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Article

Characterization of Seven New Steroidal Saponins from Korean Oat Cultivars by UPLC-QTOF-MS and UPLC-MS/MS

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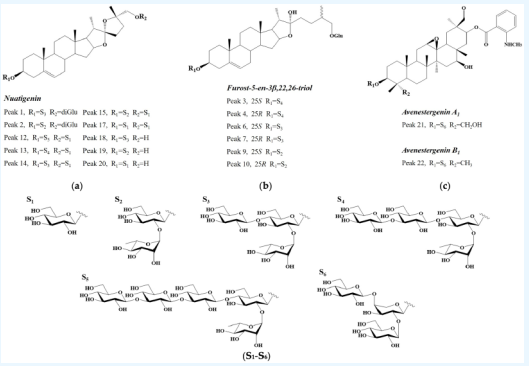
ABSTRACT: Oat saponins are composed of triterpenoid and steroidal saponins, and their potential biological activities, such as antibacterial, antifungicidal, osteogenic, and anticancer activities, have been reported. In this study, qualitative and quantitative analyses of oat saponins were conducted by using UPLC-QToF-MS and UPLC-Triple Q-MS/MS. A total of 22 saponins were analyzed in seven Korean oat cultivars. Among them, 7 saponins were identified as new compounds in this source, which were tentatively confirmed as nuatigenin-type saponins with 26-*O*-diglucoside and 3-*O*-malonylglucoside forms and (25*S*)-furost-5-en-3β,22,26-triol-type saponins. In addition, the total content of these saponins ranged from 70.61 to 141.38 mg/100 g dry weight, and it was affected by the type of oat cultivar and the presence or absence of hulling. These detailed profiles will be suggested as fundamental data for breeding superior oat cultivars, evaluating of related products, and various industries.



1. INTRODUCTION

Oats (*Avena sativa* L.) are cereal crops belonging to the Poaceae family and are ranked seventh in the world cereal production, with 26 million tons produced annually. It is mostly consumed as animal feed, and their consumption as a food is gradually increasing due to the health benefits.[1,2] This is affected by its components, such as β-glucan, alkaloids, flavonoids, phenolic acids, and saponins.[3,4] Oats are classified into two cultivated species. Covered oats are surrounded by lemmas and paleas from the kernels during harvesting and are suitable as a feed for ruminants. Whereas, naked oats, whose hulls are easily removed, have a higher proportion of nutrients, such as starch, protein, lipid, and β-glucan, than covered oats and are used as human food and feed for nonruminants.[5−8]

Saponins are nonvolatile and amphiphilic glycosides, that have soap-like foaming properties due to both hydrophilic sugar units and hydrophobic aglycone.[9] These compounds widely exist in various plants, such as soybean, ginseng, asparagus, and berries, and can be classified into triterpenoid (C_{30}) saponins and steroidal (C_{27}) saponins depending on their plant materials. In particular, triterpenoid saponins are mainly found in Dicotyledoneae, whereas steroid saponins are identified in Monocotyledoneae.[10,11] Saponins are not only used as natural surfactants to prevent microbial deterioration of food, but are also used in various industries, such as steroid drug development, soap, detergent, fire extinguisher, shampoo, beer, and cosmetics.[12] Moreover, saponins have been reported as natural substances with the potential to reduce methane gas production in ruminants.[13]

Oats contain two types of saponins: Triterpenoid saponins, including avenacins A_1, A_2, B_1, B_2, and C−K, have been reported in oat bran and roots,[14,15] whereas steroidal saponins, avenacosides A−C, and their derivatives have been reported in oat shoots,[16,17] grains,[18−20] husks,[19] brans,[14,21,22] and aerial parts[23,24] and they have bioactivities, such as antibacterial,[17] antifungicidal,[15,16] osteogenic,[20] and anticancer.[22,24] Analytical methods for these compounds have mainly focused on NMR and MS analysis, but recently, high-resolution mass spectrometric methods, such as QToF-MS, have the efficiency to isolate and identify numerous components.[25] Furthermore, previous studies on the quantification of oat saponins were mainly limited to evaluating the content of crude saponin or major components, such as avenacosides A and B.[14,19,21,22,26,27] Thus, the objective of this study is to rapidly and accurately characterize saponin derivatives based on internal and external standards. A total of 22 saponins were identified and quantified by using UPLC-QToF-MS and UPLC-Triple Q-MS/MS in seven Korean oat cultivars. These detailed profiles could be

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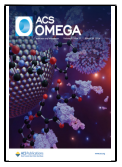

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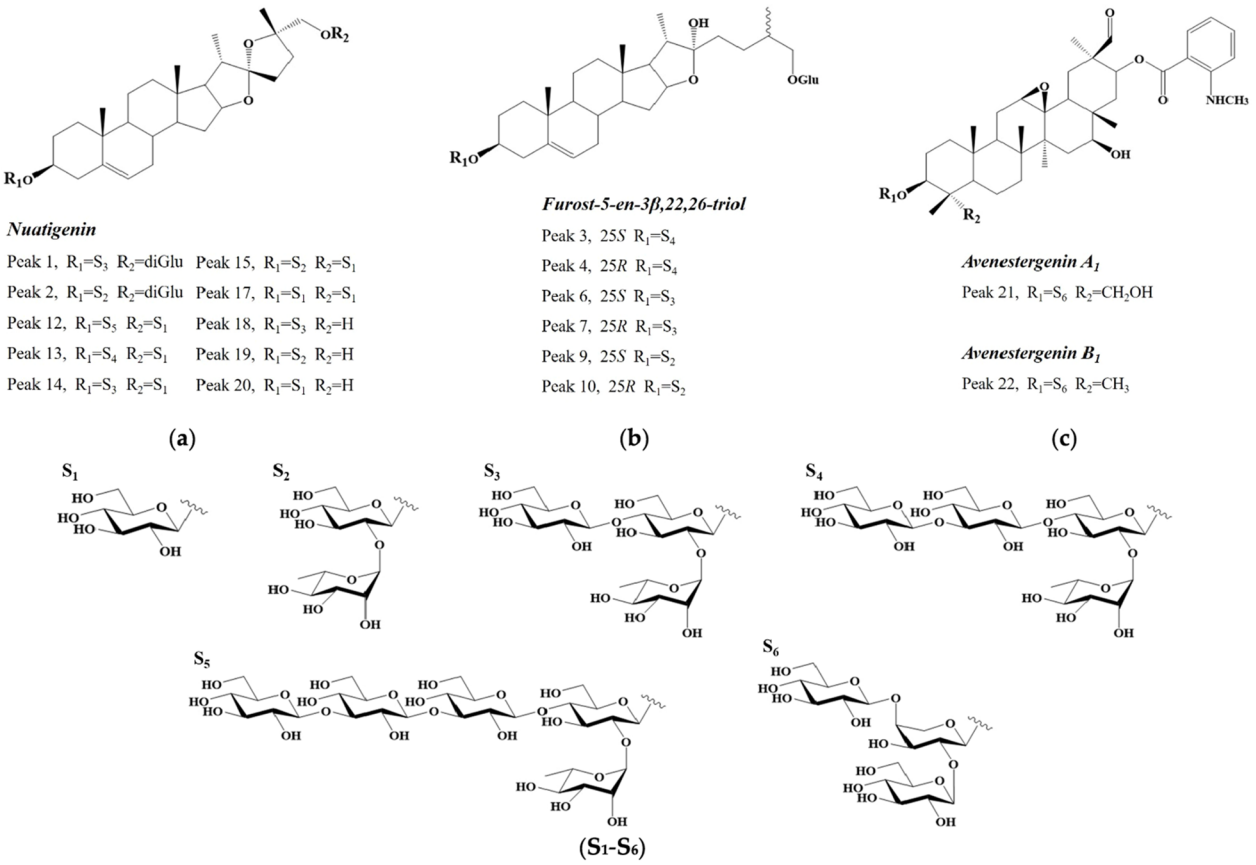

Figure 1. Chemical structures of 22 saponins depending on aglycone type. (a) Ten nuatigenin glycosides. (b) Six furost-5-en-3*β*,22,26-triol glycosides. (c) Two oleanane-type saponins. Glu, glucose; Rham, rhamnose; Ara, arabinose; (S_1−S_6) S_1 = Glu, S_2 = Rham-(1 → 2)-Glu, S_3 = Glu-(1 → 4)-[Rham-(1 → 2)]-Glu, S_4 = Glu-(1 → 3)-Glu-(1 → 4)-[Rham-(1 → 2)]-Glu, S_5 = Glu-(1 → 3)-Glu-(1 → 3)-Glu-(1 → 4)-[Rham-(1 → 2)]-Glu, S_6 = Glu-(1 → 4)-[Glu-(1 → 2)]-Ara.

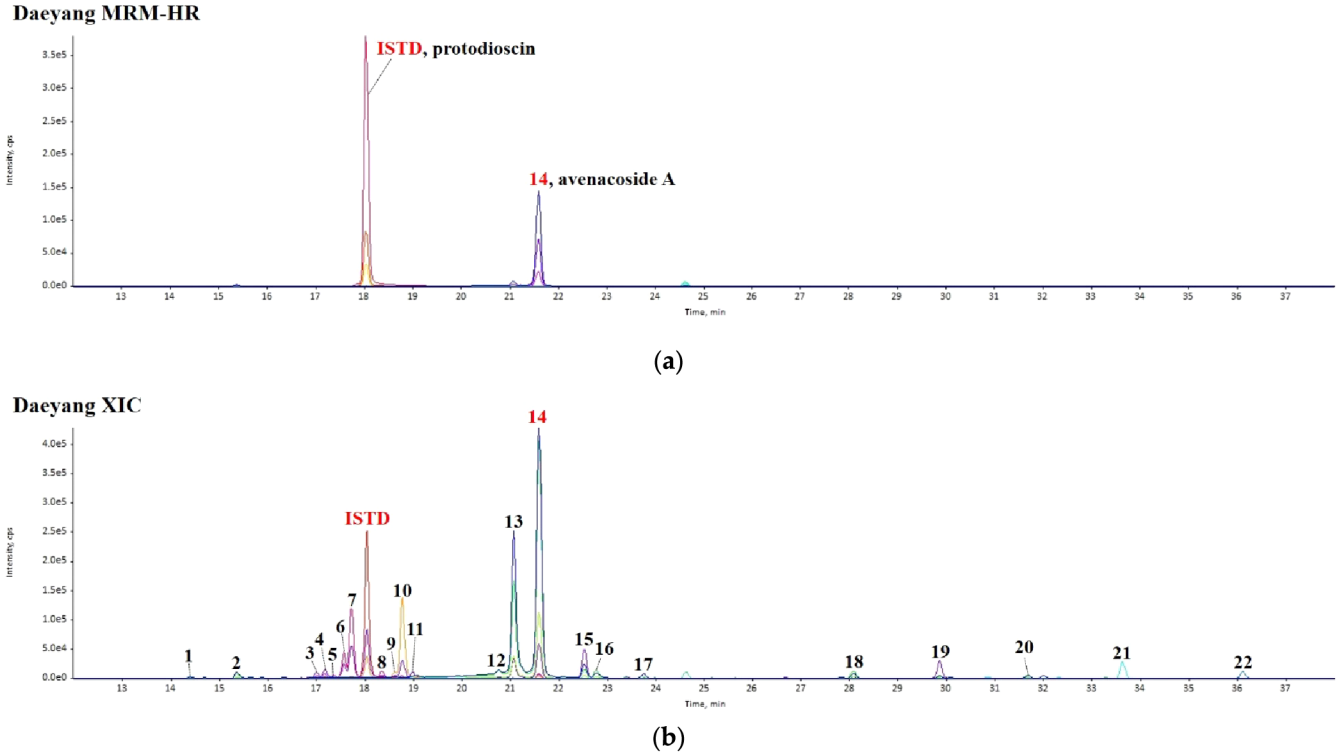

Figure 2. UPLC chromatograms of saponins in Daeyang. MRM-HR (a) and XIC (b). Internal standards (ISTD): protodioscin 25 ppm.

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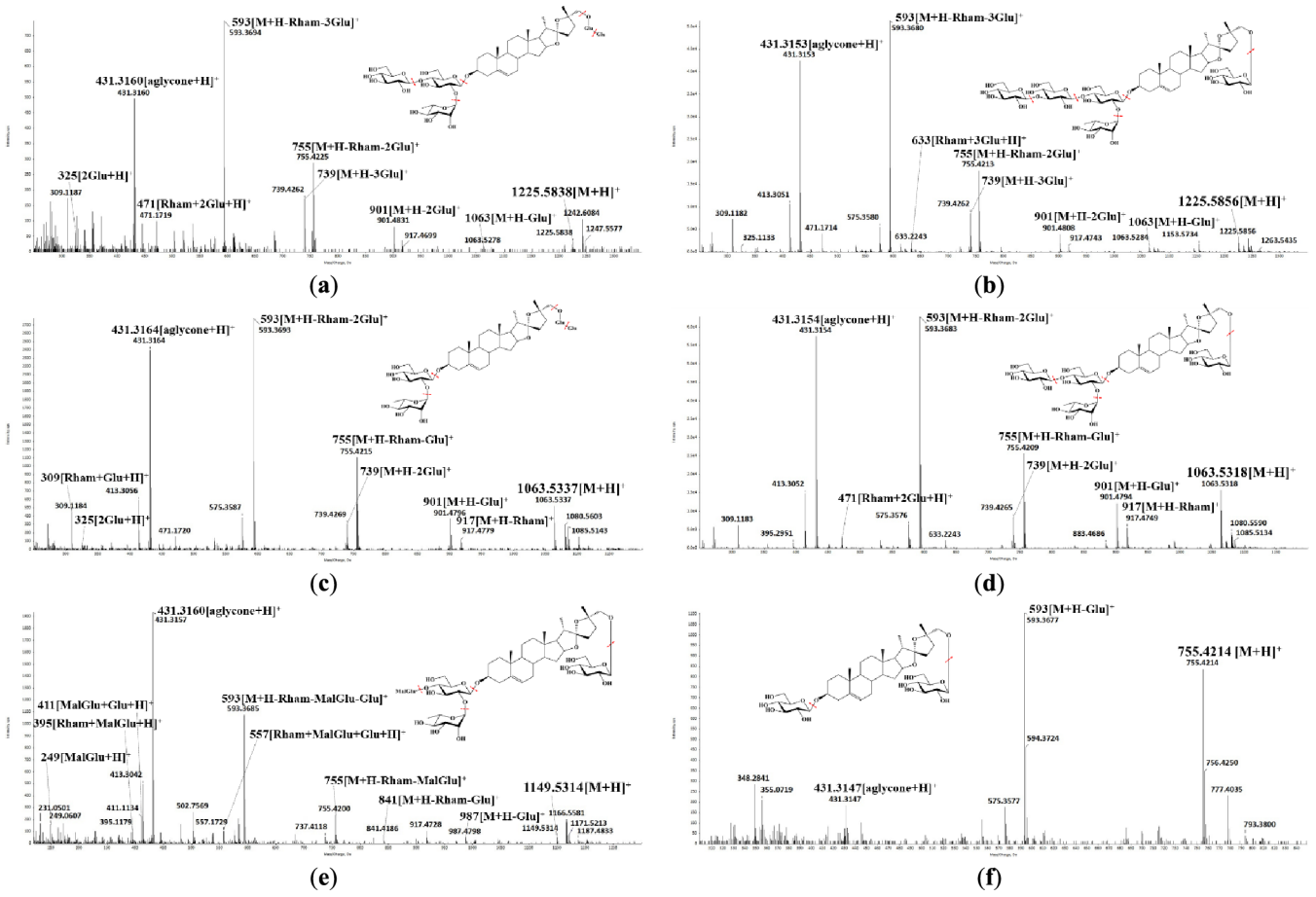

Figure 3. (+) ESI-MS spectra of nuatigenin-type saponins. Peaks **1** and **13** (a and b, m/z 1225[M + H]$^+$), peaks **2** and **14** (c and d, m/z 1063[M + H]$^+$), peak **16** (e, m/z 1149[M + H]$^+$), and peak **17** (f, m/z 755[M + H]$^+$).

suggested as fundamental results in breeding superior oat cultivars as well as in the evaluation of related products.

2. RESULTS AND DISCUSSION

2.1. Identification of Steroidal and Triterpenoid Saponins from Oats. *Avena sativa* L. includes aglycones, such as nuatigenin, (25*R*)-furost-5-en-3β,22,26-triol, and avenestergenins,[14,18,21] and twenty-two saponins containing these aglycones have been identified from oat grains. Peaks **1**, **2**, and **12**-**20** were determined to be furospirostane, peaks **3**-**11** were determined to be furostane, and peaks **21** and **22** were determined to be oleanane saponins esterified with phenolic acid (Figures 1, 2, and S1). Among the steroidal saponins, compounds **1**, **2**, and **12**-**20** exhibited nuatigenin ions at m/z 431[aglycone+H]$^+$ and 413[aglycone+H−H_2O]$^+$, whereas compounds **3**-**11** showed furost-5-en-3β,22,26-triol ions at 415[aglycone+H−H_2O]+. Compounds **21** and **22** exhibited avenestergenin A_1 and B_1 ions at m/z 638[aglycone+H]$^+$ and 622[aglycone+H]$^+$, respectively (Figures 3, 4, and S2). Based on their MS spectroscopic data and the elution order reported in the literature,[14,18,21,22,24,28] and standard (avenacoside A), peaks **4**, **7**, **10**, **12**-**15**, and **18**-**22** were identified as sativacosides B, A, and C, avenacosides D, B, A, and C, 26-desglucoavenacosides A and C, 3-*O*-glucosyl-nuatigenin, and avenacins A_1 and B_1, respectively, and are detailed in Table 1.

2.1.1. Furospirostane Saponins. Peaks **1** and **13** (avenacoside B) showed the molecular formula of $C_{57}H_{92}O_{28}$ by analyzing its protonated ions at m/z 1225.5874 and 1225.5877[M + H]$^+$, respectively, along with adduct ions at m/z 1242[M+NH_4]$^+$, 1247[M + Na]$^+$, and 1263[M+K]$^+$ in the positive mode. MS fragment ions are shown in Figure 3a,b, indicating that these compounds are isomeric and have an additional glucose unit (162 Da) than avenacoside A (peak **14**). The elution time interval between peaks **1** (Rt = 14.39) and **13** (Rt = 21.07) was similar to those of peaks **15** (avenacoside C, Rt = 22.52) and **18** (26-desglucoavenacoside A, Rt = 28.08), which were isomeric and have structures with a glucose unit attached to 26-OH or 3-OH of 3-*O*-rhamnosyl-(1 → 2)-glucosyl-nuatigenin, respectively (Figures 1 and 2). In addition, peaks **13**-**15** were closely eluted in the order of avenacosides B, A, and C, according to an additional one glucose unit at the C-3 position, while the elution time interval between peaks **1** and **14** was 7.2 min. It was indicated that the glucose unit of peak **1** was bound to the −Glu unit at the C-26 position rather than −Rham-2Glu units at the C-3 position. Previous studies have reported that saponins from plant materials belonging to Liliaceae and Solanaceae, which contain nuatigenin-type saponins, have a sophorose bound at the C-26 position.[29,30] However, in oat saponins, the exact linkage between the two glucoses could not be confirmed, and structural elucidation through 1D and 2D NMR analyses is required in the future. Therefore, peak **1** was tentatively identified as 3-*O*-glucosyl-(1 → 4)-[rhamnosyl-(1 → 2)]-glucosyl-nuatigenin 26-*O*-digluco-side and the first reported in this source.

Peak **2**, which eluted at 15.36 min following peak **1**, detected a protonated ion at m/z 1063.5340[M + H]$^+$ and was identified as

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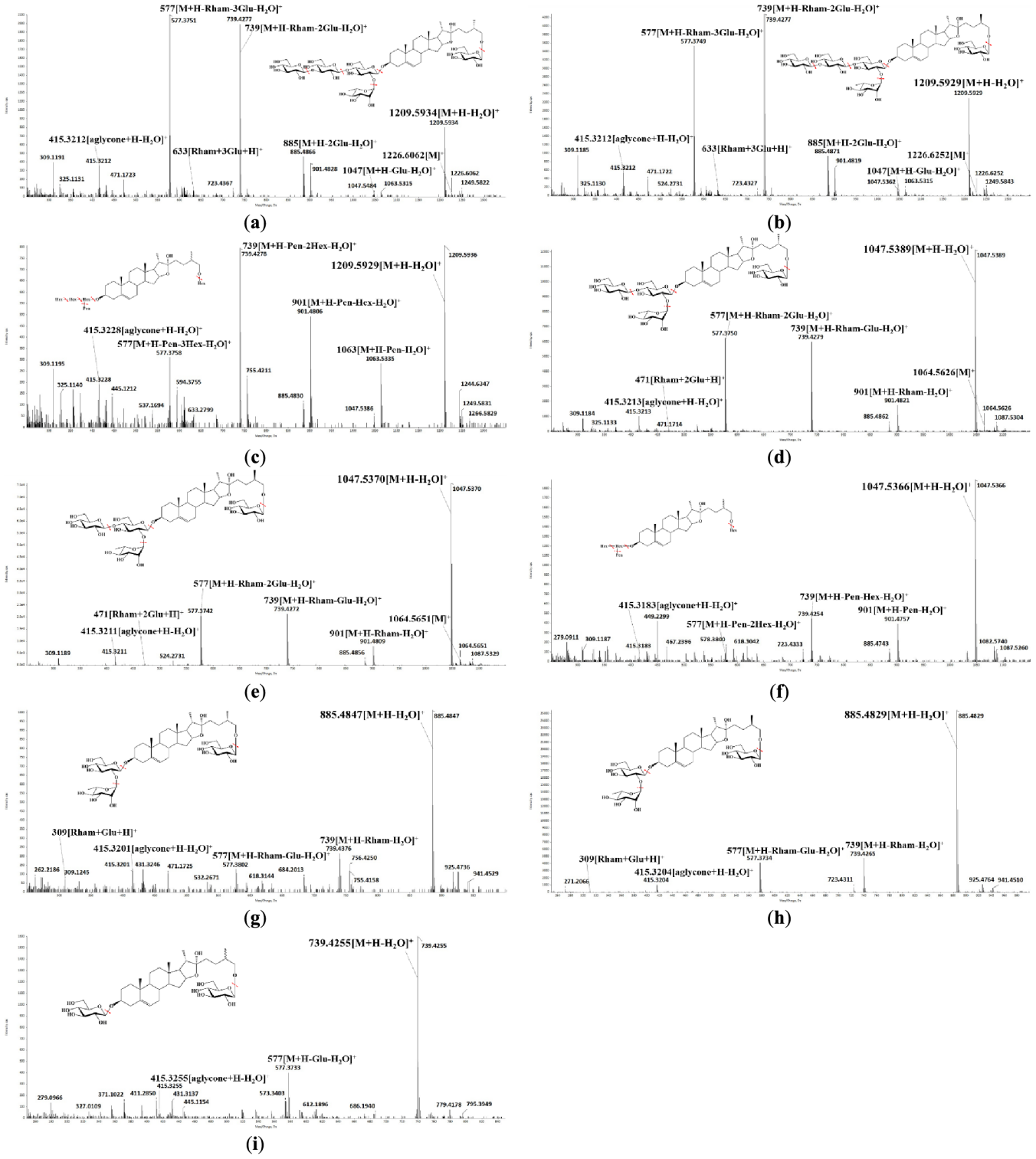

Figure 4. (+) ESI-MS spectra of furost-5-en-3β,22,26-triol-type saponins. Peaks **3**-**5** (a–c, m/z 1209[M+H−H_2O]$^+$), peaks **6**-**8** (d–f, m/z 1047[M+H−H_2O]$^+$), peaks **9** and **10** (g and h, m/z 855[M+H−H_2O]$^+$), and peak **11** (i, m/z 739[M+H−H_2O]$^+$).

an isomer of avenacoside A (Figures 3c,d and 5a). The elution time interval between peaks **1** and **2** was confirmed to be 0.97 min (Figure 2), which was estimated to be a structure in which glucose was lost in C-3 rather than in C-26. Therefore, peak **2** was tentatively identified as 3-*O*-rhamnosyl-(1 → 2)-glucosyl-nuatigenin 26-*O*-diglucoside and the first reported in this source.

Peak **16**, which possessed 86 Da more than peak **14**, showed a molecular formula of $C_{54}H_{84}O_{26}$ by detecting the protonated ion [M + H]$^+$ at m/z 1149.5314 with adduct ions at m/z 1166[M+NH_4]$^+$, 1171[M + Na]$^+$, and 1187[M+K]$^+$ in positive mode. In particular, the characteristic loss of 248 Da was observed, similar to the MS pattern of malonylglucose (MalGlu) reported in previous studies,[31,32] which was not de-esterified in the positive mode. Thus, the fragment ions were observed at m/z 1003[M+H-Rham]$^+$, 987[M+H-Glu]$^+$, 901[M+H-MalGlu]$^+$, 841[M+H-Rham-Glu]$^+$, 755[M+H-Rham-MalGlu]$^+$, 739[M+H-Glu-

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Table 1. Mass Spectrometric Data of Twenty-Two Saponins Identified in Korean Oat Cultivars

peak No.	compounds	*Rt* (min)	molecular formula	ESI(+)-QToF/MS (experimental ions, *m*/*z*)			
				$[M + H]^+$	$[M+H-H_2O]^+$	Error (ppm)[c]	adducts and fragment ions
Nuatigenin							
1[b]	3-*O*-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-nuatigenin 26-*O*-diglucoside	14.39	$C_{57}H_{92}O_{28}$	1225.5838		−0.8	1263, 1247, 1242, **1225**, 1079, 1063, 917, 901, 755, 739, 593, 575, 471, 431, 413, 395, 325, 309
2[b]	3-*O*-rhamnosyl-(1→2)-glucosyl-nuatigenin 26-O-diglucoside	15.36	$C_{51}H_{82}O_{23}$	1063.5337		1.6	1101, 1085, 1080, **1063**, 917, 901, 899, 883, 755, 739, 737, 721, 633, 593, 575, 471, 431, 413, 395, 325, 309
12	3-*O*-glucosyl-(1→3)-glucosyl-(1→3)-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-nuatigenin 26-*O*-glucoside (**avenacoside D**)	20.75	$C_{63}H_{102}O_{33}$	1387.6429		3.8	1425, 1409, 1404, **1387**, 1241, 1225, 1079, 1063, 957, 917, 901, 883, 795, 755, 739, 737, 633, 593, 575, 471, 431, 413, 395, 325, 309
13	3-*O*-glucosyl-(1→3)-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-nuatigenin 26-*O*-glucoside (**avenacoside B**)	21.07	$C_{57}H_{92}O_{28}$	1225.5856		0.7	1263, 1247, 1242, **1225**, 1079, 1063, 917, 901, 755, 739, 633, 593, 575, 471, 431, 413, 395, 325, 309
14[a]	3-*O*-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-nuatigenin 26-*O*-glucoside (**avenacoside A**)	21.59	$C_{51}H_{82}O_{23}$	1063.5318		−0.2	1101, 1085, 1080, **1063**, 917, 901, 899, 883, 755, 739, 737, 721, 633, 593, 575, 471, 431, 413, 395, 325, 309
15	3-*O*-rhamnosyl-(1→2)-glucosyl-nuatigenin 26-*O*-glucoside (**avenacoside C**)	22.52	$C_{45}H_{72}O_{18}$	901.4788		−0.4	939, 923, 918, **901**, 883, 755, 739, 737, 721, 593, 575, 470, 431, 413, 309
16[b]	3-*O*-malonylglucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-nuatigenin 26-*O*-glucoside (**malonylavenacoside A**)	22.77	$C_{54}H_{84}O_{26}$	1149.5314		−0.8	1187, 1171, 1166, **1149**, 1003, 987, 901, 841, 823, 755, 739, 593, 575, 557, 431, 413, 411, 395, 393, 249, 231
17[b]	3-*O*-glucosyl-nuatigenin 26-*O*-glucoside	23.74	$C_{39}H_{62}O_{14}$	755.4214		0.2	793, 777, **755**, 593, 575, 431, 413
18	3-*O*-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-nuatigenin (**26-desglucoavenacoside A**)	28.08	$C_{45}H_{72}O_{18}$	901.4816		2.7	939, 923, **901**, 883, 755, 739, 737, 721, 593, 575, 471, 431, 413, 325, 309
19	3-*O*-rhamnosyl-(1→2)-glucosyl-nuatigenin (**26-desglucoavenacoside C**)	29.86	$C_{39}H_{62}O_{13}$	739.4260		−0.4	777, 761, **739**, 721, 593, 575, 431, 413, 309
20	3-*O*-glucosyl-nuatigenin	31.68	$C_{33}H_{52}O_9$	593.3686		0.3	631, 615, 610, **593**, 575, 431, 413, 395
Furost-5-en-3β,22,26-triol							
3[b]	3-*O*-glucosyl-(1→3)-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-(25S)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside ((**25S**)-**sativacoside B**)	17.01	$C_{57}H_{94}O_{28}$		1209.5934	2.9	1265, 1249, 1244, **1226**, 1209, 1063, 1047, 901, 885, 739, 723, 633, 577, 471, 415, 325, 309
4	3-*O*-glucosyl-(1→3)-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-(25R)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside (**sativacoside B**)	17.18	$C_{57}H_{94}O_{28}$		1209.5929	2.5	1265, 1249, 1244, **1226**, 1209, 1063, 1047, 901, 885, 739, 723, 633, 577, 471, 415, 325, 309
5	unknown saponin 1	17.36	$C_{57}H_{94}O_{28}$		1209.5936	3.1	1265, 1249, 1244, **1226**, 1209, 1063, 1047, 901, 885, 739, 723, 633, 577, 471, 415, 325, 309
6[b]	3-*O*-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-(25S)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside ((**25S**)-**sativacoside A**)	17.56	$C_{51}H_{84}O_{23}$		1047.5389	1.8	1103, 1087, 1082, **1064**, 1047, 901, 885, 739, 577, 471, 415, 325, 309
7	3-*O*-glucosyl-(1→4)-[rhamnosyl-(1→2)]-glucosyl-(25R)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside (**sativacoside A**)	17.72	$C_{51}H_{84}O_{23}$		1047.5370	0.0	1103, 1087, 1082, **1064**, 1047, 901, 885, 739, 577, 471, 415, 325, 309
8	unknown saponin 2	18.35	$C_{51}H_{84}O_{23}$		1047.5366	−0.4	1103, 1087, 1082, **1064**, 1047, 901, 885, 739, 723, 577, 415, 309
9[b]	3-*O*-rhamnosyl-(1→2)-glucosyl-(25S)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside ((**25S**)-**sativacoside C**)	18.62	$C_{45}H_{74}O_{18}$		885.4840	0.5	941, 925, 920, 885, 739, 723, 577, 471, 415, 309
10	3-*O*-rhamnosyl-(1→2)-glucosyl-(25R)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside (**sativacoside C**)	18.77	$C_{45}H_{74}O_{18}$		885.4842	−1.5	941, 925, 920, 885, 739, 723, 577, 471, 415
11	unknown saponin 3	18.99	$C_{39}H_{64}O_{14}$		739.4255	−1.1	795, 779, 739, 577, 415
Oleanane esterified phenolic acid							
21	3-*O*-glucosyl-(1→4)-[glucosyl-(1→2)]-arabinosyl-avenestergenin A_1 (**avenacin A_1**)	33.63	$C_{55}H_{83}NO_{21}$	1094.5539		0.8	1132, 1116, **1094**, 770, 638, 620, 457, 295
22	3-*O*-glucosyl-(1→4)-[glucosyl-(1→2)]-arabinosyl-avenestergenin B_1 (**avenacin B_1**)	36.11	$C_{55}H_{83}NO_{20}$	1078.5599		1.7	1116, 1100, **1078**, 916, 754, 622, 457, 295

[a]Further confirmed in comparison with authentic standard. [b]Saponins first obtained from oat. [c]Error (ppm) indicated the mass accuracy of QToF data and its formula expressed as [(calculated ion−observed ion)/(calculated ion)] x 10^6 based on *m*/*z* $[M + H]^+$ and $[M + H-H_2O]^+$.

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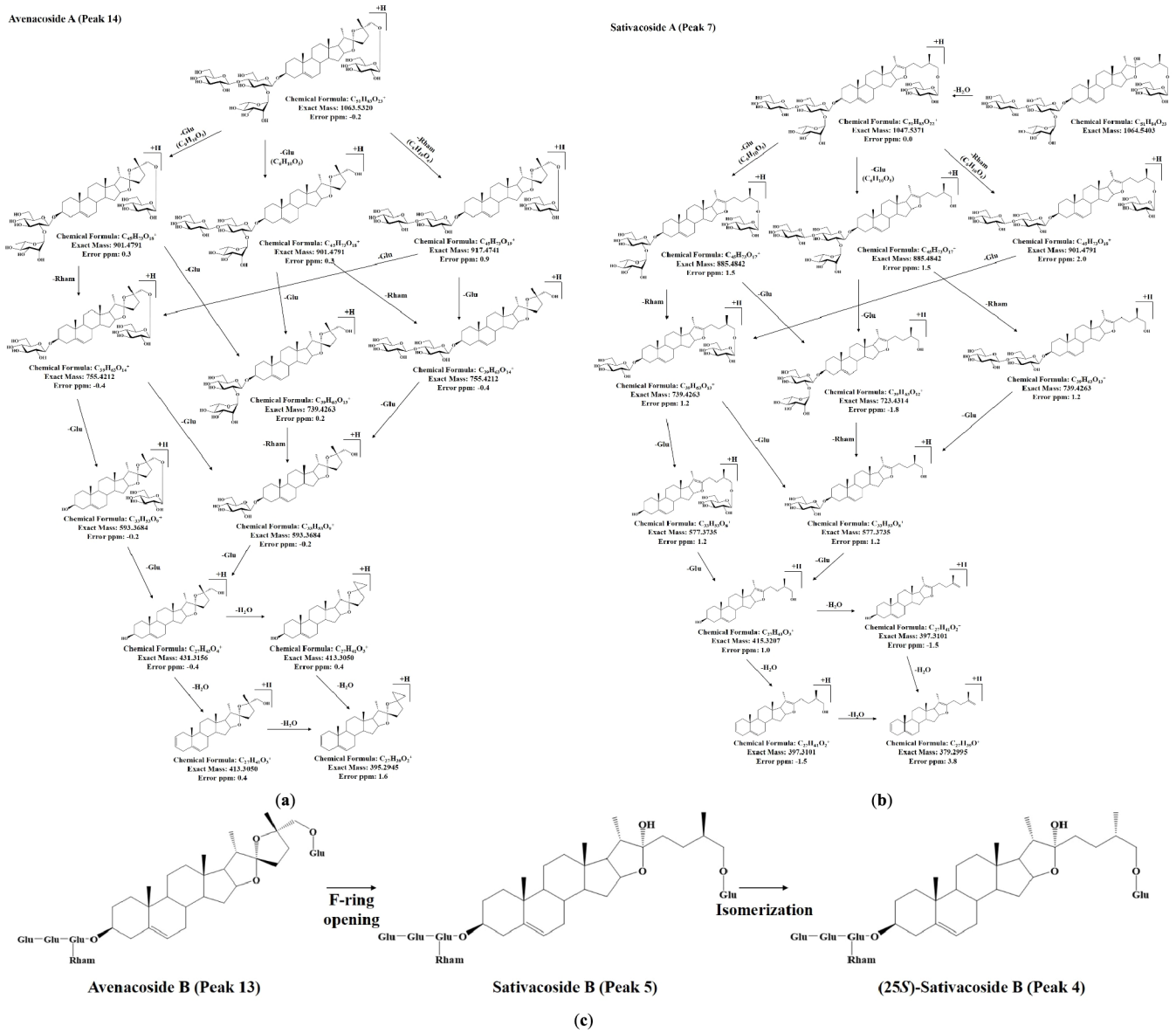

Figure 5. Proposed fragmentation patterns of avenacoside A (a) and sativacoside A (b) using ESI(+)-QToF/MS; the proposed biosynthetic pathway of sativacoside B and (25*S*)-sativacoside B from avenacoside B (c).

MalGlu]$^+$, 593[M+H-Rham-Glu-MalGlu]$^+$, and 431[M+H-Rham-Glu-MalGlu]$^+$ as well as specifically at *m*/*z* 557[Rham+Glu+MalGlu+H]$^+$, 411[Glu+MalGlu+H]$^+$, 395[Rham+MalGlu+H]$^+$, 249[MalGlu+H]$^+$, and 231[MalGlu+H−H_2O]$^+$ (Figure 3e). However, it was not possible to confirm the exact linkage between glucose and malonyl moieties; therefore, further structural elucidation is required. Therefore, peak **16** was tentatively identified as 3-*O*-malonylglucosyl-(1 → 4)-[rhamnosyl-(1 → 2)]-glucosyl-nuatigenin 26-*O*-glucoside and the first reported in this source.

Peak **17** (*Rt* = 23.74) showed the protonated ion [M + H]$^+$ at *m*/*z* 755.4214 and fragment ions at *m*/*z* 593[M+H-Glu]$^+$, 575[M+H-Glu-H_2O]$^+$, 431[M+H-2Glu]$^+$, and 413[M+H-2Glu-H_2O]$^+$ (Figure 3f), indicating a structure with one less rhamnose unit than those of peaks **15** and **18**. Thus, considering the results presented above, peak **17** was tentatively identified as 3-*O*-glucosyl-nuatigenin 26-*O*-glucoside, previously reported in the stems and leaves of the *Paris polyphylla* var. *yunnanensis*[33] and first reported in this source.

2.1.2. FUROSTANE SAPONINS

Kang et al.[34] reported that furostanol saponins were characterized by [M+NH_4]$^+$ and [M+H−H_2O]$^+$ ions in the positive mode. Similarly, peaks **3** (*Rt* = 17.01) and **4** (*Rt* = 17.18) detected a signal for the [M+H−H_2O]$^+$ ion at *m*/*z* 1209.5934 and 1209.5929, respectively. Both compounds were analyzed for [aglycone+H−H_2O]$^+$ at *m*/*z* 415.3212 (Figure 4a,b), indicating that they were furost-5-en-3*β*,22,26-triol saponins in oats. These types are induced by the F-ring opening of nuatigenin (Figure 5c) and eluted before nuatigenin types with the same sugar moieties.[18] Therefore, peaks **3** and **4** were estimated as derivatives of avenacoside B, and peak **4** was confirmed to be a more major compound than peak **3**, and was presumed to be the (25*R*)-furost-5-en-3*β*,22,26-triol type (Figures 2 and 5). Moreover, the 25*R* form eluted later than the 25*S* form, and the elution interval between the two was very close, regardless of steroidal or triterpenoid saponins.[34−36] Thus, peak **4** was identified as sativacoside B,[18,21] while peak **3** was tentatively identified as 3-*O*-glucosyl-(1 → 3)-glucosyl-(1 → 4)-[rhamno-

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Table 2. Contents (mg/100 g Dry Weight) of Individual 23 Saponins by Type of Aglycones from Seven Korean Oat Cultivars[i]

	naked oat			covered oat							
peak no.	Choyang, hulled	Daeyang, hulled	Suyang, hulled	Dahan, hulled	Dahan, whole grain	Samhan, hulled	Samhan, whole grain	Chopung, hulled	Chopung, whole grain	High-speed, hulled	High-speed, whole grain
Nuatigenin											
1	0.24 ± 0.01^{de}	0.19 ± 0.03^{ef}	0.25 ± 0.01^{d}	0.42 ± 0.03^{b}	0.14 ± 0.01^{fg}	0.51 ± 0.06^{a}	0.35 ± 0.01^{c}	0.26 ± 0.03d	0.12 ± 0.01g	0.28 ± 0.00d	0.16 ± 0.01fg
2	0.92 ± 0.03^{c}	0.80 ± 0.01^{d}	1.05 ± 0.10^{c}	1.94 ± 0.04^{a}	0.55 ± 0.04^{f}	0.94 ± 0.09^{c}	0.64 ± 0.04^{ef}	0.72 ± 0.08de	0.26 ± 0.01g	1.13 ± 0.06b	0.56 ± 0.06f
12	1.20 ± 0.05^{c}	1.42 ± 0.02^{b}	1.22 ± 0.04^{c}	1.17 ± 0.02^{c}	0.46 ± 0.06^{f}	1.67 ± 0.07^{a}	1.26 ± 0.02^{c}	1.07 ± 0.06d	0.64 ± 0.04e	1.43 ± 0.10b	0.98 ± 0.02d
13	20.63 ± 0.72^{e}	21.83 ± 0.73^{de}	19.83 ± 1.66^{e}	28.22 ± 0.30^{b}	17.23 ± 0.56^{f}	38.20 ± 2.28^{a}	30.19 ± 0.84^{b}	23.22 ± 0.57cd	16.13 ± 0.38f	24.77 ± 0.48c	17.36 ± 0.62f
14[ii]	51.09 ± 2.33^{bc}	53.94 ± 2.30^{b}	50.71 ± 2.91^{c}	65.94 ± 3.45^{a}	54.86 ± 1.49^{b}	48.59 ± 1.79^{c}	42.15 ± 0.70^{d}	43.57 ± 2.01d	35.88 ± 1.04e	52.72 ± 0.85b	43.70 ± 2.70d
15	3.92 ± 0.05^{a}	3.97 ± 0.20^{a}	3.59 ± 0.23^{b}	2.31 ± 0.06^{c}	1.62 ± 0.11^{de}	1.42 ± 0.08^{f}	1.46 ± 0.03^{ef}	1.80 ± 0.12d	1.63 ± 0.06def	1.40 ± 0.11f	1.36 ± 0.0.04f
16	1.20 ± 0.02^{b}	1.21 ± 0.04^{ab}	1.16 ± 0.02^{b}	0.37 ± 0.02^{e}	0.26 ± 0.02^{f}	0.07 ± 0.01^{g}	0.07 ± 0.00^{g}	0.53 ± 0.01d	0.37 ± 0.00e	1.29 ± 0.05a	0.89 ± 0.03c
17	0.24 ± 0.02^{de}	0.59 ± 0.04^{a}	0.34 ± 0.03^{b}	0.24 ± 0.01^{cd}	0.12 ± 0.01^{f}	0.07 ± 0.00^{g}	0.08 ± 0.01^{g}	0.29 ± 0.02c	0.19 ± 0.01e	0.08 ± 0.01g	0.07 ± 0.00g
18	0.89 ± 0.04^{ef}	1.01 ± 0.16^{de}	0.82 ± 0.05^{f}	1.72 ± 0.05^{a}	1.36 ± 0.08^{b}	0.92 ± 0.03^{ef}	1.29 ± 0.04^{b}	1.06 ± 0.08cd	1.07 ± 0.09de	0.60 ± 0.04g	1.22 ± 0.08bc
19	1.98 ± 0.07^{c}	2.55 ± 0.42^{b}	1.67 ± 0.22^{cd}	10.87 ± 0.17^{c}	2.04 ± 0.06^{c}	1.38 ± 0.04^{de}	3.25 ± 0.15^{a}	1.17 ± 0.08e	2.02 ± 0.24c	0.85 ± 0.03f	2.49 ± 0.06b
20	0.18 ± 0.03^{de}	0.54 ± 0.06^{a}	0.10 ± 0.01^{def}	0.21 ± 0.01^{cd}	0.13 ± 0.00^{efg}	0.06 ± 0.01^{g}	0.10 ± 0.02^{fg}	0.33 ± 0.04b	0.29 ± 0.00bc	0.14 ± 0.01defg	0.11 ± 0.01efg
subtotal	**83.89 ± 1.88^{cd}**	**88.02 ± 0.92^{bc}**	**80.83 ± 4.46^{de}**	**104.58 ± 3.81^{a}**	**77.74 ± 1.74^{ef}**	**93.83 ± 4.07^{b}**	**80.84 ± 0.18^{de}**	74.04 ± 2.96fg	57.98 ± 1.60h	85.30 ± 1.58bc	67.39 ± 2.66g
Furost-5-en-3β,22,26-triol											
3	0.71 ± 0.03^{ef}	0.75 ± 0.03^{ef}	0.72 ± 0.03^{ef}	1.03 ± 0.03^{bc}	0.56 ± 0.04^{g}	1.53 ± 0.21^{a}	1.16 ± 0.06^{b}	0.84 ± 0.02de	0.54 ± 0.03g	1.01 ± 0.05cd	0.63 ± 0.06fg
4	0.99 ± 0.05^{e}	1.24 ± 0.06^{d}	1.04 ± 0.06^{e}	2.78 ± 0.04^{b}	1.43 ± 0.09^{d}	1.80 ± 0.10^{c}	1.30 ± 0.02^{d}	1.82 ± 0.06c	1.24 ± 0.04d	4.14 ± 0.14a	2.83 ± 0.10b
5	0.27 ± 0.02^{e}	0.40 ± 0.03^{cd}	0.27 ± 0.04^{e}	0.57 ± 0.06^{b}	0.37 ± 0.02^{d}	0.18 ± 0.02^{f}	0.15 ± 0.01^{f}	0.36 ± 0.02d	0.28 ± 0.02e	0.63 ± 0.02a	0.44 ± 0.02c
6	3.21 ± 0.03^{de}	3.59 ± 0.13^{cd}	2.72 ± 0.31^{ef}	5.70 ± 0.29^{a}	3.20 ± 0.07^{de}	3.84 ± 0.36^{c}	2.96 ± 0.08^{ef}	2.58 ± 0.03f	1.84 ± 0.11g	5.08 ± 0.25b	3.02 ± 0.11e
7	10.70 ± 0.30^{de}	10.49 ± 0.06^{d}	9.49 ± 0.86^{ef}	17.69 ± 0.66^{c}	11.07 ± 0.53^{d}	5.28 ± 0.46^{gh}	4.33 ± 0.17^{h}	8.41 ± 0.31f	6.13 ± 0.15g	32.04 ± 1.50a	22.98 ± 0.97b
8	0.59 ± 0.06^{b}	1.07 ± 0.06^{a}	0.61 ± 0.07^{b}	0.24 ± 0.01^{e}	0.19 ± 0.02^{ef}	0.11 ± 0.01^{g}	0.10 ± 0.01^{g}	0.18 ± 0.01efg	0.12 ± 0.01fg	0.47 ± 0.02c	0.34 ± 0.01d
9	0.81 ± 0.00^{b}	0.88 ± 0.03^{a}	0.79 ± 0.07^{ab}	0.40 ± 0.03^{cd}	0.29 ± 0.01^{e}	0.26 ± 0.02^{e}	0.26 ± 0.01^{e}	0.25 ± 0.02e	0.25 ± 0.02e	0.45 ± 0.05c	0.35 ± 0.02d
10	11.68 ± 0.24^{a}	11.44 ± 0.51^{a}	10.61 ± 1.35^{a}	3.58 ± 0.19^{d}	2.50 ± 0.13^{e}	1.83 ± 0.14^{e}	1.63 ± 0.01^{e}	2.12 ± 0.13e	1.73 ± 0.05e	7.38 ± 0.47b	5.37 ± 0.16c
11	0.39 ± 0.03^{c}	0.85 ± 0.06^{a}	0.64 ± 0.05^{b}	0.26 ± 0.02^{d}	0.18 ± 0.01^{e}	0.03 ± 0.01^{g}	0.08 ± 0.01^{fg}	0.17 ± 0.02e	0.10 ± 0.02ef	0.31 ± 0.03d	0.09 ± 0.00ef
subtotal	**29.35 ± 0.49^{cd}**	**30.71 ± 0.80^{bc}**	**26.87 ± 2.72^{d}**	**32.28 ± 1.23^{b}**	**19.80 ± 0.88^{e}**	**14.87 ± 1.31^{f}**	**11.98 ± 0.36^{g}**	16.80 ± 0.57f	12.32 ± 0.38g	51.50 ± 2.45g	36.43 ± 1.41a
Oleanane esterified phenolic acid											
21	1.39 ± 0.13^{ef}	2.56 ± 0.27^{b}	1.97 ± 0.22^{d}	2.19 ± 0.18^{c}	1.17 ± 0.05^{fg}	3.05 ± 0.12^{a}	2.22 ± 0.09^{c}	1.17 ± 0.09ef	0.68 ± 0.04h	1.41 ± 0.24e	0.86 ± 0.04gh
22	0.64 ± 0.06^{de}	1.13 ± 0.06^{b}	0.87 ± 0.08^{c}	1.63 ± 0.20^{a}	0.74 ± 0.04^{cd}	1.44 ± 0.06^{a}	1.15 ± 0.05^{b}	0.56 ± 0.05e	0.33 ± 0.00f	0.81 ± 0.15c	0.57 ± 0.02e
subtotal	**2.03 ± 0.19^{d}**	**3.69 ± 0.34^{b}**	**2.87 ± 0.30^{c}**	**3.82 ± 0.37^{b}**	**1.91 ± 0.09^{d}**	**4.56 ± 0.16^{a}**	**3.37 ± 0.04^{b}**	1.71 ± 0.13de	1.01 ± 0.04f	2.45 ± 0.46f	1.43 ± 0.06e
total	**114.85 ± 1.55^{bc}**	**122.43 ± 0.00^{b}**	**110.57 ± 7.41^{cd}**	**141.38 ± 4.80^{a}**	**97.95 ± 2.26^{ef}**	**113.29 ± 5.52^{cd}**	**96.19 ± 0.44^{f}**	92.55 ± 3.63f	70.61 ± 1.78g	139.57 ± 4.14a	103.12 ± 3.15de

[i]Each value calculated as means ± SD (n = 3) using an internal standard (protodioscin). Compound names are presented according to peak numbers in Table 1. [a-h] Different small letters with mean values (n = 3) indicate a significant difference (p < 0.05) by Duncan's multiple range test. [ii]External standard (avenacoside A).

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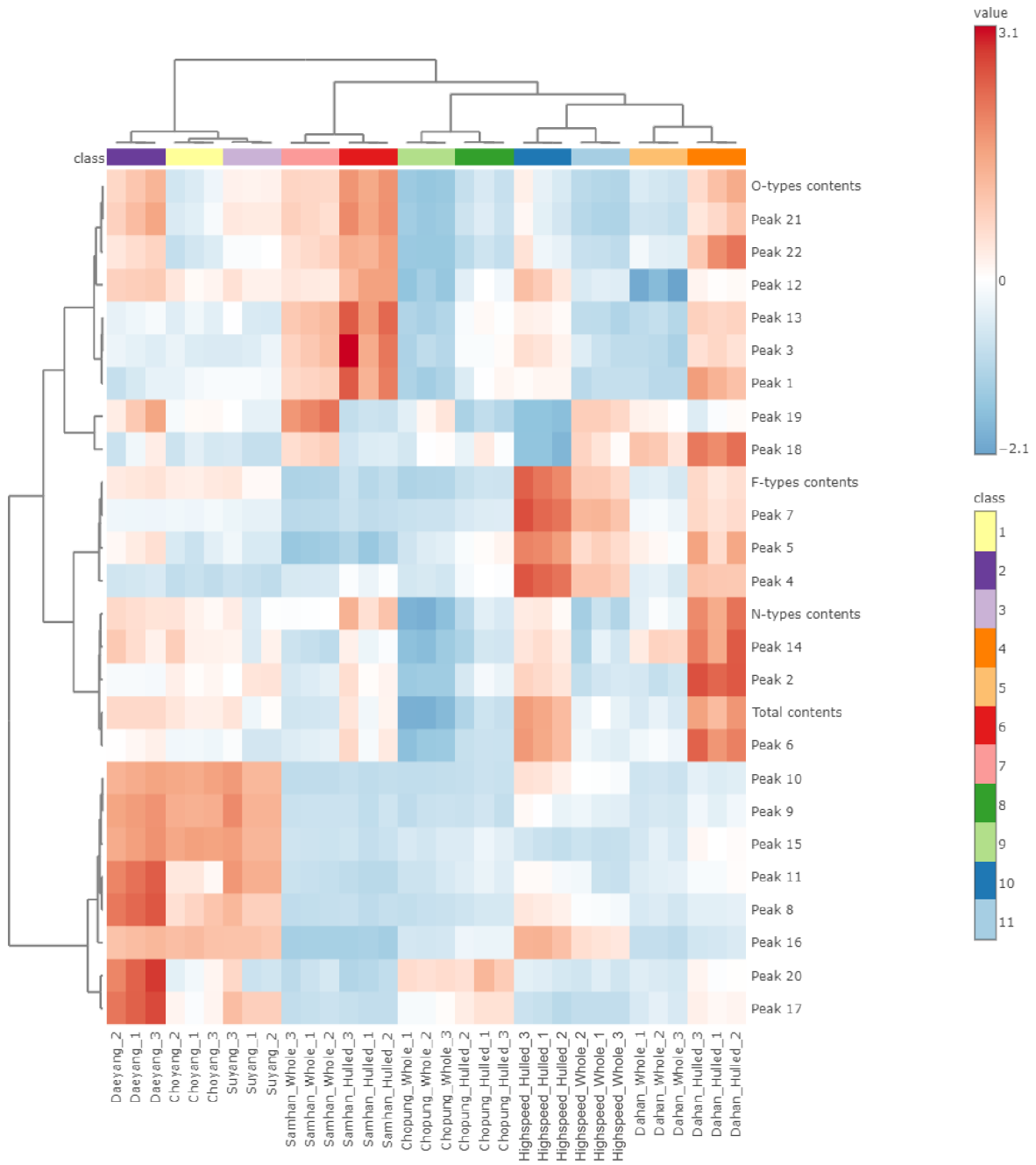

Figure 6. A hierarchical heat map of the saponin contents by cultivars. Compound names are presented according to peak numbers in Table 1. Class 1−11 are Choyang hulled, Daeyang hulled, suyang hulled, Dahan hulled, Dahan whole grain, Samhan hulled, Samhan whole grain, Chopung hulled, Chopung whole grain, High-speed hulled, and High-speed whole grain, respectively.

syl-(1 → 2)]-glucosyl-(25*S*)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside, (25*S*)-sativacoside B and the first reported in this source.

Peaks **6** and **7** were determined to have the molecular formula $C_{51}H_{84}O_{23}$ based on the fragment ions at *m*/*z* 1047.5389 and 1047.5366$[M+H-H_2O]^+$, respectively, corresponding to the loss of one glucose unit from peaks **3** and **4** (Figures 4d,e and 5b). According to the above results, the loss of one glucose unit was confirmed due to the loss at the C-3 position; therefore, peak **7** was identified as sativacoside A,[18,21] and peak **6** was tentatively identified as 3-*O*-glucosyl-(1 → 4)-[rhamnosyl-(1 → 2)]-glucosyl-(25*S*)-furost-5-en-3*β*,22,26-triol 26-*O*-glucoside, (25*S*)-sativacoside A and first reported in this source.

Peaks **5** and **8** were identified as isomers of peaks **3** and **4** and peaks **6** and **7**, respectively. Compared to the above compounds, these compounds showed higher relative abundances of *m*/*z* 1063 and 901 ions, respectively, generated by the loss of a pentose units (Figure 4c,f). Their structural differences have been tentatively suggested to be due to different sugar units or linkage between the glucose and rhamnose units at the C-3 position and will require further structural elucidation in the future.

Peaks **9**-**11** showed protonated ions $[M+H-H_2O]^+$ at *m*/*z* 885.4840, 885.4842, and 739.4255, respectively, which were attributed to the loss of 1 glucose unit or 2 sugar units (1 rhamnose and 1 glucose units, 308 Da) at the C-3 position of

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peaks **6** and **7**. The MS fragment ions of peaks **9**-**11** are shown in Figure 4g−i. Based on the elution time, MS fragmentation, and literature reports, peak **10** was identified as sativacoside C[21] and peak **9** was tentatively identified as 3-*O*-rhamnosyl-(1 → 2)-glucosyl-(25*S*)-furost-5-en-3β,22,26-triol 26-*O*-glucoside, (25*S*)-sativacoside C and was first reported in this source. However, it was not possible to determine whether the absolute configuration of peak **11** was 25*S* or 25*R*, and a follow-up study is needed to confirm the structure through one- and two-dimensional NMR analyses.

2.2. Variation in the Contents of Steroidal and Triterpenoid Saponins from Oats. A total content (mg/100 g, dry weight) of 22 saponins from seven Korean oat cultivars ranged from 70.61 to 141.38 and are summarized according to their aglycones in Table 2. These results are similar to previous data reported by Bhardwaj et al.,[27] crude saponin content: 46.9−217.2 mg/100 g; Pecio et al.,[19] avenacosides content: 55.3−90.9 mg/100 g.

Dahan was significantly identified as the most saponin-containing cultivar, followed by Daeyang, Samhan, Choyang, Suyang, and Chopung. The concentration of each saponin was different depending on the type of oat. In the naked oats, nuatigenin-type, furost-5-en-3β,22,26-triol-type, and oleanane-type saponins accounted for approximately 73.0%, 25.1%, and 2.6% of the total saponins, respectively, whereas the proportions of these saponins in covered oats were 61.1−84.0%, 12.5−36.9%, and 1.4−4.0%, respectively. In particular, in the covered oats, the content of hulled grain was significantly higher than that of whole grain by approximately 1.3 times, and this difference appeared to be due to a decrease in the overall saponin content rather than a change in the specific saponin ratio. It has been shown that the hulling process may increase the ratio of endosperm to the content and intake of oat saponins. This was similar to previous results Pecio et al.[19] and Önning et al.,[37] who reported that the saponin contents of oat grains were approximately 27 times higher than that of oat husks, and that avenacosides were primarily stored in the endosperm of oat grains.

Among nuatigenin-type saponins, avenacosides A and B were identified as primary compounds from oat grains and accounted for more than half of the total saponin content, which is consistent with previous data (avenacoside A: 24.65−55.09 mg/100 g; avenacoside B: 21.92−36.3 mg/100 g).[19,26] On the other hand, among furostane types, sativacoside C was identified as the major compound in naked oats, followed by sativacoside A, (25*S*)-sativacoside A, and sativacoside B. In contrast, the content of these saponins was confirmed in the order of sativacoside A >; (25*S*)-sativacoside A > sativacoside C > sativacoside B in covered oats except for the High-speed cultivar. The High-speed cultivar especially contained more furostane types than other cultivars (hulled: 36.9%, whole grain: 35.3% of the total saponins), of which sativacoside A accounted for about 30%. These variations can be caused by differences in cultivars, cultivation conditions, sensitivity, and precision of quantitative methods.[19]

To illustrate the contents of 22 saponins by seven cultivars, a hierarchical heat map was constructed using normalized contents (Figure 6). The red indicates the highest contents, and the blue indicates the opposite. The seven cultivars were clustered into two groups: naked oats and covered oats, and the covered oats group were then divided into two subgroups. The naked oat group mainly contained peaks **8**−**11**, and **15**, while 'Dahan' and 'Samhan' groups mainly contained peaks **3** and **13**. In particular, peak **7** might be seen as characteristic components of 'High-speed' (Table 2 and Figure 6).

Structure−activity relationship studies have been conducted on the various biological activities of steroidal saponins, including oat saponins. The antibacterial activity of oat saponins has been reported to be influenced by the presence of terminal glucose, but 26-desglucoavenacoside B (26-AB), which possesses it, has been shown to be inactive (neuramidase inhibition IC_{50}: avenacoside B, 20.5 μM; avenacoside A, >200 μM; 26-AB, >200 μM).[17] On the other hand, 26-AB was found to have higher antifungicidal activity compared to other saponins.[16] According to Yokosuka et al.,[24] 26-desglucoavenacosides A and C showed high anticancer activity. Sativacosides A and B showed stronger effects than avenacosides A and C, and the corresponding spirostane types slightly increased cytotoxicity. Therefore, it was confirmed that the furan ring opening and loss of the 26-Glu moiety of furospirostane saponin, dehydration, and pyran ring formation of furostane saponin increase anticancer activity. Similar results were confirmed in reports by Okubo et al.,[38] Beit-Yannai et al.,[39] Dai et al.[40] (furospirostane < furostane < spirostane saponin). In addition, structures containing two or more sugar chains at the C-3 position (especially the additional rhamnose units),[39,41,42] and the 25(*R*/*S*)-configuration (25*R* > 25*S*: A549, Caski, HepG2, and MCF-7;[38,43] 25*S* > 25*R*: BEL-7402, HT-29, Hela, MDA-MB-468, BT549, and SW620 cell lines[44,45]) were considered important for cytotoxicity. Moreover, the aforementioned factors also influenced anti-inflammatory,[46−49] antiatherosclerotic,[50] antidiabetic,[51] and antiosteosarcoma[52] activities. However, studies of oat saponins, including the new saponins identified in this study, are still lacking. Therefore, additional research is needed on the biological activities, bioavailability, structure−activity relationship, synergy effects of each compound, and clinical studies, including the new compounds.

3. CONCLUSIONS

Our results presented information on 7 new saponins, 12 known saponins, and 3 unknown saponins from Korean oat cultivars. Among the newly identified, peaks **1**, **2**, **16**, and **17** have structures in which glucose or malonyl units were additionally bound to 26-OH or 3-OH of the sugar moieties of avenacosides, respectively. And, another saponins were predicted as 25*S*-furost-5-en-3β,22,26-triol types (peaks **3**, **6**, and **9**), which are enantiomers of the 25*R* forms. In addition, variations in saponin content were confirmed, depending on the cultivars and hulling process of covered oats. The total saponin content ranged from 70.61 to 141.38 mg/100 g of dry weight, and Dahan was especially confirmed to be the cultivar containing the most saponin content. In naked oats, nuatigenin-types, furost-5-en-3β,22,26-triol-types, and oleanane-types accounted for approximately 73.0%, 25.1%, and 2.6% of the total saponin content, respectively. On the other hand, the proportions of these saponins in covered oats were 61.1−84.0%, 12.5−36.9%, and 1.4−4.0%, respectively. Particularly, among covered oats, the High-speed cultivar had a high proportion of furost-5-en-3β,22,26-triol types, and hulling processing affected the content of these compounds (hulled: 36.9%, whole grain: 35.3%). As interest in oats increases, providing information about the specific composition and content of oats is becoming important. However, further studies via various analysis techniques, such as NMR, IR, and ECD, are necessary to more accurately determine the structures of isomers that were not yet identified in this study. In the future, these detailed profiles can be used as

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Table 3. Regression Equation, Linear Range, Coefficient of Determination LOD and LOQ for UPLC-MS/MS MRM Analysis of Avenacoside A

compounds	calibration curve[a]	R^2	Llinear Rrange (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
avenacoside A	$y = 411873x - 19270.89259$	0.99915	0.5−50	0.27	0.82

[a] y, peak area; x, concentration, ppm.

fundamental data for further studies on composition and content changes by cultivar, harvest time, processing, and cooking conditions, as well as bioactivity evaluation of new oat saponins. Moreover, these results are suggested as usable data for breeding superior oat cultivars, ruminant feed, and various industries.

4. MATERIALS AND METHODS

4.1. Plant Materials. Oat grains of seven Korean cultivars (each 1 kg) were collected in the experimental field (May 2020, Wanju, Jeollabuk-do, Republic of Korea) of the National Institute of Crop Science, Rural Development Administration (latitude/longitude: 35°4938.37N/127°0907.78E), and composed of Naked oats (Choyang, Daeyang, and Suyang) and Covered oats (Dahan, Samhan, Chopung, and High-speed). Covered oats (50 g) were separated into grains and hulls by mechanically rubbing with a pestle in mortar (whole and hulled types).

4.2. Chemicals and Reagents. LC-MS grade solvents: methanol (MeOH) and acetonitrile (ACN) were purchased from Fisher Scientific (Fair Lawn, NJ, USA), and formic acid was obtained from Junsei Chemical (Tokyo, Japan). Avenacoside A (external standard) was supplied from Sigma-Aldrich Co. (St. Louis, MO, USA), and protodioscin (ChemFaces Biochemical Co., Wuhan, China) was used as an internal standard (ISTD).

4.3. Saponin Extraction. Saponin extraction was conducted by modifying the method described in[53] In brief, 0.1 g of the powdered grains were extracted twice with 1.5 and 1.0 mL of 70% MeOH using an ultrasonic extractor for 30 min (POWERSONIC 520, Hwasin Technology, Seoul, Korea), and then centrifuged for 15 min at 2,016 × g (LABOGENE 1580R, Bio-Medical Science Co., Seoul, Korea), respectively. The collected supernatants were filtered through a 0.2 μm PVDF syringe filter (Thermo Fisher Scientific Inc., Waltham, MA, USA, concentrated using N_2 gas, and then redissolved in 4 mL of distilled water (DW). In order to maintain stable recovery of ISTD during solid phase extraction, 0.5 mL of ISTD (25 ppm of protodioscin) was diluted with DW (6.5 mL). A C_{18} cartridge (Hypersep C_{18} 500 mg, Thermo Fisher Scientific Inc.) was conditioned with MeOH (5 mL) and DW (10 mL), then the redissolved samples and diluted ISTD were loaded into the cartridge and washed with DW (5 mL). Finally, saponins were eluted from the loaded cartridge with 70% MeOH (5 mL), then concentrated using N_2 gas, and redissolved in 70% MeOH (0.5 mL) prior to UPLC-QToF/MS and UPLC-Triple Q-MS/MS analyses.

4.4. UPLC-DAD-QToF/MS and UPLC-Triple Q-MS/MS Analysis. Saponin identification from Korean oat grain was performed using the UPLC system coupled with quadrupole time-of-flight (QToF) mass spectrometry (SCIEX X500R, SCIEX Co., MA, USA). Chromatographic conditions were set up as follows: column, CORTECS UPLC T3, 2.1 × 150 mm, 1.6 μm (Waters Co., Milford, MA, USA); precolumn, CORTECS UPLC Vanguard T3, 2.1 × 50 mm, 1.6 μm, (Waters Co.); column temperature, 30 °C; sample injection volume, 1 μL; flow rate, 0.35 mL/min; mobile phase, 0.1% formic acid in DW (A), 0.1% formic acid in ACN (B). Gradient conditions used: 0−10 min, 15% B; 10 min, 25% B; 40−45 min, 50% B; 50−60 min, 15% B. Mass spectra were multiscanned in the range of m/z 100−2000 of the positive ionization mode through an electrospray ionization (+ ESI) source, with the the following parameters: ion source gas, 50 psi; curtain gas, 30 psi; ion source temperature, 450 °C; declustering potential (DP), 80 V; collision energy (CE), 15 ± 10 V; spray voltage, 5500 V. Avenacoside A was externally quantified based on multiple-reaction monitoring (MRM) mode using UPLC-Triple Q-MS/MS (SCIEX QTRAP 4500, SCIEX CO.), whereas other saponins were internally quantified using UPLC-QToF-MS (SCIEX X500R, SCIEX CO.). Optimized MRM conditions for avenacoside A: precursor to product ion pair, 1063.2 → 1063.2; DP, 161 V; CE, 13 V.

4.5. Identification and Quantification of Steroidal and Triterpenoid Saponins. Identification of oat saponins was performed by referring to the mass fragmentation and retention times (Rt, min) of avenacoside A with the library constructed through previous literature reports of oat saponins (Table S1). Through preliminary experiments, protodioscin, which did not overlap with the sample peaks and has a structure similar to oat saponins, was selected as the ISTD, and the internal quantification was calculated by comparing the relative peak areas (based on major fragment ions) of the compounds with ISTD at 1:1 without considering the relative response factor. To quantify avenacoside A from samples, the external quantification was performed in MRM mode, and the calibration curve was constructed by plotting the peak areas of standard (0.5, 1, 5, 25, 50 ppm) versus their corresponding concentrations using a least-squares linear regression analysis: regression equation, y (peak area) = 411873 x (concentration, ppm) + 19270.89259 (R^2 = 0.99915); linear range, 0.5−50 μg/mL. The limit of detection (LOD) and quantification (LOQ) were calculated using the calibration curve data following the equation: LOQ = 3.3 x δ/S (0.27 μg/mL) and LOQ = 10 x δ/S (0.87 μg/mL), where δ and S are the standard derivation of the y-intercept and the slope of the calibration curve (Table 3), respectively.

4.6. Statistical Analysis. The triplicate results are expressed as the mean ± standard deviation. One-way ANOVA was performed with SPSS (version 28.0, SPSS Institute; Chicago, IL, USA) to determine a significant difference between individual averages using Duncan's multiple range test ($p < 0.05$). For clustering and visualization of the saponin content by cultivar, a hierarchical heat map was constructed by Pearson distance and Ward's method using MetaboAnalyst online analysis software (https//www.metaboanalyst.ca).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c10439.

Table S1. Chemical library of 26 saponins from *Avena sativa* L. (oat) based on literature sources Figure S1. UPLC chromatograms of saponins in six Korean oat cultivars. MRM-HR (a, c, e, g, I, k, m, o, q, and s) and XIC

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(b, d, f, h, j, l, n, p, r, and t). Internal standards (ISTD): protodioscin 25 ppm. Figure S2. (+) ESI-MS spectra of oat saponins. Peak **12** (a, m/z 1387$[M + H]^+$) peaks **15** and **18** (b and c, m/z 901$[M + H]^+$), peak **19** (d, m/z 739$[M + H]^+$), peak **20** (e, m/z 593$[M + H]^+$), peak **21** (f, m/z 1094$[M + H]^+$), peak **22** (g, m/z 1078$[M + H]^+$) (PDF)

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Notes

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