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Achyranthosides A and B. Novel Cytotoxic Saponins from *Achyranthes fauriei* Root

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Abstract Two new saponins, achyranthosides A and B, were isolated from *Achyranthes fauriei* root, and their structures were elucidated on the basis of chemical and physical evidences. Achyranthoside A methyl ester was found to have significant cytotoxic activity against human colon carcinoma and murine melanoma cells.

The dried root of *Achyranthes fauriei* (Amaranthaceae), one of source plants for an oriental crude drug Achyranthes root used for diuretics, tonics and remedy for blood stasis ("*Oketsu*" syndrome) such as arthralgia,[1] is known to contain phytoecdysones[2] and oleanolic acid glycosides.[1,3,4] During our phytochemical survey on this plant, two new oleanolic acid glycosides named achyranthosides A (1) and B (2) were isolated as methyl ester (yields, 0.01 and 0.002%, resp.), after methylation with diazomethane, from the crude saponin fraction obtained from the methanolic extract of *A. fauriei* root.[4] The present paper describes the structures of two achyranthosides and their cytotoxic activities against human colon carcinoma and murine melanoma cells.

Achyranthoside A methyl ester (1a), a white powder, $[\alpha]_D$ +64.3° (MeOH), was determined its molecular formula $C_{51}H_{78}O_{20}$ on the basis of the FAB-MS ($[M+Na]^+$, *m/z* 1033) and the ^{13}C NMR spectrum, which showed 51 carbon signals including those due to an esteric β-D-glucopyranosyl group (Table 1).[4] On acid hydrolysis, 1a gave D-glucose (Glc),[5] glucuronic acid (GlcA) lactone and oleanolic acid (OA), but no other components were detected in the hydrolysate even on TLC (detection: 10% H_2SO_4) in spite that the ^{13}C NMR showed nine signals due to two acetal, an isolated methylene, two ester carbonyl and four methoxyl carbons besides those due to the three components obtained. While, on treatment with crude pectinase, 1a liberated its esteric glucose to afford a prosapogenin (1b) as colorless plates, mp 262–263°, $[\alpha]_D$ +89.8° (MeOH), $C_{45}H_{68}O_{15}$ ($[M+Na]^+$, *m/z* 871), which gave OA 6-*O*-methylglucuronide[6] on partial methanolysis. The ^{13}C NMR spectrum of 1b also showed the nine signals described above in addition to those due to OA and GlcA, which were only compounds obtained from 1b on hydrolysis. On acetylation 1b gave monoacetate (1c), whose methyl ester showed no hydroxyl absorption in the IR spectrum. In order to clarify the structure, NMR spectroscopic analysis was made on 1b and 1c using homo- and hetero-nuclear 2D NMR techniques (^1H-^1H and ^{13}C-^1H COSY, NOE and HMBC spectra).[7] It was revealed that 1b was a derivative of oleanolic acid 3-*O*-β-D-glucuronopyranoside having a unique substituent composed of $C_8H_{12}O_6$ bridging between the GlcA C-3 and C-4

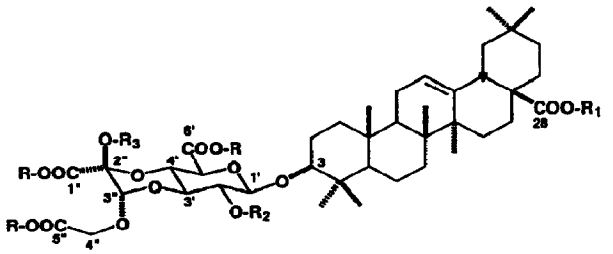
positions and that the substituent was a bis-acetal form of methyl dioxopropionate possessing *O*-methyl and *O*-methoxycarbonylmethyl (methyl glycolate) moieties at its C-2 and C-3 positions, respectively (Table 1). The stereostructure of **1b** was determined by means of X-ray crystallographic analysis (Fig. 2),[8] and thus the structure of **1a** was established as shown in Fig. 1.

Table 1. NMR Signals of Achyrantosides and Their Derivatives in C_5D_5N

	^{13}C				^1H			
	1a	**1b**	**2a**	**2b**	**1b**	**1c**	**2b**	**2c**
Oleanolic Acid[a]								
3	89.8	89.6	89.8	89.6	3.33 (dd, 4.4, 11.7)	3.21 (dd, 4.4, 11.7)	3.33 (overlapped)	3.22 (dd, 4.4, 11.7)
28	176.2	180.1	176.2	180.2	–	–	–	–
Substituent at C-28								
	Glc[b]	H	Glc[b]	H	H	H	H	H
GlcA at C-3								
1'	107.6	107.5	107.5	107.5	4.98 (d, 7.3)	5.01 (d, 7.7)	4.99 (d, 7.7)	5.03 (d, 7.7)
2'	71.9	71.6	72.0	71.8	4.11 (dd, 7.3, 8.5)	5.58 (dd, 7.7, 9.9)	4.18 (dd, 7.7, 8.1)	5.57 (dd, 7.7, 9.5)
3'	71.8	71.5	72.4	72.2	4.56 (t, 8.5)	4.70 (t, 9.9)	4.62 (t, 8.1)	4.80 (t, 9.5)
4'	69.6	69.3	69.9	69.6	4.59 (dd, 8.5, 9.3)	4.57 (dd, 9.5, 9.9)	5.12 (dd, 8.1, 9.9)	4.69 (dd, 9.0, 9.5)
5'	73.7	73.4	74.3	74.1	4.66 (d, 9.3)	4.65 (d, 9.5)	4.73 (d, 9.9)	4.66 (d, 9.0)
6'	168.5	168.8	168.8	169.2	–	–	–	–
OMe	52.8	52.5	51.2	51.5	3.72 (3H, s)	3.70 (3H, s)	3.60 (3H, s)	3.60 (3H, s)
Functional Group (UG) on GlcA								
1" (s)	166.6	166.8	169.6	169.9	–	–	–	–
OMe	52.7	52.5	52.5	52.1	3.85 (3H, s)	3.84 (3H, s)	3.55 (3H, s)],	3.84 (3H, s)
2" (s)	97.5	97.4	93.9	93.7	–	–	–	–
OMe	51.4	51.1	–	–	3.49 (3H, s)	3.45 (3H, s)	–	–
3" (d)	96.9	96.7	97.9	97.8	5.45 (s)	5.32 (s)	5.65 (s)	5.42 (s)
4" (t)	64.4	64.0	64.6	64.2	4.49, 4.54	4.44, 4.51	4.50, 4.60	4.48, 4.56
5" (s)	169.4	169.7	169.0	169.0	(ABq, 16.1)	(ABq, 16.3)	(ABq, 16.5)	(ABq, 16.1)
OMe	51.9	51.6	52.4	52.1	3.54 (3H, s)	3.63 (3H, s)	3.75 (3H, s)	3.65 (3H, s)
OAc	–	–			–	2.20 (3H, s)	–	2.14, 2.21 (3H, s)

* The signals were assigned by means of the 2D experiments.
a) C-1～C-30 signals of aglycone (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: 95.9, 74.4, 79.1, 71.4, 79.5, 62.6

Fig. 1. Structure of Achyrantosides and Their Derivatives

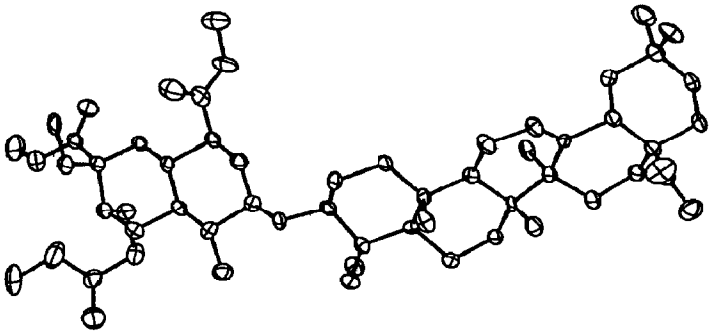


	R	R_1	R_2	R_3
1	H	Glc	H	Me
1a	Me	Glc	H	Me
1b	Me	H	H	Me
1c	Me	H	Ac	Me
2	H	Glc	H	H
2a	Me	Glc	H	H
2b	Me	H	H	H
2c	Me	H	Ac	Ac

Achyranthoside B methyl ester (**2a**), a white powder, $[\alpha]_D$ +51.7° (MeOH), was determined its molecular formula $C_{50}H_{78}O_{20}$ on the basis of the FAB-MS ($[M+Na]^+$, *m/z* 1019) and the ^{13}C

NMR spectrum, which showed 50 carbon signals similar to those of 1a but one methoxyl carbon signal (Table 1). On acid hydrolysis, 2a provided the same components as 1a. On enzymatic hydrolysis, 2a liberated the esteric glucose to afford a prosapogenin (2b), which gave diacetate (2c) on acetylation. The NMR data of 2b and 2c indicated that 2a has the same structure as 1a except the methoxy group at C-2" in 1a (Table 1). This was supported by the HMBC and NOE correlations observed in 2c[9] and by the up-field shift of the 4'-H signal in 2c compared with that in 2b on acetylation of 2"-axial-OH. Accordingly, the structure of 2a was formulated as shown in Fig 1.

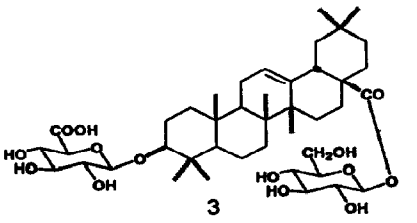
Fig. 2. ORTEP Drawing of 1b



Previously the aqueous and methanolic extracts of the oriental crude drug *Achyranthes* root were shown to have strong inhibitory activity against Ehrlich ascites carcinoma.[10] Thus, we preliminarily examined the cytotoxic activities of the two new saponins (1a and 2a) and chikusetsusaponin IV methyl ester (3a) against human colon carcinoma (HCT-116) and murine melanoma (B16-F10) cells.[11] As shown in Table 2, all the above compounds showed potent cytotoxic activities against both the human and murine cancer cell lines. Among them, the cytotoxicity of 1a was strongest with IC_{50} values of 5.2 and 8.2 μM against HCT-116 and B16-F10 cells, respectively.

Table 2. Cytotoxicities of 1a, 2a and 3a

Compound	IC50 (μM) HCT-116	B16-F10
1a	5.2	8.2
2a	94	64
3a	17	19



3

As mentioned above, 2 is a novel compound uniquely constructed with dioxopropionic acid, glycolic acid and 3, the majour saponin (0.02% as 3a) in this plant,[4] binding each other by 2,3-bis-acetal formation of dioxopropionic acid. Although 1 characterized as 2"-*O*-methyl derivative of 2 may be an artifact formed from 2 during the isolation process,[12] it is notable that its methyl ester (1a) shows remarkabe cytotoxicity and that this activity is much stronger

than that of **2a**.

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5. D-Glc was identified according to the Oshima's procedure: R.Oshima, Y.Yamauchi and J.Kumanotani, *Carbohydrate Res.* **1982**, *107*, 169.
6. The OA 6-*O*-methylglucuronide was identified as the prosapogenin obtained from **3a** in the same manner.
7. HMBC correlations in **1b**: H-3"/O-4", H_2-4"/C-3", H_2-4"/C-5", 2"-OMe/C-2", H-3"/C-3', C(6')-OMe/C-6', C(1")-OMe/C-1", C(5")-OMe/C-5". NOE correlations in **1b**: H-3"/H_2-4", H-1'/H-5', H-1'/H-3', H-1'/H-3.
8. Crystal Data of **1b**: Colorless prism (aq. MeOH), $C_{45}H_{68}O_{15}$, FW=849.02, crystal size: 0.20×0.03×0.55 mm, space group: $P2_12_12_1$ (orthorhombic), a=14.993 (4), b=32.221 (9), c=9.487 (3) A, V=4583.6 A^3, Z=4, Dc=1.230 $g \cdot cm^{-3}$, $\lambda(CuK\alpha_1)$=1.5405 A, μ=7.179 cm^{-1}, F(000)=1832, R=0.077 for 3846 unique reflections with $|Fo| = 3\sigma|Fo|$. The structure was solved by the direct method using the fully automatic method *FASE*,[13] and refined by the full matrix least squares calculation assuming anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms.
9. The HMBC correlations in **2c**: H-3"/C-4", H_2-4"/C-3", H_2-4"/C-5", H-3"/C-3', C(6')-OMe/C-6', C(1")-OMe/C-1", C(5")-OMe/C-5". NOE correlations in **2c**: H-3"/H_2-4", H-1'/H-5', H-1'/H-3', H-1'/H-3.
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11. HCT-116 and B16-F10 cells were implanted into wells of a 96-well microplate at the inoculum sizes of 6×10^4 and 3×10^4 cells/ml of McCoy's 5A and Eagle minimum essential media supplemented with fetal calf serum (10%), respectively, and incubated for 24 hours at 37° C in a humidified atmosphere of 5% CO_2 and 95% air. They were further incubated for 74 hours with test compounds. The cytotoxicity was colorimetrically determined at 540 nm after staining viable cells with 0.006% neutral red solution.
12. No chemical transformation from **2a** to **1a** (or **1b**) have been achieved up to the present: On treatment with 0.5% HCl-MeOH **2a** afforded **2b**, OA 6-*O*-methylglucuronide and OA but neither **1a** nor **1b** was detected in the reaction mixture; **2a** and **2b** did not change on treatment with CH_2N_2.
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