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Chemical Constituents of *Cynanchum wilfordii* and the Chemotaxonomy of Two Species of the Family Asclepiadacease, *C. wilfordii* and *C. auriculatum*

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Definitive identification of original plant species is important for standardizing herbal medicine. Although only the dried roots of *Cynanchum wilfordii* (Asclepiadaceae) are prescribed as Cynanchi Wilfordii Radix in Korean Pharmacopoeia, the roots of *C. wilfordii* and *C. auriculatum* are often misused in the Korean herbal market due to their morphological similarity and similar name. Therefore, it would be very useful to discover an effective chemical marker for the identification of the two species. To this end, we carried out a phytochemical study on the roots of *C. wilfordii*. As a result, twenty compounds were isolated from the roots of *C. wilfordii* and their chemical structures were identified as β-sitosterol (**1**), wilfoside C1N (**2**), wilfoside C3N (**3**), wilfoside K1N (**4**), methyleugenol (**5**), wilfoside C1G (**6**), cynauriculoside A (**7**), daucosterol (**8**), 2,4-dihydroxyacetophenone (**9**), cynandione A (**10**), 2,5-dihydroxyacetophenone (**11**), acetovanillone (**12**), *p*-hydroxyacetophenone (**13**), sucrose (**14**), conduritol F (**15**), geniposide (**16**), succinic acid (**17**), 3-(β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**18**), bungeiside A (**19**), cynanoneside B (**20**). Among them, compounds **15**, **16**, **18**, **19**, and **20** were isolated for the first time from this species. Furthermore, conduritol F (**15**) was demonstrated to be contained only in *C. wilfordii*. Therefore, it may be useful as a chemical marker to identify the two species *C. wilfordii* and *C. auriculatum*.

Key words *Cynanchum wilfordii*, *Cynanchum auriculatum*, Asclepiadaceae, Marker compound, Conduritol F

Selected by Editors

INTRODUCTION

The genus *Cynanchum* contains the largest number among the 180 species in Asclepiadacease and is distributed worldwide, including in east Africa, the Mediterranean region, the tropical zone of Europe, and the subtropical and temperate zones of Asia (Tsiang and Li, 1977b). But only 33 species have been systematically studied. Cynanchi Wilfordii Radix is prescribed as the roots of *Cynanchum wilfordii* in Korean Pharmacopoeia (KFDA, 2008). *C. wilfordii* is a perennial herb that belongs to Asclepiadacease and is 1-3 m tall. The leaves are 5-10 cm long, 4-8 cm wide and disposed oppositely, with a deltoid or deeply cordate shape. It is an inflorescence axillary umbels plant with peduncles 1-4 cm long and pedicels 5-8 mm long. The flowers are pale yellow-green in color, have 5 calyx lobes that are broadly lanceolate and acuminate, with lobed corolla, 3 mm long, incurved and fully puberulent inside, and have lanceolate follicles, 8 cm long and 1 cm wide (Liede and Tauber, 2002). Compared to *C. wilfordii*, *C.*

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auriculatum has a blade ovate leaf, which is papery with abaxial veins and similar in size (4.5-11 cm long and 2.6-7 cm wide) to that of *C. wilfordii*. The two plants have a common point in that the leaves are fully puberulent. The leaf bottom has sinus and rounded lobes, and its apex is acuminate; it has 5 basal veins and 2 or 3 pairs of lateral veins. The flower color of *C. auriculatum* is more variable than that of *C. wilfordii* and can be white, pale yellow, pink and purple, and the lanceolate follicles are similar to those in *C. wilfordii* with a size of 8 × 1 cm (Tsiang and Li, 1977a).

Cynanchi Wilfordii Radix is used to enrich vital essence and enhance immunity. Modern pharmacological studies have shown that the extract and fractions of Cynanchi Wilfordii Radix have various pharmacological actions including scavenging free radicals, enhancing immunity, reducing high serum cholesterol, and anti-tumor activity (Shan et al., 2006). To date, more than 300 compounds have been isolated from *Cynanchum* species, including steroids, alkaloids, terpenes, flavonoids, polysaccharides, and steroidal glycosides as the major constituents (Shan, 2008). The main constituents of the herb have been reported to be steroidal glycosides such as auriculosides I-IV; cynanauriculosides I and II; acetophenones such as cynandione A-D; triterpenoids such as haneoekinol and hancolupenol; flavonoids such as quercetin, tamarixetin, and 7-O-α-L-rhamnoside; and other constituents including cinnamic acid, sinapic acid, methylpalmitate, and paeonol (Shan, 2008). According to the record of "Zhong Hua Ben Cao" (The Chinese Traditional Medicine State Administration, 1999), Cynanchi Wilfordii Radix is an appellative name for the roots of *C. wilfordii* and *C. auriculatum* from the Asclepiadaceae family. Only the dried roots of *C. wilfordii* are prescribed as Cynanchi Wilfordii Radix in Korean Pharmacopoeia (KFDA, 2008). However, *C. auriculatum* has been cultivated and distributed in Korea, Japan, and China because it grows more rapidly and is more productive than *C. wilfordii*. Unfortunately, *C. wilfordii* and *C. auriculatum* are visually similar plants and result in visually similar herbal drugs. Thus, these two species are some of the most indiscriminately used herbal medicines because of their morphological similarity and similar names (Song et al., 2004). The misuse of different herbal drugs with the same appellative name may produce unexpected or even harmful effects in the patients. Therefore, a technique to distinguish between the two species is needed. In our phytochemical study, twenty compounds were isolated from the roots of *C. wilfordii*. Of these, compounds **15**, **16**, **18**, **19**, and **20** were isolated for the first time from this species. Furthermore, conduritol F (**15**) was found only in *C. wilfordii*. In this paper, we describe the isolation and structural elucidation of compounds **1-20** and the comparison of their chemical constituents by means of TLC and HPLC analyses.

MATERIALS AND METHODS

General experimental procedures

The NMR spectra were recorded on Bruker 250 MHz (DMX 250), Varian 600 MHz (VNS 600), and JEOL ECA-500 MHz spectrometers, and chemical shifts were recorded in ppm downfield from TMS as the internal standard. The FABMS spectra were performed on JMS-700 mass spectrometer (JEOL). Column chromatography was carried out on Merck Silica gel (70-230 mesh) and Merck Lichroprep RP-18 gel (40-63 μm). TLC was performed on aluminum plates precoated with Kieselgel 60 F_{254} (Merck). For preparative HPLC, LC-10AD pump (Shimadzu), SPD-10A detector (Shimadzu) and Shim-Pack Prep-ODS (20 × 250 mm) column were used, Inertsil SIL column (250 × 4.6 mm id; GL Science Inc., 5 μm).

Plant material

Each root of *C. wilfordii* and *C. auriculatum* was authenticated by Professor Je Hyun Lee (Dongguk University, Korea). The voucher specimens of *C. wilfordii* (YU00191C) and *C. auriculatum* (YU00181B) were deposited in the Natural Product Laboratory at the College of Pharmacy, Yeungnam University, Korea.

Extraction and isolation

The dried roots of *C. wilfordii* (5 kg) were extracted two times with MeOH at room temperature for a week. The MeOH extract (772 g) was suspended in H_2O and partitioned with EtOAc and BuOH, sequentially. The EtOAc-soluble fraction (179 g) was chromatographed on silica gel (70-230 mesh, column: 7 × 68 cm) and eluted with a gradient of *n*-hexane-EtOAc (100:0 to 0:100) to yield thirteen fractions (EFr.1-EFr.13) according to their TLC pattern. EFr.7 (12 g) and EFr.12 (10 g) were recrystallized in MeOH and afforded compounds **1** (2.0 g) and **8** (200 mg). EFr.8 (15 g) was chromatographed on silica gel (70-230 mesh, column: 6 × 60 cm) and eluted with a gradient of Acetone-MC (9:1 to 0:10) to obtain ten subfractions (EFr.8-1 ~ EFr.8-3). EFr.8-2 (7 g) was chromatographed on RP-18 gel (40-63 μm, column: 5 × 23 cm) with MeOH-H_2O (from 2:8 to 10:0) gradient to afford compounds **2** (91 mg) and **3** (119 mg). EFr.8-3 (6 g) also applied to RP-18 gel (40-63 μm, column: 4 × 60 cm), resulting in the isolation of compound **4** (49 mg). EFr.9 (14 g) was chromatographed

over silica gel (70-230 mesh, column: 6 × 60 cm) using a gradient of Acetone-MC (8:2 to 1:9), resulting in the isolation of compounds **5** (16 mg), **6** (98 mg), and **7** (600 mg), respectively. EFr.11 (9 g) was chromatographed on silica gel (70-230 mesh, column: 4 × 68 cm) using a gradient of Acetone-MC (7:3 to 0:10) to yield ten subfractions (EFr.11-1 ~ EFr.11-10). EFr.11-5 (3 g) was successively chromatographed on silica gel (70-230 mesh, column: 2.5 × 48 cm) using a gradient of MC-MeOH (8:2 to 0:10) to provide compound **9** (192 mg). EFr.11-7 (2 g) and EFr.11-10 (1 g) were also chromatographed on silica gel (70-230 mesh, column: 2.5 × 48 cm; 70-230 mesh, column: 1.5 × 37 cm) using a gradient of MC-MeOH (7:3 to 0:10), resulting in the isolation of compounds **10** (112 mg) and **11** (42 mg). EFr.13 (10 g) was applied to column chromatography over silica gel (70-230 mesh, column: 6.5 × 35 cm) using a gradient of MC-MeOH (2:8 to 1:9) to afford compounds **12** (40 mg) and **13** (150 mg). The BuOH-soluble fraction (30 g) was separated by silica gel column chromatography (70-230 mesh, column: 5 × 68 cm) using a gradient of EtOAc-MeOH (from 95:5 to 0:100) to yield 10 fractions (BFr.1-Fr.10) according to their TLC pattern. BFr.8 (4 g) was recrystallized in MeOH to give compound **14** (80 mg). BFr.6 (6 g) was subjected to column chromatography on silica gel (70-230 mesh, column: 4 × 60 cm) using a gradient of MC-MeOH (from 98:2 to 0:100) to yield six subfractions (BFr.6-1 ~ BFr.6-12). BFr.6-12 (2 g) was recrystallized with MeOH afforded compound **15** (791 mg). BFr.6-11 (2 g) was further purified using HPLC (20 × 250 mm, Shim-Pack Prep-ODS) with a gradient of MeOH-H_2O (from 20:80 to 50:50) to afford compound **16** (78 mg). BFr.7 (5 g) was loaded on RP-18 column (40-63 μm, column: 4 × 48 cm) and eluted with a gradient of MeOH-H_2O (from 20:80 to 60:40), resulting in the isolation of compound **17** (150 mg). BFr.3 (1 g) was fractionated by silica gel (70-230 mesh, column: 2.5 × 48 cm) column chromatog-

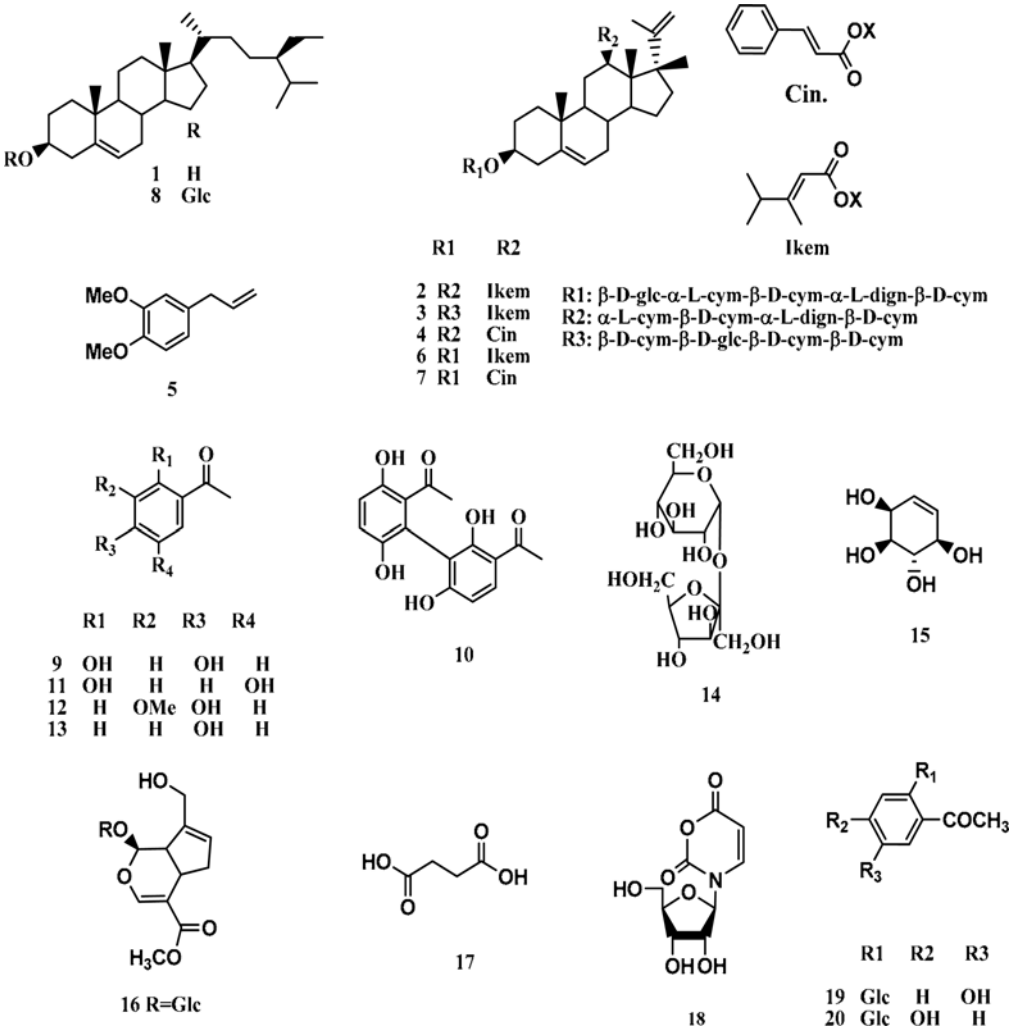

Fig. 1. Chemical structures of compounds **1**-**20** isolated from *C. wilfordii*

raphy using a gradient of MC-MeOH (99:1 to 70:30) to yield thirteen sub-fractions (BFr.3-1 ~ BFr.3-13). BFr.3-11 (250 mg) was purified using HPLC (20 × 250 mm, Shim-Pack Prep-ODS) with a gradient of MeOH-H_2O (from 5:95 to 40:60) to afford compounds **18** (61.7 mg), **19** (4.5 mg), and **20** (156 mg) (Fig. 1).

Conduritol F (15)
White solid; $[\alpha]_D^{25}$ –87 (*c* 0.1, MeOH), FABMS *m/z* 147 $[M+H]^+$; ^{1}H-NMR (250 MHz, CD_3OD): δ 5.83 (1H, ddd, *J* = 10.0, 4.9, 2.0 Hz, H-5), 5.74 (1H, dd, *J* = 10.0, 2.1 Hz, H-6), 4.18 (1H, t, *J* = 4.5 Hz, H-4), 3.96 (1H, dtd, *J* = 7.6, 2.2, 1.0 Hz, H-1), 3.66 (1H, dd, *J* = 10.5, 7.5 Hz, H-2), 3.45 (1H, dd, *J* = 10.5, 4.4 Hz, H-3); ^{13}C-NMR (63 MHz, CD_3OD): δ 133.9 (C-6), 128.0 (C-5), 74.0 (C-2), 73.9 (C-1), 72.7 (C-3), 68.1 (C-4).

Geniposide (16)
Colorless needles; ^{1}H-NMR (250 MHz, CD_3OD): δ 7.46 (1H, s, H-3), 5.74 (1H, br s, H-7), 5.13 (1H, d, *J* = 7.5 Hz, H-1), 4.67 (1H, d, *J* = 7.5 Hz, H-1'), 4.29 (1H, br d, *J* = 15.0 Hz, H-10a), 3.83 (1H, br d, *J* = 15.0 Hz, H-10b), 3.65 (3H, s, OCH3), 3.29 (1H, m, H-5), 2.70 (1H, br d, *J* = 16.1 Hz, H-6a), 2.66 (1H, br t, *J* = 7.7 Hz, H-9), 2.09 (1H, br d, *J* = 16.1 Hz, H-6b); ^{13}C-NMR (63 MHz, CD_3OD): δ 169.5 (C-11), 152.4 (C-3), 143.8 (C-8), 127.3 (C-7), 111.5 (C-4), 99.3 (C-1'), 97.2 (C-1), 77.4 (C-3'), 76.8 (C-5'), 73.8 (C-2'), 70.5 (C-4'), 61.6 (C-6'), 60.4 (C-10), 50.8 (OCH_3), 45.9 (C-9), 39.7 (C-6), 35.6 (C-5).

3-(β-D-Ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (18)
White powder; ^{1}H-NMR (250 MHz, CD_3OD): δ 8.00 (1H, d, *J* = 8.1 Hz, H-4), 5.67 (1H, d, *J* = 8.1 Hz, H-5), 5.96 (1H, d, *J* = 4.2 Hz, H-1'), 4.09 (1H, m, H-4'), 3.66 (1H, dd, *J* = 12.2, 2.6 Hz, H-5'a), 3.91 (1H, dd, *J* = 12.2, 3.0 Hz, H-5'b); ^{13}C-NMR (63 MHz, CD_3OD): δ 166.2 (C-6), 152.4 (C-2), 142.7 (C-4), 102.6 (C-5), 90.6 (C-1'), 86.3 (C-4'), 75.7 (C-3'), 71.3 (C-2'), 62.2 (C-5').

Bungeiside A (19)
White powder; ^{1}H-NMR (500 MHz, CD_3OD): δ 7.04 (1H, d, *J* = 3.1 Hz, H-6), 6.92 (1H, dd, *J* = 8.9, 3.1 Hz, H-4), 7.18 (1H, d, *J* = 8.9 Hz, H-3), 2.64 (3H, s, OCH_3), 4.88 (1H, d, *J* = 7.2 Hz, H-1'), 3.45 (1H, t, *J* = 7.5 Hz, H-2'), 3.52 (1H, m, H-3'), 3.34 (1H, m, H-4'), 3.57 (1H, m, H-5'), 3.69 (1H, dd, *J* = 10.9, 2.7 Hz, H-6'a), 3.86 (1H, dd, *J* = 10.9, 4.7 Hz, H-6'b); ^{13}C-NMR (125 MHz, CD_3OD): δ 153.8 (C-5), 151.2 (C-2), 131.5 (C-1), 121.7 (C-4), 119.6 (C-3), 116.1 (C-6), 202.6 (C=O), 32.3 (OCH_3), 103.6 (C-1'), 74.9 (C-2'), 78.2 (C-3'), 71.3 (C-4'), 78.3 (C-5'), 62.6 (C-6').

Cynanoneside B (20)
White powder; ^{1}H-NMR (500 MHz, CD_3OD): δ 6.70 (1H, d, *J* = 2.5 Hz, H-3), 6.51 (1H, dd, *J* = 7.5, 2.5 Hz, H-5), 7.71 (1H, d, *J* = 7.5 Hz, H-6), 2.52 (3H, s, OCH_3), 5.02 (1H, d, *J* = 7.5 Hz, H-1'), 3.31 (1H, t, *J* = 7.5 Hz, H-2'), 3.46 (1H, m, H-3'), 3.34 (1H, t, *J* = 8.5 Hz, H-4'), 3.54 (1H, m, H-5'), 3.76 (1H, dd, *J* = 12.0, 5.5 Hz, H-6'a), 3.94 (1H, dd, *J* = 12.0, 1.5 Hz, H-6'b); ^{13}C-NMR (125 MHz, CD_3OD): δ 121.2 (C-1), 164.5 (C-2), 103.6 (C-3), 160.9 (C-4), 110.7 (C-5), 133.4 (C-6), 200.4 (C=O), 32.1 (OCH_3), 102.3 (C-1'), 74.8 (C-2'), 78.2 (C-3'), 71.1 (C-4'), 78.3 (C-5'), 62.4 (C-6').

RESULTS AND DISCUSSION

The chemical investigation of the MeOH extract of *C. wilfordii* resulted in the isolation of twenty compounds. Their structures were characterized as β-sitosterol (**1**) (Eknamkul and Potduang, 2003), wilfoside C1N (**2**) (Tsukamoto et al., 1985a), wilfoside C3N (**3**) (Tsukamoto et al., 1985a), wilfoside K1N (**4**) (Tsukamoto et al., 1985b), methyleugenol (**5**) (Meepagala et al., 2002), wilfoside C1G (**6**) (Tsukamoto et al., 1985a, cynauriculoside A (**7**) (Zhang et al., 2006), daucosterol (**8**) (Faizi et al., 2001), 2,4-dihydroxyacetophenone (**9**) (Sun et al., 2009), cynandione A (**10**) (Lin et al., 1997), 2,5-dihydroxyacetophenone (**11**) (Akamanchi et al., 1999), acetovanillone (**12**) (Junior, 1986), *p*-hydroxyacetophenone (**13**) (Hoque, 1984), sucrose (**14**) (Chang and Su, 1986), conduritol F (**15**) (Patti et al., 1996; Kadota et al., 2001; Heo et al., 2003), geniposide (**16**) (Han et al., 1994; Lee et al., 2006; Zhou et al., 2007), Succinic acid (**17**) (Nord et al., 2004), 3-(β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (**18**) (Chwang et al., 1976), bungeiside A (**19**) (Li et al., 1992), and cynanon eside B (**20**) (Lin et al., 1997).

Compound **15** was obtained as a white solid, $[\alpha]_D^{25}$ –87, and gave a quasimolecular ion peak at *m/z* 147.15 $[M+H]^+$ in the FABMS. The ^{1}H- and ^{13}C-NMR spectra showed two olefinic proton signals at δ_H 5.83 (1H, ddd, *J* = 10.0, 4.9, 2.0 Hz, H-5) and 5.74 (1H, dd, *J* = 10.0, 2.1 Hz, H-6), which were assigned to δ_C 128.0 and δ_C 133.9, respectively. Four oxygenated methine groups were observed at δ_H 4.18 (1H, t, *J* = 4.5 Hz, H-4), 3.96 (1H, dtd, *J* = 7.6, 2.2, 1.0 Hz, H-1), 3.66 (1H, dd, *J* = 10.5, 7.5 Hz, H-2) and 3.45 (1H, dd, *J* = 10.5, 4.4 Hz, H-3), which were assigned to δ_C 68.1, δ_C 73.9, δ_C 74.0, and δ_C 72.7 by HMQC analysis. Moreover, the HMBC correlations from the olefinic proton at δ_H 5.83 (H-5) to δ_C 68.1 (C-4) and δ_C 72.7 (C-3), as well as from δ_H 5.74 (H-6) to δ_C 68.1 (C-4) and δ_C 72.7 (C-3), supported the presence of a cyclohexene moiety. Six stereoisomers are possible in the conduritol family. The above NMR

data and the optical rotation value $[\alpha]_D^{25}$ −87 were in agreement with the reported data for conduritol F $[\alpha]_D^{20}$ −71 (Kadota et al., 2001; Heo et al., 2003). Thus, the structure of **15** was identified as conduritol F.

Compound **16** was obtained as colorless needles. Its ^{1}H-NMR spectrum showed a broad singlet at δ_H 5.74 (1H, br s, H-7), a doublet at δ_H 5.13 (1H, d, J = 7.5 Hz, H-1), a characteristic of iridoids at δ_H 7.46 (1H, s, H-3), a methoxy group at δ_H 3.65 (3H, s, OCH3), and a methyl group signals at δ_H 4.29 (1H, br d, J = 15.0 Hz, H-10a) and at δ_H 3.83 (1H, br d, J = 15.0 Hz, H-10b). The ^{13}C spectrum showed one carbonyl carbon at δ_C 169.5, and signals of a sugar moiety at δ_C 99.3 (C-1'), 77.4 (C-3'), 76.8 (C-5'), 73.8 (C-2'), 70.5 (C-4'), 61.6 (C-6') supported the presence of glucose. The anomeric proton at δ_H 4.67 (H-1') correlated with the carbon at C-1 in an HMBC experiment, providing the linkage of the sugar moiety at C-1 position of the aglycon. Thus, the structure of **16** was identified as geniposide (Han et al., 1994; Lee et al., 2006; Zhou et al., 2007).

Compound **18** was obtained as a white powder. The ^{1}H-NMR spectrum showed two olefinic proton peaks at δ_H 8.00 (1H, d, J = 8.1 Hz, H-4) and 5.67 (1H, d, J = 8.1 Hz, H-5), and showed characteristic signals attributed to a β-D-ribofuranosyl at δ_H 5.96 (1H, d, J = 4.2 Hz, H-1'), 4.09 (1H, m, H-4'), 3.66 (1H, dd, J = 12.2, 2.6 Hz, H-5'a), 3.91 (1H, dd, J = 12.2, 3.0 Hz, H-5'b). The ^{13}C spectrum data showed two carbonyl signals at δ 166.22 (C-6) and 152.4 (C-2), and the signals of a β-D-ribofuranosyl moiety were observed at δ_C 90.6 (C-1'), δ_C 86.3 (C-4'), δ_C 75.7 (C-3'), δ_C 71.3 (C-2'), δ_C 62.2 (C-5'). In the HMBC spectrum, correlations from δ_H 5.96 (H-1') to δ_C 75.7 (C-3') and 152.4 (C-2), from δ_H 8.00 (H-4) to δ_C 90.6 (C-1'), δ_C 102.6 (C-5), δ_C 152.4 (C-2) and δ_C 166.2 (C-6), and from δ_H 5.67 (H-5) to δ_C 166.2 (C-6) were observed. Accordingly, the structure of **18** was identified as 3-(β-D-ribofuranosyl)-2,3-dihydro-6*H*-1,3-oxazine-2,6-dione (Chwang et al., 1976).

Compound **19** was obtained as a white powder. The ^{1}H-NMR spectrum showed the presence of an acetyl group at δ_H 2.64 (3H, s, OCH_3), an ABX system of phenyl protons at δ_H 6.92 (1H, dd, J = 8.9, 3.1 Hz, H-4), 7.04 (1H, d, J = 3.1 Hz, H-6), and 7.18 (1H, d, J = 8.9 Hz, H-3). A sugar moiety was observed at δ_H 3.69 (1H, dd, J = 10.9, 2.7 Hz, H-6'a), 3.86 (1H, dd, J = 10.9, 4.7 Hz, H-6'b), and 4.88 (1H, d, J = 7.2 Hz, H-1'). The ^{13}C-NMR data of **19** showed a carbonyl carbon at δ_C 202.6, three aromatic methane carbons at 121.7 (C-4), 119.6 (C-3), 116.1 (C-6), six sugar signals at 103.6 (C-1'), 74.9 (C-2'), 78.2 (C-3'), 71.3 (C-4'), 78.3 (C-5'), and 62.6 (C-6'), and a acetyl group at δ_C 32.3 (OCH_3). In the HMBC spectrum, correlations were observed from δ_H 7.04 (H-6), 6.92 (H-4) and 4.88 (H-1') to δ_C 151.2 (C-2), from δ_H 2.64 (OCH_3) and 7.04 (H-6) to δ_C 202.6 (C=O). On the basis of the above results, the structure of **19** was identified as bungeiside A (Li et al., 1992).

Compound **20** was obtained as a white powder. The ^{1}H-NMR spectrum showed a similar pattern to that of **19**, except for a few signals due to the ABX system of

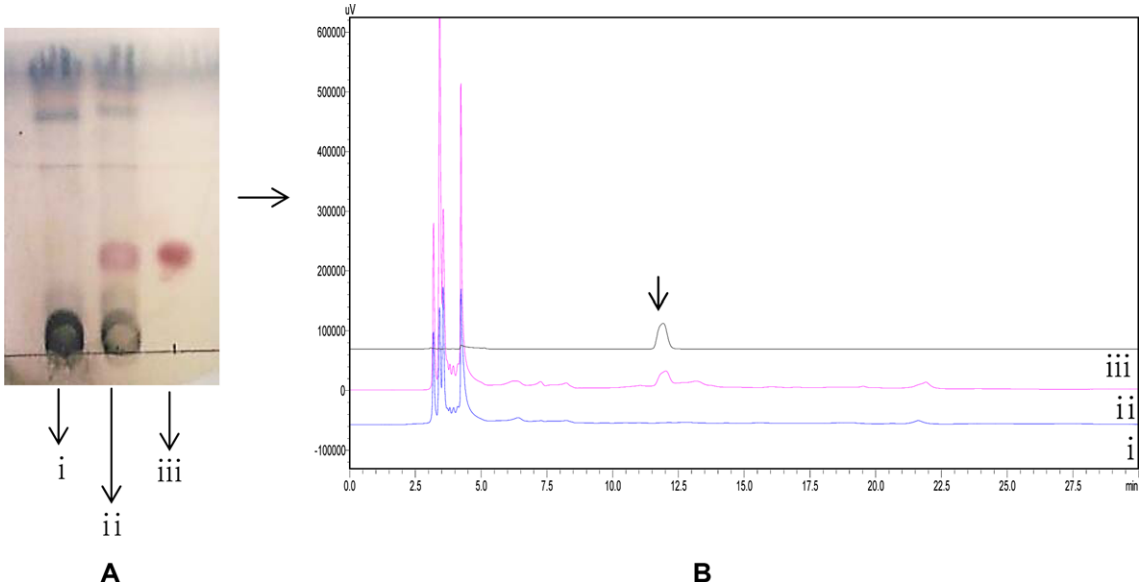

Fig. 2. TLC of compound **15** (iii), the MeOH extract of *C. wilfordii* (ii) and *C. auriculatum* (i). TLC R_f = 0.3 ($CHCl_3$-MeOH-H_2O = 15:6:0.5) (**A**). HPLC of compound **15** (iii), the MeOH extract of *C. wilfordii* (ii), and *C. auriculatum* (i). Experimental condition: Inertsil SIL column (250 × 4.6 mm id; GL Science Inc, 5 μm); solvent: acetonitrile-H_2O = 98:2; UV detection: 205 nm; flow rate: 1.0 mL/min (**B**).

benzene ring protons at δ_H 6.70 (1H, d, J = 2.5 Hz, H-3), 6.51 (1H, dd, J = 7.5, 2.5 Hz, H-5), and 7.71 (1H, d, J = 10 Hz, H-6). The differences in the chemical shift in the NMR data suggested that **20** is an isomer of **19**. The structure was further confirmed by an HMBC experiment: proton signal correlations from δ_H 5.02 (1H, d, J = 7.5 Hz, H-1') to δ_C 164.5 (C-2) and from δ_H 7.71 (1H, d, J = 10 Hz, H-6) to δ_C 200.4 (C=O) were observed. From these spectral data, the structure of **20** was identified as cynanoneside B. (Lin et al., 1997).

Of the twenty compounds isolated, compounds **15**, **16**, **18**, **19**, and **20** were isolated for the first time from *C. wilfordii*. Furthermore, we proved that conduritol F (**15**) was contained only in *C. wilfordii* by the comparison and analysis of experimental results, particularly TLC and HPLC analyses. As shown in Fig. 2A, the TLC analysis was performed on a silica gel plate (20 × 20 cm) and developed with $CHCl_3$-MeOH-H_2O (15:6:0.5). Under this TLC condition, conduritol F (**15**) developed as a red spot at the R_f value of 0.3 and was detected only in the MeOH extract of *C. wilfordii*. In addition, HPLC analysis was performed on an GL Science Inc. silica column (250 × 4.6 mm i.d.; GL Science Inc., 5 μm) with the an isocratic solvent system at a flow rate of 1.0 mL/min and detected at 205 nm. As shown in Fig. 2B, conduritol F (**15**) at the retention time of 11.5 min was detected only in the MeOH extract of *C. wilfordii*. These results suggest that conduritol F (**15**) can be used as a chemical marker for the identification of two species, *C. wilfordii* and *C. auriculatum*.

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