Chinese Journal of Chemistry, **2004**, *22*, 1200—1203 **Article**

Cyano- and Nitro-containing Compounds from the Roots of *Semiaquilegia adoxoides*

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Four cyano-containing compounds, (1*E*,4*α*,5*β*,6*α*)-4,5,6-trihydroxy-2-cyclohexen-1-ylideneacetonitrile (**1**), lithospermoside (**2**), 4-hydroxy-2-*β*-*D*-glucopyranosyl oxyphenylacetonitrile (**3**) and 4-[*β*-*D*-apiofuranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyl oxy]phenylacetonitrile (**4**), and a nitro-containing one, 4-[*β*-*D*-xylopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyloxy]-1-(2-nitroethyl)benzene (**5**), as well as eleven other types of compounds, were isolated from the roots of *Semiaquilegia adoxoides*. Their structures were elucidated mainly by spectroscopic methods. Among them, **1** and **4** are new isolated compounds. The cyano- and nitro-containing compounds are very rare in plants and their isolation from one traditional Chinese medicine is really interesting.

Keywords *Semiaquilegia adoxoides*, cyano- and nitro-containing compounds, cyanogenic glucoside

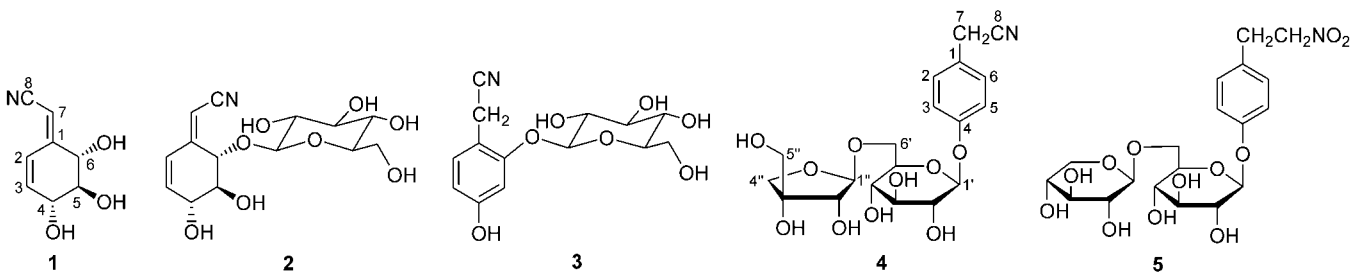
Introduction

Semiaquilegia adoxoides (DC.) Makino, which occurs widely in China,[1] is a perennial herbaceous plant of the Ranunculaceae family. Both the aerial parts and the roots are used as traditional Chinese medicines (TCM) with different indications.[1-3] The roots are applied to the treatment of inflammation, snake bite, bruises and injuries.[1] A flavonoid glucoside was isolated from the aerial parts,[2] and only one cyanogenic glucoside and a few other types of compounds were obtained from its roots.[4] Thus, a further chemical study was necessary. Our reinvestigation on its roots has led to the isolation of four cyano-containing compounds **1**—**4** and a nitro-containing one **5** (see Scheme 1), as well as eleven other known compounds. Two of them are new compounds, namely, (1*E*,4*α*,5*β*,6*α*)-4,5,6-trihydroxy-2-cyclohexen-1-ylideneacetonitrile (**1**) and 4-[*β*-*D*-apiofuranosyl-(1→6)-*O*-*β*-*D*-glucopyranosloxy]phenylacetonitrile (**4**). Their structures were mainly elucidated by spectroscopic methods, especially 2D NMR techniques. The isolation of cyano- and nitro-containing compounds from one TCM plant is very rare. Some cyano-containing compounds, such as sarmentosin[5] and sarmentosin epoxide,[6] possess significant biological activities. Herein, we report the isolation and structural elucidation of the two new compounds **1** and **4**.

Results and discussion

Compound **1** was assigned the molecular formula $C_8H_9NO_3$ by HREIMS at *m/z* 167.0575 $[M]^+$ (calcd 167.0582). A sharp IR absorption band at 2216 cm^{-1} suggested the presence of a conjugated nitrile,[7] which was supported by the observation of a quaternary carbon signal at δ 118.3 in the ^{13}C NMR spectrum. A strong broad IR absorption band at 3300 cm^{-1} was indicative of hydroxyl groups. The HMQC spectrum made it possible to address each of the proton signals to the carbons where the protons are attached. Three coupled proton signals at δ 4.22 (br.d, J=8.1 Hz, 1H, H-4), 3.36 (dd,

Scheme 1



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J=10.6, 8.1 Hz, 1H, H-5) and 4.13 (dd, J=10.6, 2.0 Hz, 1H, H-6) were assigned to the hydroxyl-bearing carbons at δ 73.9 (C-4), 79.5 (C-5) and 73.8 (C-6) respectively, suggesting the presence of a structural fragment —CH(OH)CH(OH)CH(OH)—. The olefinic proton signals at δ 6.56 (dd, J=10.1, 2.4 Hz, 1H, H-2), 6.14 (br.d, J=10.1 Hz, 1H, H-3) and 5.63 (br.s, 1H, H-7), and the carbon signals at δ 124.7 (C-2), 141.6 (C-3), 93.6 (C-7) and 161.6 (C-1) were attributable to the presence of two double bonds. For five degrees of unsaturation in its molecule, a cyano-group and two double bonds accounted for the four degrees of unsaturation, and the remaining degree of unsaturation was assumed to represent the alicyclic ring in compound **1**. The aforementioned functionalities and structural fragment have led to a rational planar structure of **1**.

The large coupling constants between H-4 and H-5 (8.1 Hz), and between H-5 and H-6 (10.6 Hz) indicated that three hydroxyl groups were all located to equatorial positions, which was identical with the assignments for the hydroxyls in the aglycone moiety of the isolated known compound **2**. Comparison of the ^{1}H and ^{13}C NMR data of **1** with those of **2**[8,9] inferred that **1** was most likely a steric isomer of the aglycone of **2** different at the C-7. The H-2 proton signal of **1** was more down field shifted than that of the aglycone moiety of **2,** suggesting that compound **1** was *E*-isomer of cyanomethylene-cyclohexene,[10] which was also confirmed by the NOESY experiment, showing strong correlation between H-7 and H-6. The structure of compound **1** was thus elucidated as (1*E*,4α,5β,6α)-4,5,6-trihydroxy-2-cyclohexen-1-ylideneacetonitrile, which is an isomer of huazhongilexol.[11]

Compound **4** was assigned the molecular formula $C_{19}H_{25}NO_{10}$ by positive ESIMS at *m/z* 450 $[M+Na]^+$ and negative ESIMS at *m/z* 426 $[M-H]^-$, and HREIMS at *m/z* 295.1061 $[M-api]^+$ (calcd 295.1056) and 133.0535 $[M-glc-api]^+$ (calcd 133.0527). A sharp IR absorption band at 2256 cm^{-1} indicated the presence of a CN group[7] and this was proved by the presence of a quaternary carbon signal at δ 120.4 (C-8) in the ^{13}C NMR spectrum. The proton signals at δ 7.29 (d, J=8.4 Hz, 2H, H-2,6) and 7.11 (d, J=8.4 Hz, 2H, H-3,5), along with the carbon signals at δ 130.8 (C-2,6) and 118.8 (C-3,5) were typical of a 1,4-substituted aromatic ring. In the HMBC spectrum (Figure 1), H-2 and H-6 were correlated with the carbon signal of C-7 at δ 23.3, and H-7 (δ 3.82, 2H, s) was correlated with both C-1 and C-8, indicating that a —CH_2CN group was attached to C-1 at δ 126.5. HREIMS, ^{1}H and ^{13}C NMR (Table 1) spectra showed the existence of a sugar linkage of β-*D*-apiofuranosyl-(1→6)-*O*-β-*D*-glucopyranosyl, which was confirmed by an HMBC correlation (Figure 1) from the proton signal at δ 4.97 (H-1") to the carbon signal at δ 69.3 (C-6'). Comparison of the ^{1}H and ^{13}C NMR data for the sugar moiety of **4** with those of 2-(4-hydroxyphenyl)ethyl-β-*D*-apiofuranosyl-(1→6)-*O*-β-*D*-glucopyranoside (osmanthuside H)[12] also indicated that both compounds had the same sugar sequence. On the basis

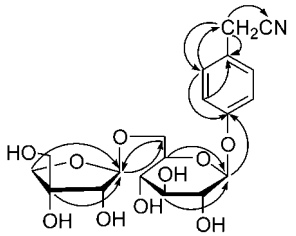

Figure 1 Selected HMBC correlations of **4**.

Table 1 ^{1}H and ^{13}C NMR data of compounds **1** and **4**[a]

Position	**1**		**4**	
	δ_C	δ_H	δ_C	δ_H
1	161.6		126.5	
2	124.7	6.56 (dd, J=10.1, 2.4)	130.8	7.29 (d, J=8.4)
3	141.6	6.14 (br.d, J=10.1)	118.8	7.11 (d, J=8.4)
4	73.9	4.22 (br.d, J=8.1)	159.2	
5	79.5	3.36 (dd, J=10.6, 8.1)	118.8	7.11 (d, J=8.4)
6	73.8	4.13 (dd, J=10.6, 2.0)	130.8	7.29 (d, J=8.4)
7	93.6	5.63 (br.s)	23.3	3.82 (s)
8	118.3		120.4	
1'			102.8	4.86 (d, J=7.0)
2'			75.3	3.45 (m)
3'			78.4	3.45 (m)
4'			72.0	3.34 (m)
5'			77.5	3.63 (m)
6'			69.3	4.02 (d, J=9.5) 3.63 (m)
1"			111.5	4.97 (d, J=1.9)
2"			78.5	3.92 (d, J=1.9)
3"			81.0	
4"			75.4	3.97 (d, J=9.9) 3.75 (d, J=9.9)
5"			66.0	3.58 (s)

[a] Spectral data were measured in CD_3OD with δ values in ppm and J values in Hz. The assignation of the signals was solved by HMQC experiment.

of biogenetic consideration and the fact of natural occurrence of the same sugar sequence as in osmanthuside H,[12] two sugars in **4** were therefore tentatively assigned as *D*-apiose and *D*-glucose. The sugar moiety was substituted at C-4 of the phenyl ring according to the HMBC correlation between H-1' (δ 4.86) and C-4 (δ 159.2). The structure of compound **4** was thus elucidated as 4-[β-*D*-apiofuranosyl-(1→6)-*O*-β-*D*-glucopyranosyloxy]phenylacetonitrile.

Fourteen known compounds are lithospermoside (**2**),[4,8,9] 4-hydroxy-2-β-*D*-glucopyranosyloxyphenylacetonitrile (**3**),[13] 4-[β-*D*-xylopyranosyl-(1→6)-*O*-β-*D*-glucopyranosyloxy]-1-(2-nitroethyl)benzene (**5**),[14] 1-β-*D*-ribofuranosyluracil,[15] β-sitosterol, daucosterol, 5-hydroxymethyl furfural,[16] griffonilide,[4,8,9] (+)-syringaresinol,[17] (+)-pinoresinol,[17] salidroside,[18] 4-β-*D*-gluco-

pyranosyloxybenzoic acid,[19] *n*-butyl-*β*-*D*-fructopyranoside[20] and 5-hydroxy-methyl-2-furoic acid.[21] Their structures were identified by ^{1}H, ^{13}C NMR, EIMS and ESIMS spectra.

Experimental

General

IR spectra were recorded on a Perkin-Elmer 577 spectrometer. Optical rotations were determined on a Perkin-Elmer 341 polarimeter. NMR spectra were measured on a Bruker AM-400 spectrometer. EIMS (70 eV) was carried out on a Finnigan MAT 95 mass spectrometer, and ESIMS was recorded on a Finnigan LCQ^{DECA} Mass spectrometer. All solvents used were of analytical grade. Silica gel (200—300 mesh) was used for column chromatography, and pre-coated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, China) were used for TLC. RP-18 silica gel (150—200 mesh, Merck) and MCI gel CHP20P (75—150 μ, Mitsubishi Chemical Industries Ltd.) were also used for column chromatography.

Plant material

The roots of *Semiaquilegia adoxoides* were collected from Anhui province of China in August of 2001, and were identified by Zeng-Tao Wang of Shanghai Traditional Chinese Medical University. A voucher specimen has been deposited in Shanghai Institute of Materia Medica (accession number TKZ-2001-1YZ).

Extraction and isolation

10.0 kg of air-dried powder of the roots of *S. adoxoides* was extracted with 95% EtOH. The crude extract (230.0 g) was dissolved in water (2 L), and partitioned with petroleum ether, EtOAc and *n*-BuOH to give three parts PE, EA and BU, respectively.

The PE part (16.0 g) was subjected to a column of MCI gel eluted with 40% MeOH in water to give a major fraction, which was then separated over a silica gel column eluted with petroleum ether-acetone (10 ∶ 1, *V*/*V*) to afford *β*-sitosterol (10 mg) and daucosterol (29 mg).

The EA part (39.0 g) was fractionated on a column of MCI gel to give four major fractions (Frs. 1—4). Fr. 1 was extensively separated over columns of silica gel and MCI gel to obtain 5-hydroxymethyl furfural (128 mg) and griffonilide (85 mg). Fr. 3 was purified by column chromatography of silica gel (eluted with petroleum ether-acetone, 1 ∶ 1, *V*/*V*) and RP-18 silica gel (MeOH-H_2O, 3 ∶ 1, *V*/*V*) to give (+)-syringaresinol (8 mg) and (+)-pinoresinol (7 mg).

The BU part (20.0 g) was subjected to a column of MCI gel eluted with H_2O-MeOH (1 ∶ 0, 4 ∶ 1, 1 ∶ 1, *V*/*V*, each 500 mL) to offer three major fractions (Frs. 1—3). Fr. 1 was separated on a silica gel column eluted with EtOAc-MeOH (1 ∶ 0, 20 ∶ 1, 10 ∶ 1, 5 ∶ 1, 2 ∶ 1, 1 ∶ 1, 0 ∶ 1, *V*/*V*) to obtain 10 elutes (Frs. 1a—10a). Frs. 1a and 2a were purified by RP-18 silica gel column to obtain salidroside (5 mg) and compound **1** (12 mg), respectively. Frs. 3a and 4a were purified by using column chromatographies of Sephadex LH-20 (MeOH) and followed by RP-18 silica gel column (MeOH-H_2O, 4 ∶ 1, *V*/*V*) to afford 4-*β*-*D*-glucopyranosyloxybenzoic acid (10 mg) and compound **3** (37 mg), respectively. Frs. 5a—7a were purified by using column chromatographies of MCI gel, Sephadex LH-20 and RP-18 silica gel to give *n*-butyl-*β*-*D*-fructopyranoside (8 mg), 1-*β*-*D*-ribofuranosyluracil (7 mg) and compound **4** (16 mg), respectively. After Sephadex LH-20 gel column chromatography (MeOH), Frs. 8a—9a were further purified by preparative TLC ($CHCl_3$-MeOH-HCO_2H, 3 ∶ 1 ∶ 0.02, *V*/*V*) to obtain 5-hydroxymethyl-2-furoic acid (5 mg) and compound **2** (8 mg), respectively. Fr. 2 was extensively purified by column chromatography of silica gel and RP-18 silica gel to give 4-[*β*-*D*-xylopyranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyloxy]-1-(2-nitroethyl) benzene (**5**) (105 mg).

(1*E*,4*α*,5*β*,6*α*)-4,5,6-Trihydroxy-2-cyclohexen-1-ylideneacetonitrile (1)

White amorphous powder, $[\alpha]_D^{20}$ +54.3 (*c* 1.00, MeOH); ^{1}H and ^{13}C NMR see Table 1; IR (KBr) *ν*: 3300, 2216, 1630, 1124, 1067, 1034, 785 cm^{-1}; EIMS (70 eV) *m/z* (%): 167 (M^+, 5), 149 (52), 120 (87), 94 (100), 65 (77); HREIMS calcd for $C_8H_9NO_3$: 167.0582 ($[M]^+$), found 167.0575.

4-[*β*-*D*-Apiofuranosyl-(1→6)-*O*-*β*-*D*-glucopyranosyloxy]phenylacetonitrile (4)

White amorphous powder, $[\alpha]_D^{20}$ −68.5 (*c* 1.00, MeOH); ^{1}H and ^{13}C NMR see Table 1; IR (KBr) *ν*: 3500—3200, 2256, 1612, 1512, 1371, 1234, 1067, 824 cm^{-1}; EIMS (70 eV) *m/z* (%): 295 ($[M-api]^+$, 5), 133 ($[M-api-glc]^+$, 100), 132 (20), 106 (10), 94 (10), 85 (18); positive ESIMS *m/z*: 450 ($[M+Na]^+$) and negative ESIMS *m/z*: 426 ($[M-H]^-$); HREIMS calcd for $C_{14}H_{17}NO_6$: 295.1056 ($[M-api]^+$), found 295.1061, and calcd for C_8H_7NO: 133.0527 ($[M-api-glc]^+$), found 133.0535.

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