H

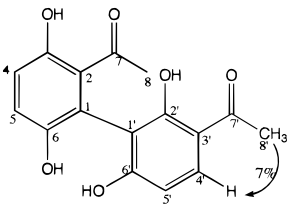
8 R=Ac



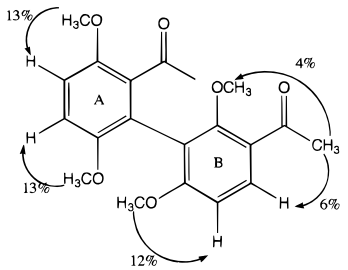
4 R_1 = glc $(OH)_4$, R_2=H

6 R_1 = glc $(OAc)_4$, R_2= H

7 $R_1 = R_2$= H



9



10

Experimental Section

General Experimental Procedures. Melting points were determined with Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 781 spectrophotometer. UV spectra were measured on a Hitachi U-3200 spectrophotometer. Optical rotations were measured on a JASCO DIP-370 instrument. FABMS spectra were obtained on a JEOL SX-102A spectrometer. ^{1}H- and ^{13}C-NMR, NOESY, and HMBC spectra were run on a Bruker AC-300 spectrometer. Elementary analyses were run on a Perkin-Elmer 2400, EA instrument.

Plant Material. The root of *Cynanchum taiwanianum* Yamazaki was bought from Cha-Yi, Taiwan, in May 1993. The plant material was identified by Dr. Ih-Sheng Chen, School of Pharmacy, Kaohsiung Medical College, and a voucher specimen has been deposited at the Herbarium of the Department of Botany of the National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The roots of *C. taiwanianum* (5 kg) were extracted twice with EtOH (30 L) at 50 °C. The EtOH extract was evaporated *in vacuo*, yielding a black residue, which was suspended in H_2O (1 L). The aqueous solution was extracted with EtOAc and *n*-BuOH, successively. The EtOAc and BuOH fractions were evaporated under reduced pressure to dryness, and repeated separation and purification on Si gel and C_{18} reversed-phase column chromatography afforded nine pregnane oligoglycosides,[18] β-sitosterol, β-amyrin, 4-hydroxyacetophenone, 3,4-dihydroxyacetophenone, sitosteryl-3-*O*-β-D-glucopyranoside, cynanoneside A (325 mg), cynanoneside B (160 mg), and cynandione A (3.65 g).

Cynanoneside A (3): amorphous solid; $[\alpha]^{20}_D$ −5.0 (*c* 1.0, EtOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.14), 327 (3.66) nm; IR (dry film) ν_{max} 3400 (OH), 1660 (C═O, ketone), 1600, 1500 (aromatic), 1200, 1080, 1000 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6, 300 MHz, 75 MHz) Table 1; FABMS (negative) *m*/*z* [M − 1]$^-$ 313 (16), 199 (30), 168 (27), 153 (100), 152(66), 151 (61), 122 (21); *anal*. C 53.49%, H 5.80%, calcd for $C_{14}H_{18}O_8$, C 53.50%, H 5.73%.

Cynanoneside B (4): amorphous solid; $[\alpha]^{20}_D$ −11.0 (*c* 1.0, EtOH); UV (MeOH) λ_{max} (log ϵ) 227 (3.75), 269 (3.64), 296 (3.48) nm; IR (dry film) ν_{max} 3400 (OH), 1665 (C═O, ketone), 1604, 1497 (aromatic), 1203, 1085, 1000 cm^{-1}; ^{1}H and ^{13}C NMR (DMSO-d_6, 300MHz, 75 MHz) Table 1; FABMS (negative) *m*/*z* (M − 1)$^-$ 313 (15), 199 (36), 168 (30), 153 (100), 152 (68), 151 (62), 122 (19); *anal*. C 53.56%, H 5.78%, calcd for $C_{14}H_{18}O_8$, C 53.50%, H 5.73%.

Acetylation of 3 and 4. A solution of cynanoneside A (**3**) (10 mg) or cynanoneside B (**4**) (10 mg) in pyridine (0.5 mL) and Ac_2O (0.5 mL) was left at room temperature overnight. The reaction mixture was poured into iced H_2O and then extracted with EtOAc (30 mL × 3). The EtOAc layer was treated in the usual manner and

purified by Si gel to yield pentaacetate (**5**) (12 mg) or tetraacetate (**6**) (11 mg). Pentaacetate **5**: mp 123−124 °C; IR (dry film) ν_{max} 1755, 1740, 1595, 1492, 1230, 1070, 1032, 945 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.01, 2.03, 2.05, 2.06, 2.26 and 2.52, (each 3H, s), 3.84 (1H, m, H-5′), 4.13 (1H, dd, J = 12.1, 2.5 Hz, Ha-6′), 4.26 (1H, dd, J = 12.1, 5.2 Hz, Hb-6′), 5.12 (1H, d, J = 7.6 Hz, H-1′), 5.17 (1H, dd, J = 9.1, 7.6 Hz, H-2′), 5.28 (1H, t, J = 9.1 Hz, H-3′), 5.31 (1H, t, J = 9.1 Hz, H-4′), 7.06 (1H, d, J = 9.0 Hz), 7.16 (1H, dd, J = 9.0, 3.0 Hz), 7.36 (1H, d, J = 3.0 Hz). Tetraacetate **6**: mp 115−116 °C; IR (dry film) ν_{max} 3350, 1735, 1605, 1494, 1248, 1130, 1045, 955 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.01, 2.02, 2.03, 2.05 and 2.47 (each 3H, s), 3.88(1H, m, H-5′), 4.17 (1H, dd, J = 12.4, 2.1 Hz, Ha-6′), 4.25 (1H, dd, J = 12.4, 5.2 Hz, Hb-6′), 5.15 (1H, dd, J = 9.1, 7.6 Hz, H-2′), 5.28 (1H, t, J = 9.1 Hz, H3′), 5.31 (1H, t, J = 9.1 Hz, H-4′), 7.06 (1H, d, J = 9.0 Hz, H-5), 7.16 (1H, dd, J = 9.0, 3.0 Hz, H-6), 7.36 (1H, d, J = 3.0 Hz, H-2).

Acidic Hydrolysis 3 and 4. A mixture of cynanoneside A (**3**) (30 mg) and TsOH (10 mg) in H_2O (5 mL) was heated at 60 °C for 3 h. Then the reaction mixture was extracted with EtOAc (10 mL × 3). After purification, the organic layer gave 3,4-dihydroxyacetophenone (**1**) (11 mg) [mp, 114−116 °C (lit.[22]; mp, 116 °C)] and glucose was detected from the aqueous layer. The same treatment of cynanoneside B (**4**) gave 2,4-dihydroxyacetophenone (**7**) [mp, 145−147 °C (lit[25]; mp 147 °C)] and glucose.

Methylation of 9. A mixture of **9** (40 mg), K_2CO_3 (100 mg), and MeI (1 mL) in 5 mL of butanone was heated under reflux. After 4 h, the solvent was evaporated *in vacuo*; 30 mL of H_2O was poured into the residue, then extracted with ether (30 mL × 3). After purification, the organic layer yielded tetramethoxy ether derivative **10** (28 mg): colorless needles (MeOH); mp 121−122 °C; IR (KBr) ν_{max} 1705, 1670, 1587, 1495, 1280, 1090, 1038, 810, 710 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.32, 2.56 (each 3H, s), 3.47, 3.67, 3.73, 3.81 (each 3H, s), 6.69, 7.73 (each 1H, d, J = 8.8 Hz, H-5′, H-4′), 6.92, 6.94 (each 1H, d, J = 9.0 Hz, H-4, H-5); ^{13}C NMR ($CDCl_3$) δ 30.5 (q, C-8′), 30.9 (q, C-8), 55.9 (q, 6′-OMe), 56.0 (q, 3-OMe), 56.6 (q, 6-OMe), 61.3 (q, 2′-OMe), 106.4 (d, C-5′), 111.3 (d, C-4), 112.7 (d, C-5), 118.7(s, C-3′), 121.5 (s, C-1′), 125.7 (s, C-1), 131.5 (d, C-4′), 133.1 (s, C-2), 150.2 (s, C-6), 151.7 (s, C-3), 159.4 (s, C-6′), 161.0 (s, C-2′), 198.7 (s, C-7′), 203.1 (s, C-7).

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