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# Two Novel Oleanolic Acid Saponins Having a Sialyl Lewis X Mimetic Structure from *Achyranthes fauriei* Root

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**Abstract** Two novel triterpene glycosides, achyranthosides E and F, were isolated as methyl esters from the root of *Achyranthes fauriei*, an antiinflammatory medicinal plant. Their structures were characterized as oleanolic acid glucuronides having unique substituents composed of $C_6H_9O_5$ and $C_9H_{15}O_7$, respectively, at the C-3 position of glucuronic acid. These compounds are active components which can inhibit the excess recruiting of neutrophiles to injured tissues 1,000 times more potently than sialyl Lewis X.


Many mimetics of sialyl Lewis X (sLe$^x$), which can inhibit the excess interaction between neutrophiles and E-selectin much more potently than sLe$^x$ itself, have been researched for some years as candidates for use as antiinflammatory agents for clinical tests.[1] *Achyranthes* root has been utilized in East Asia from ancient times to cure arthritis caused by rheumatism. We have previously reported four novel triterpene glycosides, achyranthosides A (**1**), B (**2**),[2] C (**3**) and D (**4**),[3] in addition to known saponins including chikusetsusaponin IVa (**5**)[4] from the dried root of *Achyranthes fauriei* LEVEILLE *et* VANIOT (Amaranthaceae). Based on the background that these glycosides have oleanolic acid as an aglycon, which was used to design a sLe$^x$ mimic by linking with a fucose derivative,[1] we confirmed the existence of components having an activity like sLe$^x$ mimetics in this plant. In fact, a fraction consisting of two components was found to have a potent inhibitory activity as a result of the random assay of the partially purified saponins from *A. fauriei* roots. This fraction was, after methylation with diazomethane, separated into the two compounds by means of chromatography on silica gel and ODS silica gel, and further purification of them afforded two new saponins, achyranthosides E (**6**) and F (**7**) as methyl ester (**6a** and **7a**) in 0.012 and 0.004% yield.[5] Here, we would like to describe their structures and the inhibitory activity on the interaction between neutrophiles and E-selectin.

The molecular formula of achyranthoside E methyl ester (**6a**), a powder, $[\alpha]_D$ +1.4° (*c* 0.5, MeOH), was determined to be $C_{49}H_{76}O_{19}$ based on the positive FAB-MS ($[M+Na]^+$, *m/z* 991) and $^{13}C$ NMR spectrum. The $^{13}C$ NMR spectrum showed 49 signals ascribable to 28-*O*-β-D-glucopyranosyl oleanolate 3-*O*-β-D-glucuronopyranoside methyl ester (**5a**) moiety[2] and six ones due to carbons of two methoxy groups (δ 51.8, 52.1), two esteric carbonyls (δ 168.1, 170.6), an acetal methine (δ 99.5) and a carbinyl methylene (δ 63.2) (Table 1).[3] On acid hydrolysis, **6a** gave only D-glucose (Glc),[6] the γ-lactone of D-glucuronic acid (GluA), and oleanolic acid (OA), but no other components were detected from the hydrolysate on TLC (detection: 10%

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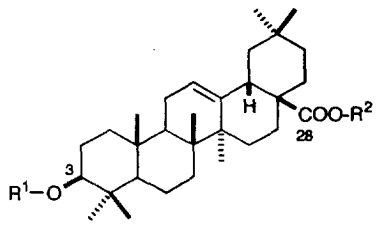


**1** : $R^1 = S^1$ (R=H), $R^2$ = Glc
**2** : $R^1 = S^1$ (R=Me), $R^2$ = Glc
**3** : $R^1 = S^2$ (R=H), $R^2$ = Glc
**4** : $R^1 = S^2$ (R=Glc), $R^2$ = Glc
**5** : $R^1$ = GluA, $R^2$ = Glc
**5a**: $R^1$ = GluA methylester, $R^2$ = Glc
**6** : $R^1 = S^3$ (R=H), $R^2$ = Glc
**6a** : $R^1 = S^3$ (R=Me), $R^2$ = Glc
**6b** : hexaacetate of **6a**
**7** : $R^1 = S^4$ (R=H), $R^2$ = Glc
**7a** : $R^1 = S^4$ (R=Me), $R^2$ = Glc
**7b** : $R^1 = S^4$ (R=Me), $R^2$ = H

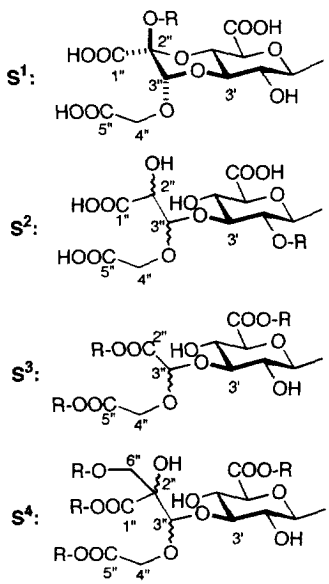


**Table 1.** $^{13}C$ NMR Signals[8] of **6a**, **6b**, **7a** and **7b**

| | **6a** | **6b**[10] | **7a** | **7b** |
|---|---|---|---|---|
| OA[9] | | | | |
| 3 | 89.4 | 90.6 | 89.3 | 89.3 |
| 28 | 176.4 | 175.6 | 176.4 | 180.1 |
| GluA at the C-3 of OA | | | | |
| 1' | 106.8 | 102.9 | 106.6 | 106.6 |
| 2' | 74.9 | 72.5 | 74.7 | 74.7 |
| 3' | 84.3 | 79.7 | 84.9 | 84.9 |
| 4' | 71.2 | 68.1 | 71.8 | 71.8 |
| 5' | 76.8 | 72.7 | 76.7 | 76.7 |
| 6' | 170.3 | 167.5 | 170.2 | 170.2 |
| OMe | 52.5 | 52.4 | 52.1 | 52.1 |
| Functional Group at the C-3 of GluA | | | | |
| 1" | -- | -- | 172.5 | 172.5 |
| OMe | -- | -- | 52.1 | 52.4 |
| 2" | 168.1 | 166.3 | 81.8 | 81.8 |
| OMe | 52.1 | 51.9 | -- | -- |
| 3" | 99.5 | 97.9 | 104.9 | 104.8 |
| 4" | 63.2 | 62.0 | 64.7 | 64.7 |
| 5" | 170.6 | 169.8 | 171.2 | 171.1 |
| OMe | 51.8 | 52.8 | 51.3 | 51.3 |
| 6" | -- | -- | 74.3 | 74.4 |
| OMe | -- | -- | 59.5 | 59.5 |
| Glc at C-28 of OA | | | | |
| 1'" | 95.8 | 91.6 | 95.7 | -- |
| 2'" | 74.2 | 70.1 | 74.1 | -- |
| 3'" | 79.0 | 79.7 | 78.9 | -- |
| 4'" | 71.1 | 70.4 | 71.1 | -- |
| 5'" | 79.3 | 72.6 | 79.3 | -- |
| 6'" | 62.3 | 61.6 | 62.2 | -- |

$H_2SO_4$) in spite of the six carbon signals mentioned above as in the case of **4** methyl ester (**4a**).[3] On acetylation, **6a** gave hexaacetate (**6b**), which showed $[M+Na]^+$ peak at *m/z* 1243 ($C_{61}H_{88}O_{25}$: 1220) in the MS spectrum and no hydroxyl absorption in the IR spectrum. The NMR signals of **6a** and **6b** were assigned as shown in Table 1 by means of the 2D NMR techniques ($^1H$-$^1H$ and $^{13}C$-$^1H$ COSY, NOE and HMBC). The location of the GluA at the C-3 hydroxyl group of OA was determined based on the NOE and HMBC correlations in **6b**: H-3 (δ 3.04, dd, *J*=4.4, 11.6)/C-1', H-1' (δ 4.46, d, *J*=8.1)/C-3. All the *J* values of the GluA and Glc H-1 - H-5 signals exhibited the stereochemistry of both GluA and Glc to be β-pyranose with the $^4C_1$-conformation. The GluA H-2 and H-4 signals (δ 5.10, 5.11) of **6b** appeared at lower field than those of **6a** (δ 4.03, 4.32) by acetylation shift, indicating the $C_6$ moiety consist of $C_6H_9O_5$ to be located at the GluA C-3 hydroxyl group. This was supported by the HMBC correlations between the GluA C-3 and the acetal positions in **6b**: H-3' (δ 4.00, t, *J*=9.9)/C-3", H-3" (δ 5.20, s)/C-3'. The following HMBC correlations were also observed in **6b**: H-3"/C-2", H-3"/C-4", $H_2$-4" (δ 4.20, 4.37, ABq, *J*=16.5)/C-3", and $H_2$-4"/C-5". These results revealed the structure of $C_6H_9O_5$ moiety in **6b** to be MeOOC-CH(OR)-O-$CH_2$-COOMe (R = **5a** moiety). Thus, the structures of **6b**, **6a** and achyranthoside E (**6**) were formulated as shown in the figure.

The molecular formula of achyranthoside F methyl ester (**7a**), a white powder, $[\alpha]_D$ +14.3°, was determined to be $C_{52}H_{82}O_{21}$ by negative FAB-MS ($[M-H]^-$, *m/z* 1041) and $^{13}C$ NMR spectrum. The $^{13}C$ NMR spectrum showed 52 signals ascribable to 28-*O*-β-D-glucopyranosyl oleanolate 3-*O*-β-D-glucuronopyranoside methyl ester (**5a**) moiety[2] and nine due to three methoxyl groups (δ 51.3, 52.1, 59.5), two esteric carbonyls (δ 171.2, 172.5), an acetal methine (δ 104.9), two methylene (δ 64.7, 74.4), and a quaternary carbon (δ 81.8) (Table 1). On acid hydrolysis, **7a** gave Glc, the γ-lactone of GluA, and OA, but no other components were detected from the hydrolysate on TLC in spite of the nine carbon signals mentioned above as in the case of **6a**. Compound **7a** liberated its esteric Glc on treatment with crude pectinase[7] to afford a prosapogenin (**7b**; $[M-H]^-$ at *m/z* 879, $C_{46}H_{72}O_{16}$: 880) whose C-28 signal appeared at lower field (δ 180.1) than that of **7a** (δ 176.4) as shown above. The prosapogenin (**7b**) still showed the nine carbon signals described above besides those of OA 3-*O*-β-D-glucuronopyranoside methyl ester (**5b**) moiety in the $^{13}C$ NMR spectrum and provided only GluA and OA on acid hydrolysis. The NMR signals of **7a** and **7b** were ascribed as shown in Table 1 by means of the 2D NMR techniques mentioned above. The location of the GluA at the C-3 hydroxyl group of OA was determined based on the NOE and HMBC correlations in **7b**: H-3 (δ 3.28)/C-1', H-1' (δ 4.82, d, *J*=7.7)/C-3. All the *J* values of the GluA H-1 - H-5 signals exhibited the stereochemistry of the GluA to be β-pyranose with $^4C_1$-conformation. The observation of HMBC correlations between the GluA C-3 and the acetal positions in **7b** (H-3' (δ 4.29, t, *J*=8.8)/C-3", H-3" (δ 5.86, s)/C-3') indicated that the $C_9$ moiety consisting of $C_9H_{15}O_7$ is located at the GluA C-3 hydroxyl group as in **6b**. The following HMBC correlations were also observed in **7b**: H-3"/C-4", H-4" (δ 4.84, 5.08, ABq, *J*=16.5)/C-3", H-3"/C-1", H-6" (δ 4.08, 4.30, ABq, *J*=9.6)/C-2", H-6"/$OCH_3$ (δ 59.5). Thus, the structure of the $C_9H_{15}O_7$ moiety in **7b** was shown to be MeOOC-C(OH)($CH_2$OMe)-CH(OR)-O-$CH_2$-COOMe (R = **5b** moiety), and the structure of **7b**, **7a**, and achyranthoside F (**7**) were formulated as shown in the figure.[11]

The effect of the fraction composed from **6** and **7** on the adhesion of polymorphonuclear leukocytes (PMNs) to bovine aortic endotherial cells (BAECs) was measured.[12] The grown BAECs in 24-well culture plates to near confluence were treated with TNF-α (10 ng/ml) for 4 hours, and a suspension of rat PMNs was pre-incubated with lipopolysaccharide (10 ng/ml) for 30 min. The PMNs ($8x10^5$) cells and the fraction diluted

with phosphate buffered saline (PBS) in various concentration were added into each well containing BAECs monolayer. After 30 min incubation, the supernatants were aspirated and the wells were rinsed twice with PBS to remove non-adherent PMNs. PMNs that remained adherent to BAECs were countered under a phase-contrast in versed light microscope at a 200 times magnification. The measured $IC_{50}$ value of the farction was 15 μg/ml, which is 1,000 times more potent than $sLe^x$.

As mentioned above, achyrnathoside E (**6**) and F (**7**) from *A. fauriei*, which were characterized as novel glucuronide saponins having unique substituents at the GluA C-3 position, potently inhibited the interaction between PMNs and E-selectin. Study of the stereochemistry of the substituents in **6a** and **7a** is in progress.

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3. Ida, Y.; Satoh, Y.; Katoh, M.; Katsumata, M.; Nagasao, M.; Yamaguchi, K.; Shoji, J. *Chem. Pharm. Bull.*, **1995**, *43*, 896. Achyranthoside D (**5**) was also isolated from Chinese *A. bidentata* thought to be identical with Japanese *A. fauriei*: Yoshikawa, M.; Murakami, T.; Matsuda H.; Murakami, H. The 115th Annual Meeting of Japanese Society of Pharmaceutical Sciences, Sendai (1995), Abstract Vol. 2, p. 252. Achyranthoside C (**4**) from *Beta vulgaris*: Yoshikawa, M.; Murakami, T.; Kakuya, M.; Matsuda, H.; Murakami, H. *idem.*, p. 253.
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5. Compounds **5a**, **6a**, and **7a** were homogeneous on silica gel and reverse-phase TLCs. The *Rf* values of **5a**, **6a** and **7a** on a silica gel TLC [solv.: $CHCl_3$-MeOH-$H_2O$ (15:5:1, v/v)] were as follows: **5a**, 0.42; **6a**, 0.72; **7a**, 0.71.[3]
6. D-Glc was identified according to Oshima's procedure: Oshima, R.; Yamauchi, Y.; Kumanotani, J. *Carbohydr. Res.*, **1982**, *107*, 169.
7. Satoh, Y.; Sakai, S.; Katsumata, M.; Nagasao, M.; Miyakoshi, M.; Ida, Y.; Shoji, J. *Phytochemistry*, **1994**, *36*, 147.
8. $C_5D_5N$ was used as the solvent except for **6b** ($CDCl_3$).
9. The other signals (C-1 - C-30) of OA : δ 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.
10. The signals attributed to the acetyl groups were observed at δ 168.6, 168.9, 169.4, 169.5, 170.1, 170.6, 20.52, 20.54, 20.56, 20.59, 20.64, 20.90.
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