ACHYRANTHOSIDES C AND D, NOVEL GLUCURONIDE SAPONINS FROM *ACHYRANTHES FAURIEI* ROOT

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Two novel saponins, achyranthosides C and D, were isolated from *Achyranthes fauriei* root, and their structures were characterized based on the chemical and spectroscopic evidence.

KEYWORDS Amaranthaceae; Achyranthes fauriei root; glucuronide saponin; oleanolic acid; achyranthoside

In the previous papers, we reported four known oleanolic acid saponins, chikusetsusaponins IVa (1), V, 28-desgluco-chikusetsusaponin V, pseudoginsenoside RT_1,[1] and two novel cytotoxic saponins, achyranthosides A (2) and B (3), from the dried root of *Achyranthes fauriei* Leveille et Vaniot (Amaranthaceae).[2] On continuation of the phytochemical survey on the methylated saponin fraction of the title plant, two new saponins named achyranthosides C (4) and D (5) were isolated as methyl ester with the yield of 0.01 and 0.007%, respectively, in addition to the above six compounds.[3] We describe here their characterization.

Achyranthoside C methyl ester (**4a**), a powder, $[\alpha]_D$ +13.7° (MeOH), was determined in its molecular formula $C_{50}H_{78}O_{20}$ based on the FAB-MS ($[M+Na]^+$, *m/z* 1021) and ^{13}C NMR spectra. The ^{13}C NMR spectrum showed 43 signals ascribable to a 28-*O*-β-D-glucopyranosyl oleanolate 3-*O*-β-D-glucuronopyranoside methyl ester (**1a**) moiety[2] and seven due to carbons of two methoxyl groups (δ 52.0, 52.4), two esteric carbonyls (δ 172.0, 170.9), an acetal methine (δ 104.8), a carbinyl methine (δ 74.1) and a carbinyl methylene (δ 64.5) (Table 1).[2] On acid hydrolysis, **4a** gave only D-glucose (Glc),[4] D-glucuronic acid (GluA) γ-lactone and oleanolic acid (OA), but no other components were detected from the hydrolysate even on TLC (detection: 10% H_2SO_4) in spite of the seven carbon signals mentioned above, as in the case of **3a**.[2] On treatment with crude pectinase,[5] **4a** liberated its esteric Glc to afford a prosapogenin[6] whose C-28 signal appeared in a down field (δ 180.1) compared with that of **4a** (δ 176.2). The prosapogenin (**4b**) still showed the seven carbon signals described above as well as those due to an OA 3-*O*-β-D-glucuronopyranoside methyl ester (**1b**) moiety in the ^{13}C NMR spectrum, and afforded only GluA and OA on acid hydrolysis. When treated with 0.5N methanolic hydrochloric acid for 30 min,[2] **4b** provided the prosapogenin of **1a** (**1b**).[2] On acetylation, **4b** gave triacetate (**4c**), whose methyl ester showed no hydroxyl absorption in the IR spectrum. In order to clarify the structure, NMR spectroscopic analysis was carried out on **4b** and **4c** using homo- and hetero-nuclear 2D NMR techniques (^1H-^1H and ^{13}C-^1H COSY, NOE and HMBC spectra), and the signals were assigned as shown in Table 1.

The location of the GluA at the C-3 hydroxyl group of OA was determined by the NOE and HMBC correlations in **4b**: H-3/C-1', H-1'/C-3. All the J values of the GluA H-1～H-5 signal exhibited the stereochemistry of the GluA to be β-pyranose with the $^4C_1$-conformation. The GluA H-2 and H-4 signals (δ 5.54, 5.65) of **4c** appeared in a lower field than those of **4b** (δ 4.03, 4.42) by acetylation shift. Hence, the C_7 moiety consisting of $C_7H_{11}O_6$ was shown to be located to the GluA C-3 hydroxyl group. This was

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Table 1. NMR Signals of Achyranthosides C, D and Their Derivatives*

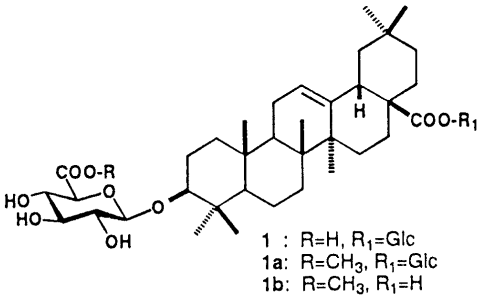
	^{13}C				^{1}H			
	4a	4b	5a	5b	4b	4c	5b	5c
OA[a]								
3	89.8	89.6	89.5	89.6	3.31 (dd, 4.4, 11.7)	3.30 (dd, 4.4, 11.7)	3.28 (overlapped)	3.08 (dd, 4.7, 11.4)
28	176.2	180.1	176.4	180.2	–	–	–	–
Substituent at the C-28 of OA								
	Glc[b]	H	Glc[b]	H	H	H	H	H
GluA at the C-3 of OA								
1'	106.8	106.7	104.9	104.9	4.91 (d, 7.8)	4.96 (d, 8.1)	5.01 (d, 7.7)	4.48 (d, 7.7)
2'	74.9	74.5	78.3	78.3	4.03 (dd, 7.8, 9.8)	5.54 (dd, 8.1, 9.5)	4.49 (dd, 7.7, 9.9)	3.88 (dd, 7.7, 9.5)
3'	84.9	84.7	82.6	82.7	4.32 (t, 9.8)	4.86 (t, 9.5)	4.37 (t, 9.9)	4.04 (t, 9.5)
4'	72.2	71.9	72.3	72.3	4.42 (t, 9.8)	5.65 (t, 9.5)	4.47 (t, 9.9)	5.11 (t, 9.5)
5'	77.1	76.8	76.5	76.5	4.53 (d, 9.8)	4.67 (d, 9.5)	4.51 (d, 9.9)	3.90 (d, 9.5)
6'	170.0	170.3	170.0	170.1	–	–	–	–
OMe	51.9	52.1	51.8	51.8	3.69 (3H, s)	3.80 (3H, s)	3.64 (3H, s)	3.74 (3H, s)[c]
Functional Group at the C-3 of GluA								
1'' (s)	172.0	172.4	172.3	172.2	–	–	–	–
OMe	52.0	51.7	52.0	51.3	3.71 (3H, s)	3.73 (3H, s)	3.43 (3H, s),	3.71 (3H, s)[c]
2'' (d)	74.1	73.8	72.5	72.6	5.16 (1H, d, 3.4)	5.53 (d, 4.8)	5.28 (d, 2.2)	5.24 (d, 3.2)
3'' (d)	104.8	104.6	104.1	104.1	6.03 (1H, d, 3.4)	5.67 (d, 4.8)	6.11 (d, 2.2)	5.35 (d, 3.2)
4'' (t)	64.5	64.0	64.4	64.6	4.73, 5.22	4.69, 4.74	4.62, 5.04	4.20, 4.32
5'' (s)	170.9	171.2	171.0	171.0	(ABq, 16.6)	(ABq, 16.5)	(ABq, 16.5)	(ABq, 16.1)
OMe	52.4	51.4	51.3	52.0	3.51 (3H, s)	3.65 (3H, s)	3.76 (3H, s)	3.79 (3H, s)[c]
Glc at the C-2 of GluA								
1'''	–	–	103.5	103.5	–	–	5.61 (d, 7.7)	4.99 (overlapped)
2'''	–	–	76.2	76.1	–	–	4.15 (dd, 7.7, 8.6)	4.99 (overlapped)
3'''	–	–	78.2	78.2	–	–	4.29 (t, 8.6)	5.21 (t, 9.5)
4'''	–	–	72.5	72.4	–	–	4.18 (dd, 8.6)	5.08 (t, 9.5)
5'''	–	–	78.1	78.1	–	–	3.95 (m)	3.74 (br d, 9.5)
6'''	–	–	63.1	63.2	–	–	4.36 (br d, 12.0)	4.08 (dd, 2.4, 12.1)
							4.53 (br d, 12.0)	4.29 (dd, 4.4, 12.1)
OAc	–	–	–	–	–	2.09, 2.18, 2.30 (3H each, s)	–	1.99, 2.02, 2.05, 2.06, 2.07, 2.22 (3H each, s)

* C_5D_5N was used as solvent for the compounds except for 5c ($CDCl_3$). The signals were assigned by means of the 2D experiments (^1H-^1H and ^{13}C-^1H COSY and HMBC).

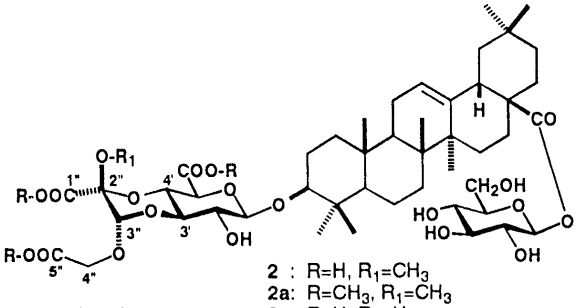
a) C-1~C-30 signals of OA (28-COO-R):
R=H: 39.0, 27.1, 90.1, 40.1, 56.2, 19.0, 33.7, 40.2, 48.4, 37.4, 24.3, 122.8, 144.8, 42.6, 28.8, 24.3, 47.1, 42.4, 46.9, 31.4, 34.7, 33.6, 28.2, 16.9, 16.1, 17.9, 26.7, 179.9, 33.7, 24.3
R=Glc: 39.1, 27.0, 89.5, 39.9, 56.1, 19.0, 33.6, 40.3, 48.4, 37.4, 23.9, 122.8, 144.0, 42.5, 28.7, 24.3, 47.4, 42.2, 46.6, 31.2, 34.5, 33.0, 28.6, 17.4, 16.0, 18.0, 26.6, 176.1, 33.6, 24.1

b) C-1~C-6 signals of Glc at C-28: 95.7, 74.2, 78.9, 71.1, 79.3, 62.2

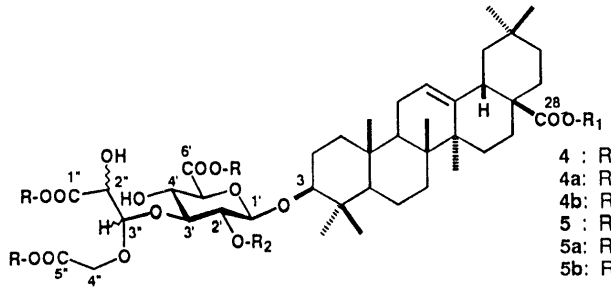
c) Assignment may be reversed.



1 : R=H, R_1=Glc
1a: R=CH_3, R_1=Glc
1b: R=CH_3, R_1=H



2 : R=H, R_1=CH_3
2a: R=CH_3, R_1=CH_3
3 : R=H, R_1=H
3a: R=CH_3, R_1=H



4 : R=H, R_1=Glc, R_2=H
4a: R=CH_3, R_1=Glc, R_2=H
4b: R=CH_3, R_1=R_2=H
5 : R=H, R_1=R_2=Glc
5a: R=CH_3, R_1=R_2=Glc
5b: R=CH_3, R_1=H, R_2=Glc

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supported by the HMBC correlations between the GluA C-3 and the acetal positions in **4b**: H-3'/ C-3", H-3"/C-3'. The H-3" signal (δ6.03) was coupled with the methine proton signal (H-2", δ5.16) in an AB spin system with J=3.4 Hz, suggesting a partial structure of $>C_{(2")}H-C_{(3")}H<$. The H-2" signal (δ5.16) in **4b** was shifted to down field at δ5.53 in **4c** by the acetylation shift, indicating the methine was of secondary hydroxyl group. The further HMBC correlations were observed in **4b** and **4c** as follows: H-2"/C-1", H-2"/C-3", H-3"/C-4", H_2-4"/C-3" and H_2-4"/C-5". These results revealed the structure of the $C_7H_{11}O_6$ moiety in **4b** to be MeOOC-CH(OH)-CH(O-R)-O-CH_2-COOMe (R=**1b** moiety). Thus, the structure of **4b**, **4a** and achyranthoside C (**4**) were formulated as shown in the figure.

Achyranthoside D methyl ester (**5a**), a powder, $[\alpha]_D$ +5.0° (MeOH), was determined in its molecular formula $C_{56}H_{88}O_{25}$ by the FAB-MS ($[M+Na]^+$, *m/z* 1183) and ^{13}C NMR spectra. The ^{13}C NMR spectrum showed 56 signals, suggesting that **5a** was composed of **4a** and a hexose (Table 1). On acid hydrolysis, **5a** provided the same components as **4a**. On enzymatic hydrolysis, **5a** liberated the esteric Glc to afford a prosapogenin (**5b**)[6] whose C-28 carbon signal appeared at δ180.2 as in the case of **4b**. The hydrolysis products obtained from **5b** were same as those from **5a**. Hence the hexose was to be Glc. When treated with 0.5N methanolic hydrochloric acid for 30 min, **5b** yielded **1b** as in the case of **4b**. On acetylation **5b** gave hexaacetate (**5c**), whose methyl ester showed no hydroxyl absorption in the IR spectrum.

The NMR spectroscopic analysis was also undertaken on **5b** and **5c**, as mentioned above, and the signals were assigned as listed in Table 1. The H-1 ~ H-5 signals of both Glc and GluA indicated their structures to be β-pyranose with the $^4C_1$-form. We also observed the same NMR signals due to the $C_7H_{11}O_6$ moity as in the case of **4b** and **4c** (Table 1). Of the GluA H-1 ~ H-5 signals of **5c**, only the H-4 signal was affected by the acetylation shift, suggesting that the GluA had two substituents at its C-2 and C-3 positions. The locations of the $C_7H_{11}O_6$ and Glc moieties were determined to be at the GluA C-3 and C-2 positions, respectively, based on the following HMBC correlations: H-3'/C-3", H-3"/C-3', H-2'/C-1" and H-1"/C-2'. Thus, the structures of **5b**, **5a** and achyranthoside D (**5**) were formulated as shown in the figure.

As mentioned above, achyranthosides C (**4**) and D (**5**) were characterized as a novel glucuronide saponin having a unique $C_5H_7O_6$ substituent, composed of 2-hydroxy-1-oxopropionic and glycolic acid, at the GluA C-3 position. The stereochemistry of the $C_5H_7O_6$ moiety in both **4** and **5** is now under investigation. An analogous compound missing the -CH_2-COOH group at the C-3" position of **4** was recently isolated from *Beta vulgaris*.[7]

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3. The *Rf* values of **1a**-**5a** on a silica gel TLC [solv.: $CHCl_3$-MeOH-H_2O (15:5:1, v/v)] were as follows: **1a**, 0.42; **2a**, 0.72; **3a**, 0.66; **4a**, 0.66; **5a**, 0.35.[2]
4. D-Glc was identified according to the Oshima's procedure: Oshima R., Yamauchi Y., Kumanotani J., *Carbohydrate Res.*, 107, 169-176 (1982).
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6. The molecular formula was determined as follows based on the Fab-MS and ^{13}C NMR spectra : **4b**, *m/z* 859 ($[C_{44}H_{68}O_{15}+Na]^+$); **5b**, *m/z* 1021 ($[C_{50}H_{78}O_{20}+Na]^+$).
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