Four New Oleanane Saponins from *Anemone anhuiensis*

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Four new oleanane triterpene saponins, anhuienosides C—F, together with three known saponins, were isolated from the rhizomes of *Anemone anhuiensis* (Ranunculaceae). The structures of anhuienosides C—F were elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-β-D-xylopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, respectively.

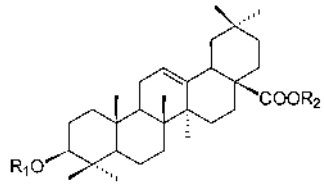
Key words *Anemone anhuiensis*; Ranunculaceae; anhuienoside; oleanane saponin

Anemone anhuiensis Y. K. YANG, N. WANG *et* W. C. YE (Ranunculacese) is a newly identified species growing in the southeastern part of Anhui Province of China.[1)] The rhizome of this plant is used in folk medicine for the treatment of rheumatism and phlebitis. Recently we have reported several known triterpene glycosides from this plant.[2—4)] Further investigation of an ethanol extract of this plant has now led to the isolation of four new oleanane saponins, designated as anhuienosides C (**1**), D (**2**), E (**3**) and F (**4**), together with three known saponins (**5**—**7**). This paper deals with the isolation and structural elucidation of these compounds.

An EtOH extract of the rhizomes was chromatographed on D101 macroporous resin to afford a fraction rich in saponins. The saponin mixture was subjected to silica gel and C_{18} reverse-phased column chromatography, followed by high-perfromance liquid chromatography (HPLC) to yield seven analytically pure compounds. Compounds **1**—**4** were determined to be new saponin structures, whereas the other three isolates were identified as cussonoside B (**5**),[5)] flaccidoside II (**6**),[6)] and flaccidoside III (**7**)[7)] by comparison of their NMR data and physical properties with literature values.

Anhuienoside C (**1**) was obtained as an amorphous powder. The fast atomic bombardment mass spectrometry (FAB-MS) of **1** displayed quasi-molecular ions $[M+H]^+$ and $[M+Na]^+$ at *m/z* 1059 and 1081, respectively, consistent with a molecular formula $C_{53}H_{86}O_{21}$. Acid hydrolysis of **1** yielded oleanolic acid, D-glucose, L-rhamnose and D-xylose. The sugars were identified by direct comparison with authentic samples using HPLC and optical rotation measurement. The ^{1}H- and ^{13}C-NMR data of **1** suggested the presence of β-xylopyranosyl, α-rhamnopyranosyl and two β-glucopyranosyl moieties, as shown by four anomeric proton signals at δ 4.80 (d, J=7.6 Hz), 6.50 (br s), 5.01 (d, J=7.6 Hz) and 6.24 (d, J=8.0 Hz), as well as the corresponding anomeric carbons at δ 106.3, 102.2, 105.3 and 95.8. The ^{13}C-NMR signals at δ 88.3 (C-3) and 176.7 (C-28) were characteristic for oleanolic acid glycosylated at both C-3 and C-28 positions.[5)] Subsequently, the NMR signals arising from the sugar portions were assigned by DQF-COSY (double quantum-filtered phase-sensitive correlation spectroscopy) and HMQC (^{1}H-detected heteronuclear one-bond spectroscopy) experiments. The interglycosidic linkages of the sugar chain could be deduced from an HMBC (^{1}H-detected heteronuclear multiple-bond spectroscopy) experiment. Thus, long-range correlation signals were observed between H-1 (δ 6.50) of rhamnose and C-2 (δ 79.8) of xylose, between H-1″ (δ 5.01) of the terminal glucose and C-6′ (δ 69.6) of the bridging glucose, as well as between H-1′ (δ 6.24) of the bridging glucose and C-28 (δ 176.7) of the aglycon. Moreover, the HMBC spectrum revealed correlations between H-1 (δ 4.80) of xylose and C-3 (δ 88.3) of the aglycon. Based on these findings, the structure of anhuienoside C (**1**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. The ^{13}C-NMR data of **1** are shown in Table 1.

Anhuienoside D (**2**) was obtained as an amorphous powder. The FAB-MS of **2** displayed quasi-molecular ions $[M+H]^+$ and $[M+Na]^+$ at *m/z* 1059 and 1081, respectively, indicating the same molecular formula as **1**. The hydrolysis reaction further suggested the presence of same compositions of sugar residues and aglycon as in **1**. However, a comparison of the ^{13}C-NMR and HMBC data indicated that the sugar chain structures between **1** and **2** are different. Thus, in the HMBC spectrum of **2**, correlation signals were observed be-



1. R_1 = -xyl(2→1)rha R_2 = -glc'(6→1)glc"
2. R_1 = -xyl R_2 = -glc'(6→1)glc"(4→1)rha'
3. R_1 = -glc(2→1)rha R_2 = -glc'(6→1)glc"(4→1)rha'
4. R_1 = -xyl(2→1)rha(3→1)glc R_2 = -glc'(6→1)glc"(4→1)rha'
5. R_1 = -H R_2 = -glc'(6→1)glc"(4→1)rha'
6. R_1 = -xyl(2→1)rha R_2 = -glc'(6→1)glc"(4→1)rha'
7. R_1 = -xyl(2→1)glc R_2 = -glc'(6→1)glc"(4→1)rha'

Fig. 1. Structures of Compounds **1**—**7**

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tween H-1 (δ 5.83) of rhamnose and C-4″ (δ 78.4) of the central glucose, between H-1″ (δ 4.97) of the central glucose and C-6′ (δ 69.4) of the inner glucose. Moreover, the HMBC spectrum revealed correlations between H-1 (δ 4.81) of xylose and C-3 (δ 88.8) of the aglycon, as well as between H-1′ (δ 6.22) of the inner glucose and C-28 (δ 176.6). These findings led to the assignment of anhuienoside D (**2**) as 3-*O*-β-D-xylopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. The ^{13}C-NMR data of **2** are shown in Table 1.

Anhuienoside E (**3**) was obtained as an amorphous powder. The FAB-MS of **3** displayed quasi-molecular ions $[M+H]^+$ and $[M+Na]^+$ at *m/z* 1235 and 1257, respectively, consistent with a molecular formula $C_{60}H_{98}O_{26}$. Acid hydrolysis of **3** yielded oleanolic acid, D-glucose and L-rhamnose. The ^{1}H- and ^{13}C-NMR spectra suggested anhuienside E (**3**) was an oleanane bisdesmoside containing five sugar residues. The ^{1}H- and ^{13}C-NMR signals could be assigned by DEPT (distortionless enhancement by polarization transfer), DQF-COSY and HMQC spectra, and the sequence and glycosylating sites were determined by an HMBC experiment. Thus, in the HMBC spectrum of **3**, correlation signals were observed between H-1 (δ 5.76) of rhamnose and C-4″ (δ 78.4) of the central glucose, between H-1″ (δ 4.90) of the central glucose and C-6′ (δ 69.4) of the inner glucose, as well as between H-1 (δ 6.56) of rhamnose (at C-3 sugar chain) and C-1 (δ 80.1) of glucose. Moreover, the HMBC spectrum revealed correlations between H-1 (δ 4.85) of glucose and C-3 (δ 89.2) of the aglycon as well as between H-1′ (δ 6.14) of the inner glucose and C-28 (δ 176.6) of the aglycon. From the above evidence, anhuienoside E (**3**) was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. The ^{13}C-NMR data of **3** are shown in Table 1.

Anhuienoside F (**4**) was obtained as an amorphous powder. The FAB-MS of **4** displayed quasi-molecular ions $[M+H]^+$ and $[M+Na]^+$ at *m/z* 1367 and 1389, respectively, consistent with a molecular formula $C_{65}H_{106}O_{30}$. The ^{1}H- and ^{13}C-NMR spectra revealed the presence of oleanolic acid and six sugar residues, which consisted of xylose, two rhamnose and three glucose residues. The carbon signals at δ 89.2 (C-3) and 177.0 (C-28) suggested a bisdesmoside structure. Analysis of the NMR data indicated that **4** had an identical glycosidic chain located on C-28 as in **2** and **3**. Comparison of the ^{13}C-NMR data between **4** and flaccidoside II (**6**) further revealed the former possessed an additional glucose residue on the sugar chain located on C-3 of the aglycon. The HMBC experiment supported the above findings. In the HMBC spectrum of **4**, correlation signals were observed between H-1 (δ 5.43) of glucose and C-3 (δ 83.3) of the central rhamnose, between H-1 (δ 6.39) of the central rhamnose at C-3 sugar chain and C-2 (δ 79.6) of xylose, as well as between H-1 (δ 5.79) of rhamnose at C-28 sugar chain and C-4″ (δ 78.8) of the central glucose, between H-1″ (δ 5.00) of the central glucose and C-6′ (δ 69.4) of the inner glucose. Moreover, the HMBC spectrum revealed correlations between H-1 (δ 4.78) of xylose and C-3 (δ 89.2) of aglycone as well as between H-1′ (δ 6.21) of the inner glucose and C-28 (δ 177.0) of aglycone. Based on these findings, anhuienoside F (**4**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-

Table 1. ^{13}C-NMR Chemical Shifts of Anhuienosides C (**1**), D (**2**), E (**3**) and F (**4**) (Pyridine-d_5, ppm)[a)]

Carbon	1	2	3	4
1	39.4	39.1	39.4	39.5
2	28.7	28.6	28.6	28.8
3	**88.3**	**88.8**	**89.2**	**89.2**
4	39.9	39.9	39.8	40.2
5	56.5	56.1	56.4	56.6
6	19.0	19.5	19.1	19.1
7	33.5	33.4	33.5	33.6
8	40.3	40.2	40.2	40.4
9	48.4	48.4	48.3	48.6
10	37.4	37.4	37.3	37.6
11	23.8	23.7	23.7	23.9
12	123.0	123.0	123.0	123.2
13	144.3	144.2	144.2	144.5
14	42.5	42.4	42.5	42.7
15	27.2	27.1	27.1	27.4
16	24.2	24.2	24.1	24.4
17	46.6	46.5	46.6	46.8
18	42.1	41.9	42.0	42.2
19	47.4	47.3	47.3	47.6
20	31.1	31.1	31.1	31.3
21	34.4	34.3	34.3	34.5
22	32.9	32.8	32.8	33.0
23	28.4	28.5	28.4	28.8
24	17.5	17.3	17.5	17.8
25	16.1	16.0	16.0	16.3
26	17.9	17.9	17.9	18.1
27	26.5	26.4	26.4	26.7
28	**176.7**	**176.6**	**176.6**	**177.0**
29	33.5	33.5	33.5	33.7
30	24.1	24.2	24.0	24.3
C-3				
xyl 1	106.3	107.8		106.6
2	**79.8**	75.7		**79.6**
3	78.4	78.8		78.4
4	71.8	71.5		71.9
5	67.2	67.4		67.3
rha 1	102.2		101.9	101.9
2	72.6		72.6	71.8
3	72.8		72.8	**83.3**
4	74.4		74.4	73.1
5	70.1		69.9	70.3
6	19.1		19.1	19.3
glc 1			105.6	106.7
2			**80.1**	76.1
3			78.0	78.8
4			72.4	71.8
5			78.3	78.9
6			63.1	62.9
C-28				
glc 1′	95.8	95.8	95.8	96.0
2′	74.2	74.0	74.1	74.3
3′	78.9	78.9	78.9	78.9
4′	71.0	71.0	71.0	71.0
5′	78.2	78.2	78.3	78.4
6′	**69.6**	**69.4**	**69.4**	**69.4**
glc 1″	105.3	105.0	105.0	105.0
2″	75.4	75.6	75.6	75.7
3″	78.6	76.7	76.7	76.9
4″	71.7	**78.4**	**78.4**	**78.8**
5″	78.7	77.4	77.4	77.4
6″	62.8	61.5	61.5	61.7
rha 1′		102.9	102.9	103.1
2′		72.8	72.8	72.9
3′		73.0	73.0	73.1
4′		74.2	74.2	74.3
5′		70.6	70.6	70.8
6′		18.8	18.9	19.1

a) Assignments were established by interpretation of the ^{13}C DEPT, HMQC and HMBC spectra. Values given in boldface indicated the glycosidic positions.

rhamnopyranosyl-(1→2)-β-D-xylopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester. The ^{13}C-NMR data of **4** are shown in Table 1

Experimental

Melting points were measured on a Leica Galen III micro melting point apparatus and uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. ^{1}H,- ^{13}C- and two dimensional (2D)-NMR spectra were recorded on a JEOL JNM-EX400 spectrometer. FAB-MS spectra were determined in positive ion mode on a Finnigan MAT TSQ7000 spectrometer. Column chromatography was carried on D101 macroporous resin (15—30 mesh, Tianjing, China), silica gel (200—300 mesh) and ODS (10—40 μm). TLC was conducted on Silica gel 60 F_{254} and RP-18 F_{254} S plates Merck. Vanillin–H_2SO_4 was used as staining reagents. HPLC was performed using an ODS column (Waters, NOVA-Pak C_{18}, 3.9×300 mm).

Plant Materials The rhizomes of *A. anhuiensis* were collected in Qingyang County, Anhui Province, China in March of 1995. Voucher specimen (95028) has been deposited in the Herbarium of China Pharmaceutical University.

Extraction and Isolation Air-dried rhizomes of *A. anhuiensis* (0.223 kg) were extracted three times with 95% EtOH for 3 h each under reflux. The EtOH extract was concentrated and subjected to D101 resin column chromatography using EtOH–H_2O (0 : 100→25 : 75→5 : 95) as eluant to afford a saponin fraction (95% EtOH elution, 20.5 g). The total saponins were subjected to silica gel column chromatography (500 g) using $CHCl_3$–MeOH (99 : 1→50 : 50) as solvents. Fractions 54—66 were combined (6.5 g) and purified by ODS column chromatography (*ca.* 200 g) using MeOH–H_2O (4 : 6→9 : 1) as solvents to afford **1** (35 mg), **3** (40 mg), **6** (1.5 g) and **7** (2.5 g). Fractions 44—50 were combined and separated by HPLC (mobile phase: MeCN–H_2O, 30 : 70; detector: waters 996 photodiode array detector; flow rate: 1 ml/min; detection wavelength: 204 nm) to afforded **2** (42 mg) and **5** (50 mg). Compound **4** (54 mg) was purified from the mixture containing **4**, **6** and **7** by HPLC (MeCN–H_2O, 29 : 71).

Anhuienoside C (**1**): White powder (MeOH), mp 238—241°C, $[\alpha]_D^{20}$ −8.6° (c=0.42, MeOH). IR (KBr) cm^{-1}: 3410 (OH), 2940, 1734 (COOR), 1634 (C=C), 1388, 1076 (C–O–C), 581. FAB-MS *m/z*: 1059 $[M+H]^+$, 1081 $[M+Na]^+$. *Anal*. Calcd for $C_{53}H_{86}O_{21}$: C, 60.11; H, 8.13. Found: C, 60.07; H, 8.16. ^{1}H-NMR (C_5D_5N) δ: 0.86 (3H, s, Me-25), 0.88 (6H, s, Me-29, −30), 1.08 (3H, s, Me-24), 1.17 (3H, s, Me-26), 1.24 (6H, s, Me-23, -27), 1.69 (3H, d, J=5.6 Hz, Me of rhamnose), 3.29 (1H, dd, J=11.6, 4.0 Hz, 3-(H), 4.80 (1H, d, J=7.6 Hz, 1-H of xylose), 5.01 (1H, d, J=7.6 Hz, 1-H of glucose″), 5.37 (1H, br s, 12-H), 6.24 (1H, d, J=8.0 Hz, 1-H of glucose′), 6.50 (1H, br s, 1-H of rhamnose); ^{13}C-NMR data, see Table 1.

Anhuienoside D (**2**): White powder (MeOH), mp 251—253°C, $[\alpha]_D^{20}$ −63.6° (c=0.33, MeOH). IR (KBr) cm^{-1}: 3412 (OH), 2941, 1734 (COOR), 1635 (C=C), 1386, 1075 (C–O–C), 580. FAB-MS *m/z*: 1059 $[M+H]^+$, 1081 $[M+Na]^+$. *Anal*. Calcd for $C_{53}H_{86}O_{21}$: C, 60.11; H, 8.13. Found: C, 60.04; H, 8.18. 1H-NMR (C_5D_5N) δ: 0.87 (6H, s, Me-29, -30), 0.88 (3H, s, Me-25), 0.98 (3H, s, Me-26), 1.08 (3H, s, Me-24), 1.23 (3H, s, Me-23), 1.28 (3H, s, Me-27), 1.68 (3H, d, J=6.4 Hz, Me of rhamnose′), 3.33 (1H, dd, J=11.6, 3.6 Hz, 3-(H), 4.81 (1H, d, J=7.6 Hz, 1-H of xylose), 4.97 (1H, d, J=7.6 Hz, 1-H of glucose″), 5.40 (1H, br s, 12-H), 5.83 (1H, br s, 1-H of rhamnose), 6.22 (1H, d, J=8.0 Hz, 1-H of glucose′); ^{13}C-NMR data, see Table 1.

Anhuienoside E (**3**): White powder (MeOH), mp 224—226°C. FAB-MS *m/z*: 1235 $[M+H]^+$, 1257 $[M+Na]^+$. *Anal*. Calcd for $C_{60}H_{98}O_{26}$: C, 58.35; H, 7.94. Found: C, 58.29; H, 8.01. IR (KBr) cm^{-1}: 3410 (OH), 2940, 1734 (COOR), 1636 (C=C), 1388, 1076 (C–O–C), 580. ^{1}H-NMR (C_5D_5N) δ: 0.84 (3H, s, Me-25), 0.86 (3H, s, Me-29), 0.87 (3H, s, Me-30), 1.06 (3H, s, Me-24), 1.18 (3H, s, Me-26), 1.22 (3H, s, Me-23), 1.23 (3H, s, Me-27), 1.68 (3H, d, J=6.0 Hz, 1-H of rhamnose′), 1.70 (3H, d, J=5.2 Hz, 1-H of rhamnose), 3.34 (1H, dd, J=11.6, 4.0 Hz, 3-(H), 4.85 (1H, d, J=7.8 Hz, 1-H of glucose), 4.90 (1H, d, J=7.6 Hz, 1-H of glucose″), 5.39 (1H, br s, 12-H), 5.76 (1H, br s, 1-H of rhamnose′), 6.14 (1H, d, J=8.0 Hz, 1-H of glucose′), 6.56 (1H, br s, 1-H of rhamnose); ^{13}C-NMR data, see Table 1.

Anhuienoside F (**4**): White powder (MeOH), mp 235—236 °C, FAB-MS *m/z*: 1367 $[M+H]^+$, 1389 $[M+Na]^+$. *Anal*. Calcd for $C_{65}H_{106}O_{30}$: C, 57.10; H, 7.76. Found: C, 57.02; H, 7.82. IR (KBr) cm^{-1}: 3410 (OH), 2942, 1734 (COOR), 1635 (C=C), 1385, 1073 (C–O–C), 579. ^{1}H-NMR (C_5D_5N) δ: 0.86 (3H, s, Me-25), 0.88 (3H, s, Me-29), 0.90 (3H, s, Me-30), 1.07 (3H, s, Me-24), 1.19 (3H, s, Me-26), 1.25 (3H, s, Me-23), 1.37 (3H, s, Me-27), 1.63 (3H, d, J=6.0 Hz, Me of rhamnose), 1.68 (3H, d, J=6.0 Hz, Me of rhamnose′), 3.30 (1H, dd, J=10.6, 4.0 Hz, 3-(H), 4.78 (1H, d, J=5.6 Hz, 1-H of xylose), 5.00 (1H, d, J=7.6 Hz, 1-H of glucose″), 5.42 (1H, br s, 12-H), 5.43 (1H, d, J=8.0 Hz, 1-H of glucose), 5.79 (1H, br s, 1-H of rhamnose′), 6.21 (1H, d, J=8.0 Hz, 1-H of glucose′), 6.39 (1H, br s, 1-H of rhamnose); ^{13}C-NMR data, see Table 1.

Acid Hydrolysis of 1—4 A solution of each compound (30.0 mg in 5 ml 10% HCl) was refluxed for 5 h. The reaction solution was concentrated under reduced pressure and the residue was diluted with 30 ml H_2O. The solution was neutralized with Ag_2CO_3 and extracted with EtOAc. The EtOAc extract was evaporated and chromatographed on a Sephadex LH-20 column (*ca.* 30 g) using MeOH (700 ml) as eluant to afford the aglycon, which was analyzed by IR, NMR and by comparison with an authentic sample. The H_2O layer was concentrated, filtered, and passed through a NOVA-Pak C_{18} cartridge (Waters) and then repeatedly separated by HPLC [HPLC conditions: mobile phase: MeCN–H_2O (3 : 1); flow rate: 0.6 ml/min; detection: refractive index (RI)] to afford D-glucose (7.2 mg in **1**, 7.4 mg in **2**, 9.8 mg in **3**, 8.6 mg in **4**, 16.8 min), D-xylose (2.8 mg in **1**, 2.6 mg in **2**, 1.5 mg in **4**, 16.2 min) and L-rhamnose (3.6 mg in **1**, 3.3 mg in **2**, 6.3 mg in **3**, 5.1 mg in **4**, 10.9 min.). The optical rotation values of the above monosaccharides indicated these sugars to be D-glucose {$[\alpha]_D^{25}$ +52.5° (c=0.72, H_2O) in **1**, +53.0° (c=0.74 , H_2O) in **2**, +53.2° (c=0.98, H_2O) in **3**, +52.3° (c=0.86, H_2O) in **4**; lit. value[8] +52.7°}, D-xylose {$[\alpha]_D^{25}$ +18.6° (c=0.28 , H_2O) in **1**, +18.9° (c=0.26, H_2O) in **2**, +18.4° (c=0.21, H_2O) in **4**; lit. value[8] +18.8°}, and L-rhamnose {$[\alpha]_D^{25}$ +6.6° (c=0.36, H_2O) in **1**, +6.9° (c=0.33, H_2O) in **2**, +7.3° (c=0.63, H_2O) in **3**, +7.2° (c=0.51, H_2O) in **4**; lit. value[8] +8.2°}.

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