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Antioxidant Flavonols and Phenolic Compounds from *Atraphaxis frutescens* and Their Inhibitory Activities against Insect Phenoloxidase and Mushroom Tyrosinase

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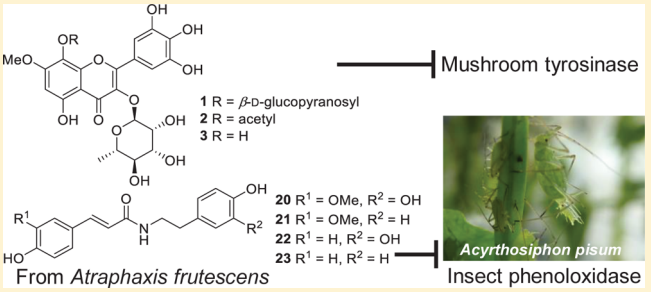
Supporting Information

ABSTRACT: Chemical investigation of the aerial parts of *Atraphaxis frutescens* resulted in the isolation of five 7-methoxyflavonols with pyrogallol B-ring moieties (**1**–**5**), a fisetinidol glucoside (**13**), and a benzyl glycoside (**18**), together with 26 known compounds including flavonoids, phenylpropanoid amides, anthraquinone glycosides, lignans, and a benzyl derivative. The principal chemical structural feature of the isolated compounds was either a pyrogallol or catechol B-ring moiety, and they showed potent 1,1-diphenyl-2-picrylhydrazyl radical scavenging activities. To assess the effects of these antioxidants on biological enzymes, their inhibitory effects against an insect phenoloxidase and a mushroom tyrosinase were evaluated. This study indicated that insect phenoloxidase was inhibited by phenylpropanoid amides and that mushroom tyrosinase was inhibited by the characteristic 7-methoxyflavonol 3-*O*-rhamnopyranosides.



Atraphaxis frutescens (L.) K. Koch is a shrub and a member of the Polygonaceae family that is found in the Mongolian Gobi.[1,2] The *Atraphaxis* genus consists of 30 species, and 25 of these grow in Central Asia, Russia, Kazakhstan, China, and Mongolia.[1] Most *Atraphaxis* plants are typical desert plants; *A. bracteata*, *A. compacta*, *A. pungens*, *A. spinosa*, *A. virgata*, and *A. frutescens* all grow in the Gobi desert.[2] This is the largest desert region in Asia, with a landscape characterized by a dry climate (annual precipitation of between 50 and 150 mm) and extreme temperature differences.[3]

Flavonoids and phenolic compounds with antioxidant activity may play key roles in protecting plants from oxidative stress. Therefore, the levels of these antioxidant compounds may increase when plants are subjected to drought- and cold-induced stress.[4] *Atraphaxis* species growing in the Gobi desert are known to be a rich source of flavonoids and have traditionally been used to treat various human and domestic animal diseases. Thus, herbivores and humans can be protected from oxidative stress by products derived from these plants.

The dried aerial parts of *A. frutescens* provide a traditional Mongolian medicine that is used for detoxification and to treat lymph disorders, bacterial fevers, throat infections, and eye diseases, including cataracts.[5] A previous study has reported the constituents of *A. frutescens*: quercetin, kaempferol, catechin, 4,5-dihydroxy-3-methoxybenzoic acid, myricetin, myricetin 3-*O*-*β*-D-glucopyranoside, quercetin 3-*O*-*β*-D-glucopyranoside, and kaempferol 3-*O*-*β*-D-glucopyranoside.[6] Even though the current information relating to *A. frutescens* is limited to the above study, other species of *Atraphaxis* have been investigated extensively. For instance, anthraquinones and antibacterial naphthalene derivatives have been identified in *A. laetevirens*,[7] 7-*O*-methylgossypetin and its glycosides were isolated from *A. laetevirens*[8] and *A. pyrifolia*,[9−13] and other flavonoids and phenolic compounds were extracted from *A. muschketovii*[14] and *A. spinosa*.[9,15]

In the present study, five new 7-methoxyflavonols with pyrogallol B-ring moieties (**1**–**5**), a new fisetinidol glucoside (**13**), and a new benzyl glycoside (**18**) were isolated from the aerial parts of *A. frutescens*, together with 26 known compounds. Compounds **1**–**17** had either pyrogallol or catechol B-ring moieties, which are known to be important for antioxidant activities.[16] In fact, almost all of the compounds isolated herein showed good radical scavenging activity, as demonstrated using 1,1-diphenyl-2-picrylhydrazyl (DPPH). Antioxidant agents play important roles in various mechanisms in the human and animal body, producing both beneficial and adverse effects.[17] Oxidase enzymes are involved in these biological redox reactions, and their activities can be modulated by antioxidants.

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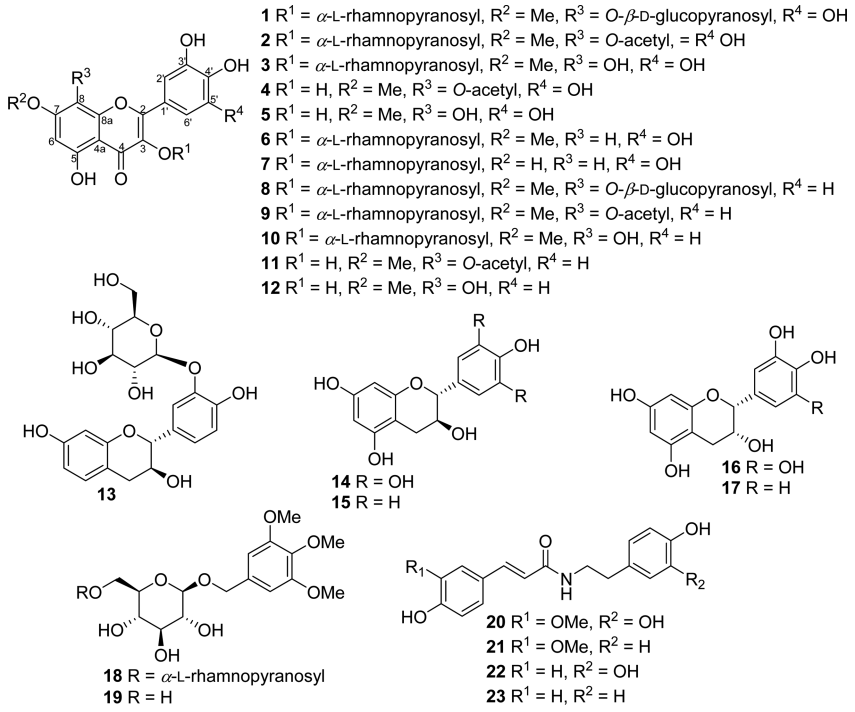
Chart 1



To estimate the effects of the isolated compounds on oxidase activity, their inhibitory effects on an insect phenoloxidase from *Acyrthosiphon pisum* and a mushroom tyrosinase were assayed. These are both copper-containing enzymes that catalyze the oxidation of mono- and diphenols to dopaquinones, melanin being produced by the oxidative polymerization of dopaquinones.[18,19]

Phenoloxidase is a component of the insect innate immune system, which defends against foreign substances by producing melanin.[19,20] This enzyme is activated from pro-phenoloxidase by a serine protease at one of the final steps in the activation of the insect innate immune system.[21] In this study, an assay was developed for phenoloxidase activity using *A. pisum*, an aphid that is an important agricultural pest in legume crops. This aphid is found throughout the temperate zone and is widely used in laboratory studies as a basic insect model species; furthermore, its genome sequence has been determined.[22] Tyrosinase catalyzes melanogenesis in human skin and plays an important role in protection against UV damage. The control of tyrosinase is a target for pharmaceutical and cosmetic research because excess activity can cause freckles and age spots.[23]

RESULTS AND DISCUSSION

An acetone−H_2O extract of the aerial parts of *A. frutescens* was dissolved in H_2O and extracted in Et_2O. This aqueous extract was applied to an octadecylsilyl (ODS) column and separated using HPLC to obtain 33 compounds; these included three new flavonol glycosides (**1**−**3**), two new flavonols (**4** and **5**), a new fisetinidol glucoside (**13**), and a new benzyl glycoside (**18**). The known compounds were identified by comparison of their chemical characteristics and spectroscopic data with reported data. These were europetin 3-*O*-α-L-rhamnopyranoside (**6**),[24] myricitrin (**7**),[24] fisetinidol,[25] gallocatechin (**14**),[26] catechin,[27] afzelechin (**15**),[27] aromadendrin,[28] epigallocatechin (**16**),[29] epicatechin (**17**),[29] nikoenoside (**19**),[30] *N-trans*-feruloyldopamine (**20**),[31] *N-trans*-feruloyltyramine (**21**),[32] *N-p-trans*-coumaroyldopamine (**22**),[31] *N-p-trans*-coumaroyltyramine (**23**),[33] emodin 8-*O*-β-D-glucopyranoside,[34] emodin 8-*O*-(6′-*O*-malonyl)glucoside,[34,35] torachrysone 8-*O*-β-D-(6′-*O*-malonyl)glucopyranoside,[36] syringaresinol,[37] dehydroconiferyl alcohol,[38] 3,4,5-trimethoxyphenyl 1-*O*-β-D-glucopyranoside,[39] and methyl syringate.[40] Compounds **8**−**12** had been isolated previously by Russian research groups and were identified as 8-*O*-β-D-glucopyranosyl-7-*O*-methyl-3-*O*-α-L-rhamnopyranosylgossypetin (**8**),[13] 8-*O*-acetyl-7-*O*-methyl-3-*O*-α-L-rhamnopyranosylgossypetin (**9**),[12] 7-*O*-methyl-3-*O*-α-L-rhamnopyranosylgossypetin (**10**),[12] 8-*O*-acetyl-7-*O*-methylgossypetin (**11**),[10,12] and 7-*O*-methylgossypetin (**12**).[11] These compounds were identified using NMR data based on a comparison with the data for compounds **1**−**5**.

Compounds **1**−**5** were obtained as yellowish, amorphous solids. In the ^1H and ^{13}C NMR spectra, these compounds shared the spectroscopic features of an aromatic A-ring proton singlet (δ_H 6.47−6.69, 1H, H-6), a two-proton B-ring singlet (δ_H 6.86−7.35, 2H, H-2′ and H-6′), and 14 aromatic carbon resonances, suggesting that **1**−**5** had flavonoid skeletons with a pyrogallol B-ring. The acid hydrolysis of compounds **1**−**3** produced aglycone **5**.

The molecular formula of **5** was established as $C_{16}H_{12}O_9$ based on the HRFABMS data (*m/z* 349.0562 $[M + H]^+$; calcd for $C_{16}H_{13}O_9$, 349.0559); this included one CH_2 more than in hibiscetin, a 3,3′,4′,5,5′,7,8-heptahydroxyflavone. In the ^{13}C NMR spectrum of **5**, an oxygenated carbon resonance at δ_C 135.5 (C-3) and a carbonyl carbon resonance at δ_C 176.2 (C-4) were consistent with a flavonol moiety. The ^1H NMR spectrum revealed a hydrogen-bonded hydroxy proton resonance (δ_H 12.04, s), aromatic proton singlets (δ_H 6.55, 1H, H-6; 7.35, 2H, H-2′ and H-6′), four singlet hydroxy protons (δ_H 8.77, 1H, br s; 8.83, 1H, br s; 9.26, 2H, br s; 9.34, 1H, br s), and a methoxy singlet (δ_H 3.91, 3H). In the HMBC spectra, the methoxy protons were long-range coupled with C-7 (δ_C 153.2), H-2′ and H-6′ were long-range coupled with C-2, C-1′, C-3′, C-4′,

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and C-6′, and the OH-5 proton was long-range coupled with C-5, C-6, and C-4a (Table S1, Supporting Information). Collectively these data identified **5** as 3,3′,4′,5,5′,8-hexahydroxy-7-methoxyflavone.

The molecular formula of **1** was determined as $C_{28}H_{32}O_{18}$ based on HRFABMS data (*m/z* 657.1682 $[M + H]^+$; calcd for $C_{28}H_{33}O_{18}$, 657.1666). Eleven oxygenated sp^3 carbon resonances (δ_C 105.8, 104.1, 78.2, 77.8, 75.7, 73.4, 72.1, 72.0, 71.9, 71.2, 62.4) and an aliphatic carbon resonance (δ_C 17.7) were observed in the ^{13}C NMR spectrum of **1**, suggesting the presence of two glycosyl moieties. The aliphatic carbon and its corresponding methyl doublet resonance in the HMQC spectrum (δ_H 1.06, 3H, d, *J* = 6.5 Hz, H-Rha-6) suggested the presence of a rhamnopyranosyl moiety. The six carbon resonances (δ_C 105.8, 75.7, 77.8, 71.2, 78.2, 62.4, C-Glc-1−6) and the anomeric proton resonance (δ_H 4.85, overlapping, H-Glc-1) with its correlated proton resonance in the COSY spectrum at δ_H 3.57 (dd, 1H, *J* = 9.5, 8.0 Hz, H-Glc-2) suggested the presence of a glucopyranosyl moiety. In the sugar analysis using HPLC after acid hydrolysis of **1**, peaks of D-glucopyranose and L-rhamnopyranose derivatives were detected, indicating the presence of D-glucopyranosyl and L-rhamnopyranosyl moieties.[41] The anomeric proton coupling constant of 8.0 and 1.5 Hz, respectively, indicated the *β*- and *α*-orientations of the D-glucopyranosyl and L-rhamnopyranosyl moieties. In the HMBC spectrum, the anomeric proton of the *β*-D-glucopyranosyl moiety (H-Glc-1) was long-range coupled with C-8 (δ_C 126.6) and the anomeric proton of the *α*-L-rhamnopyranosyl (δ_H 5.16, 1H, br s) unit was long-range correlated with C-3 (δ_C 136.5). From these data, **1** was established as 8-*β*-D-glucopyranosyloxy-3′,4′,5,5′-tetrahydroxy-7-methoxy-3-*α*-L-rhamnopyranosyloxyflavone.

The molecular formulas of **2** and **3** were determined as $C_{24}H_{24}O_{14}$ (*m/z* 537.1249 $[M + H]^+$; calcd for $C_{24}H_{25}O_{14}$, 537.1244) and $C_{22}H_{22}O_{13}$ (*m/z* 494.1159 $[M + H]^+$; calcd for $C_{22}H_{23}O_{13}$, 495.1138), respectively, on the basis of the molecular ions present in the HRFABMS data. The NMR spectroscopic features of these two compounds were similar. Neither compound showed the ^1H and ^{13}C NMR resonances of the *β*-D-glucopyranosyl moiety observed in **1**. For compound **2**, the resonances of an acetoxy group (δ_H 2.45, 3H, s; δ_C 19.9, 168.4) were observed and the C-8 resonance (δ_C 118.4) was shifted to higher field compared to that of **3** (δ_C 126.0). Although the proton resonances of OH-3′, OH-4′, OH-5, and OH-5′ were observed in the spectra of both **2** and **3**, the OH-8 proton resonance was observed only in the spectrum of **3** (δ_H 8.84, 1H, br s). These data demonstrated that **2** was the 8-*O*-acetyl derivative of **3**. The L-rhamnopyranosyl configuration of **2** and **3** was determined using the same procedure employed for **1**. This identified **2** and **3** as 8-acetoxy-3′,4′,5,5′-tetrahydroxy-7-methoxy-3-*α*-L-rhamnopyranosyloxyflavone and 3′,4′,5,5′,8-pentahydroxy-7-methoxy-3-*α*-L-rhamnopyranosyloxyflavone, respectively.

The molecular formula of **4** was determined as $C_{18}H_{14}O_{10}$ based on the HRFABMS data (*m/z* 391.0653 $[M + H]^+$; calcd for $C_{18}H_{15}O_{10}$, 391.0665), which represented an additional $C_2H_2O_1$ structural moiety as compared with **5**. The NMR spectra of **4** were similar to those of **5**, except for acetoxy resonances [δ_H 2.45 (3H, s), δ_C 20.0, 168.4] in the spectra of **4**. The C-8 (δ_C 118.4) resonance suggested that **4** was the 8-*O*-acetyl derivative of **5**. From these data, the structure of **4** was assigned as 8-acetoxy-3,3′,4′,5,5′-pentahydroxy-7-methoxyflavone.

The NMR spectroscopic features of **8**−**12** (Table S2, Supporting Information) were similar to those of **1**−**5**, respectively. In the ^1H NMR spectrum, an ABX system was observed for the B-ring proton resonances, rather than the two-proton singlets of **1**−**5**. These data demonstrated that **8**−**12** had a catechol B-ring instead of the pyrogallol moiety in **1**−**5**. The structures of **8**−**12** were reported previously,[8,12,13] and these compounds were identified as 8-*O*-*β*-D-glucopyranosyl-7-*O*-methyl-3-*O*-*α*-L-rhamnopyranosylgossypetin (**8**), 8-*O*-acetyl-7-*O*-methyl-3-*O*-*α*-L-rhamnopyranosylgossypetin (**9**), 7-*O*-methyl-3-*O*-*α*-L-rhamnopyranosylgossypetin (**10**), 8-*O*-acetyl-7-*O*-methylgossypetin (**11**), and 7-*O*-methylgossypetin (**12**).

Compound **13** was assigned the molecular formula $C_{21}H_{24}O_{10}$, as determined from its molecular ion $[M + Na]^+$ at *m/z* 459.1272 (calcd for $C_{21}H_{24}O_{10}Na$, 459.1266). In the ^{13}C NMR spectra, resonances of a glucopyranosyl moiety were observed (δ_C 103.9, 74.9, 77.7, 71.2, 78.3, 62.4). The hydrolysis of **13** and sugar analysis using HPLC showed the D-glucopyranose derivative peak. This result, and the coupling constant of an anomeric proton resonance at δ_H 4.79 (1H, d, *J* = 7.5 Hz, H-Glc-1), suggested that **13** had a *β*-D-glucopyranosyl moiety. The oxygenated proton resonance at δ_H 4.04 (1H, m, H-3) was correlated with the next oxygenated proton resonance at δ_H 4.67 (1H, d, *J* = 7.5 Hz, H-2) and aliphatic proton resonances (δ_H 2.89, 1H, dd, *J* = 16.0, 5.5 Hz, H-4*α*; 2.71, 1H, dd, *J* = 16.0, 8.5 Hz, H-4*β*) in the COSY spectrum. The coupling constants of 7.5, 5.5, and 8.5 Hz, indicated axial protons of H-2, H-3, and H-4*β* and an equatorial proton of H-4*α*.[25,42] These protons suggested that **13** had a flavan-3-ol moiety, while the other ^1H and ^{13}C NMR data for the aglycone moiety were similar to those of fisetinidol,[25] except for the B-ring. The deshielded aromatic proton doublet at δ_H 7.20 (1H, d, *J* = 2.0 Hz, H-2′) correlated with the anomeric proton (H-Glc-1) in the NOE spectrum (Figure 1), and the anomeric

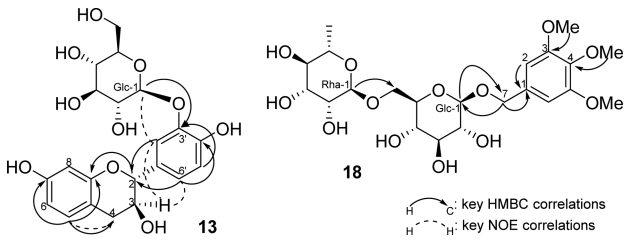

Figure 1. Key HMBC and NOE correlations of compounds **13** and **18**.

proton was long-range coupled with C-3′ in the HMBC spectrum (Figure 1). The *trans*-oriented C-2 phenyl and C-3 hydroxy groups were determined by the NOE correlation from H-3 to H-2′ and H-6′ (Figure 1) and the coupling constant of H-2 (δ_H 4.67, 1H, d, *J* = 7.5 Hz).[25,27] The negative Cotton effect around 280 nm in the ECD spectrum indicated that this compound was in the 2*R*,3*S*-configuration.[25,43] Therefore, **13** was established as fisetinidol 3′-*O*-*β*-D-glucopyranoside.

The NMR spectroscopic features of compounds **18** and nikoenoside (**19**)[30] were similar, indicating that **18** was a simple derivative of **19**. Thus, the 3,4,5-trimethoxybenzyl moiety was established by the resonances of a two-proton aromatic singlet (δ_H 6.75, 2H, s, H-2 and H-6), three methoxy protons (δ_H 3.84, 6H, s; 3.74, 3H, s), and two methylene protons (δ_H 4.82, 1H, d, *J* = 12.0 Hz, H-7; 4.61, 1H, d, *J* = 12.0 Hz, H-7). The Glc-6 carbon and proton resonances were shifted to lower field, as

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compared to those of **19**.[30] Five oxygenated and one methyl carbon resonance (δ_C 103.1, 72.3, 72.5, 74.1, 69.9, 18.1, C-Rha-1−6) were observed in the ^{13}C NMR spectra of **18**, but not in that of **19**. These carbons, the anomeric proton (δ_H 4.79, 1H, d, J = 1.5 Hz, H-Rha-1), and the HPLC sugar analysis[41] suggested the presence of an α-L-rhamnopyranosyl moiety. The anomeric H-Rha-1 resonance was long-range coupled with the Glc-6 carbon at δ_C 68.3 in the HMBC spectrum (Figure 1). The molecular formula of **18** was determined as $C_{22}H_{34}O_{13}$ based on HRFABMS (m/z 507.2090 $[M + H]^+$; calcd for $C_{22}H_{35}O_{13}$, 507.2078). This also indicated that **18** had an additional rhamnosyl moiety, as compared with **19**. From these data, the structure of **18** was assigned as 3,4,5-trimethoxybenzyl α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

The antioxidant activities of the isolated compounds were tested using the DPPH radical scavenging method and compared with a positive control, Trolox, which had an IC_{50} value of 28.2 μM. Flavonoids with either pyrogallol or catechol B-ring moieties, including flavonol glycosides (**1**−**3**, **6**−**10**), aglycones (**4**, **5**, **11**, **12**), and catechins (**14**, **16**, **17**), showed potent activity (IC_{50} 7.8−31.3 μM). The phenylpropanoid amides (**20**, IC_{50} 30.7 μM; **21**, IC_{50} 41.2 μM) also had radical scavenging activity. Thus, flavonol glycosides containing either hydrogen or β-D-glucopyranosyloxy moieties at C-8 and with a pyrogallol B-ring (**1**−**5**) showed significant radical scavenging activity.

The inhibitory effects of the compounds against the two types of phenoloxidase, insect phenoloxidase and mushroom tyrosinase, are shown in Table 1. Gallocatechin (**14**) and epigallocatechin (**16**) showed inhibitory activities against both insect phenoloxidase (IC_{50} 37.5 and 83.8 μM, respectively) and mushroom tyrosinase (IC_{50} 0.16 and 0.7 mM, respectively). For the other compounds, the structure−activity correlations in the insect phenoloxidase assay were simple and clear, as compared to those determined by the tyrosinase assay. Insect phenoloxidase was weakly inhibited by phenylpropanoid amides (**20**−**23**) (IC_{50} 92.4−291 μM), while mushroom tyrosinase was inhibited by the characteristic flavonol 3-*O*-rhamnopyranosides **1**−**3**, **6**−**8**, and **10** (IC_{50} 0.9−4.7 mM). Radical scavenging compounds such as thymol are considered to inhibit melanogenesis by inhibiting redox reactions, without any interaction with tyrosinase.[44] However, the differences in the inhibition of the two test enzymes suggested that these active compounds affected different stages in melanin production and that each compound may have direct effects on the enzyme. Furthermore, although the aglycones **4**, **5**, **11**, and **12** did not inhibit tyrosinase, almost all flavonol glycosides with a 3-*O*-rhamnopyranosyl moiety, **1**−**3**, **6**−**8**, and **10**, showed equivalent weak to moderate activities. This suggested that the 3-*O*-rhamnopyranosyl moiety in flavonols might make an important contribution to tyrosinase enzyme inhibition. *N*-Phenylthiourea, which was used as a positive control, is a competitive inhibitor of phenoloxidase[45] that strongly inhibits *A. pisum* phenoloxidase and also inhibits tyrosinase. Although an insect lepidopteran larval phenoloxidase was previously reported to be inhibited by kojic acid, which is an effective tyrosinase inhibitor,[46] the present study of *A. pisum* phenoloxidase found that gallocatechin, epigallocatechin, and phenylpropanoid amides showed higher activity than kojic acid.

A. frutescens contained additional flavonols with a pyrogallol B-ring moiety. These and many of the isolated flavonoids and phenylpropanoid amides had potent radical scavenging activities, which could protect plants and animals from oxidative stress and support their health. Some of the identified antioxidants also inhibited insect phenoloxidase and tyrosinase. These activities may produce both beneficial and detrimental effects. Investigation of plant inhibitors of insect phenoloxidase may improve the understanding of the interactions between plant and insect innate immune systems, and the novel tyrosinase inhibitors identified in the present study indicate that *A. frutescens* constituents may have pharmacological effects that are suitable for cosmetic applications.

Table 1. Activities of DPPH Radical Scavenging and Inhibition against Insect Phenoloxidase and Tyrosinase for Isolated Compounds from *Atraphaxis frutescens*

	DPPH radical scavenging activity	insect phenoloxidase inhibition activity	tyrosinase inhibition activity
compound	IC_{50} (μM) ± SEM[a]	IC_{50} (μM) ± SEM[b]	IC_{50} (mM) ± SEM[a]
1	26.2 ± 0.3	ND[d]	0.9 ± 0.02
2	12.9 ± 0.9	ND[d]	4.7 ± 0.5
3	9.9 ± 0.04		1.2 ± 0.03
4	13.6 ± 1.0	ND[d]	ND[e]
5	15.4 ± 0.3		ND[e]
6	23.2 ± 0.2	ND[d]	1.1 ± 0.07
7	19.8 ± 0.1	ND[d]	2.0 ± 0.03
8	9.5 ± 1.1	ND[d]	4.0 ± 0.06
9	16.0 ± 0.2	ND[d]	ND[e]
10	7.8 ± 0.1	ND[d]	2.2 ± 0.3
11	11.2 ± 1.1	ND[d]	ND[e]
12	13.9 ± 0.04	ND[d]	ND[e]
14	31.3 ± 1.2	37.5 ± 5.13	0.16 ± 0.002
15	138.8 ± 1.8	230 ± 0.04	2.2 ± 0.03
16	23.6 ± 0.2	83.8 ± 3.29	0.7 ± 0.002
17	15.0 ± 0.2	176 ± 2.51	ND[e]
18	74.1 ± 2.6	ND[d]	ND[e]
19	110.9 ± 0.4	ND[d]	ND[e]
20	30.7 ± 1.9	291 ± 7.77	ND[e]
21	41.2 ± 1.9	162 ± 8.39	ND[e]
22	72.1 ± 0.2	92.8 ± 2.86	4.3 ± 0.7
23	ND[c]	92.4 ± 4.53	ND[e]
Trolox	28.2 ± 0.3	ND[d]	0.7 ± 0.02
kojic acid	ND[c]	ND[d]	0.088 ± 0.002
N-phenylthiourea	ND[c]	0.058 ± 0.002	0.011 ± 0.002

[a]The treatments were replicated 3 times. [b]The treatments were replicated 4 times. [c]Not determined: IC_{50} value >200 μM. [d]Not determined: IC_{50} value >300 μM. [e]Not determined: IC_{50} value >5 mM.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured with a JASCO P-2300 polarimeter (JASCO, Tokyo, Japan). ECD spectra were recorded on a JASCO J-700 spectropolarimeter. UV spectra were recorded with a Shimadzu MPS-2450 (Shimadzu, Kyoto, Japan). ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded using a JEOL JNM-AL400 FT-NMR spectrometer (JEOL, Tokyo, Japan), and the chemical shifts were reported as δ values with tetramethylsilane as an internal standard at 30 °C (measured in methanol-d_4, DMSO-d_6, and acetone-d_6). Inverse-detected heteronuclear correlations were measured using HMQC (optimized for $^1J_{C-H}$ = 145 Hz) and HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulsed-field gradient. HRFABMS data were obtained using a JEOL JMS700 mass spectrometer, with a glycerol matrix. Preparative HPLC was performed using a JASCO

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2089 with UV detection at 210 nm, using the following columns: Ultra Pack ODS-SM-50C-M (Yamazen, Osaka, Japan, 37 × 100 mm), TSKgel ODS-120T (Tosoh, Tokyo, Japan, 21.5 × 300 mm), Cosmosil 5C_{18}-AR-II (Nacalai tesque, Kyoto, Japan, 20 mm × 250 mm), Mightysil RP-18 GP (Kanto Chemical, Tokyo, Japan, 10 mm × 250 mm), and Develosil C_{30}-UG-5 (Nomura Chemical, Aichi, Japan, 20 × 250 mm).

Plant Material. The aerial parts of *Atraphaxis frutescens* were collected in Dornogobi Province, Sainshand soum, on the way to Khamriin Hiid (N 47°37.349′; E 110°11.096′, at an altitude of 779 m), Mongolia, in September 2012. The plant material was identified by Dr. Ts. Jamsran, Department of Biology, School of Arts and Sciences, National University of Mongolia. Voucher specimens (31.4.4.12A) were deposited at the herbarium of the Laboratory of Bioorganic Chemistry and Pharmacognosy, National University of Mongolia.

Extraction and Isolation. The aerial parts of *A. frutescens* (480 g) were cut into small pieces and extracted with acetone−H_2O (4:1) (3 × 5 L) at 60 °C. The extract (40 g) was suspended in H_2O (1 L) and extracted with Et_2O (3 × 1 L). The aqueous layer extract (44 g) was passed through a porous polymer gel (Mitsubishi HP-20, 70 mm × 470 mm) column eluted with H_2O (fr. 1A, 17.8 g), MeOH−H_2O (1:1) (fr. 1B, 17.8 g), and MeOH (fr. 1C, 3.5 g). Fr. 1C was applied to the reversed-phase column using an ODS-packed column ODS-SM-50C-M (Yamazen, 37 mm × 100 mm) and eluted with MeOH−H_2O (2:3) (frs. 2A−2E) and MeOH−H_2O (1:1) (frs. 2F−2I). Fr. 2C was subjected to HPLC to yield compounds **7** (1.9 mg) [column, RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (11:29)], **10** (7.6 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (11:29)], **20** (9.3 mg), **21** (8.9 mg), **23** (1.7 mg), syringaresinol, (3.6 mg), dehydroconiferyl alcohol (5.3 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (3:7)], aromadendrin (1.0 mg), methyl syringate (0.4 mg) [RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (3:7)], and **22** (4.3 mg) [RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (1:4)]. Fr. 2E was subjected to HPLC to yield compounds **2** (19.5 mg), **3** (2.5 mg), **4** (64.7 mg), **5** (4.8 mg) [5C_{18}-AR-II, CH_3CN−H_2O containing 0.2% TFA (11:29)], and emodin 8-*O*-6′-*O*-malonylglucoside (3.0 mg) [RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (3:7)]. Fr. 2F was subjected to HPLC to yield compounds **6** (1.1 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (3:7)], **9** (2.1 mg), emodin 8-*O*-(6′-*O*-malonyl)glucoside (6.7 mg) [RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (3:7)], **12** (1.2 mg), torachrysone 8-*O*-*β*-D-(6′-*O*-malonyl)-glucopyranoside (0.7 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (7:13)], and emodin 8-*O*-*β*-D-glucopyranoside (12.0 mg) [ODS-120T, CH_3CN−H_2O containing 0.2% TFA (3:7)]. Fr. 2G was subjected to HPLC to yield compounds **9** (103.9 mg) [ODS-120T, CH_3CN−H_2O containing 0.2% TFA (3:7)], **10** (22.3 mg), and **11** (19.8 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (3:7)]. Fr. 1B was passed through a reversed-phase column using ODS (Cosmosil 140 C_{18}-OPN, Nacalai tesque, 60 × 100 mm) and eluted with MeOH−H_2O (1:4) (fr. 3A, 9.6 g and fr. 3B, 2.5 g), MeOH−H_2O (3:7) (fr. 3C, 0.8 g), MeOH−H_2O (2:3) (fr. 3D, 0.15 g), MeOH−H_2O (3:2) (fr. 3E, 0.2 g), and MeOH (fr. 3F, 90 mg). Fr. 3B was subjected to HPLC to yield compounds **1** (17.9 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (1:4)], **13** (1.2 mg), **19** (2.1 mg), fisetinidol (0.4 mg), 3,4,5-trimethoxyphenyl 1-*O*-*β*-D-glucopyranoside (0.8 mg) [RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (3:17)], **14** (9.4 mg), catechin (22.1 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (1:9)], **15** (4.8 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (3:7)], **16** (3.5 mg) [RP-18 GP, CH_3CN−H_2O containing 0.2% TFA (1:9)], and **17** (7.6 mg) [5C_{18}-AR-II, CH_3CN−H_2O containing 0.2% TFA (3:7)]. Fr. 3C was subjected to HPLC to yield compounds **8** (47.9 mg) and **18** (3.5 mg) [C_{30}-UG-5, CH_3CN−H_2O containing 0.2% TFA (3:7)].

8-β-D-Glucopyranosyloxy-3′,4′,5,5′-tetrahydroxy-7-methoxy-3-α-L-rhamnopyranosyloxyflavone (**1**): yellowish, amorphous solid; $[\alpha]^{22}_D$ −9 (*c* 0.1, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) *δ* 7.17 (1H, s, H-2′ and 6′), 6.47 (1H, s, H-6), 5.16 (1H, br s, H-Rha-1), 4.85 (overlapping, H-Glc-1), 4.20 (1H, dd, *J* = 3.0, 1.5 Hz, H-Rha-2), 3.92 (3H, s, 7-OMe), 3.87 (1H, dd, *J* = 9.5, 6.5 Hz, H-Rha-5), 3.84 (1H, dd, *J* = 9.5, 3.0 Hz, H-Rha-3), 3.76 (1H, dd, *J* = 12.0, 2.0 Hz, H-Glc-6), 3.70 (1H, dd, *J* = 12.0, 4.5 Hz, H-Glc-6), 3.57 (1H, dd, *J* = 9.5, 8.0 Hz, H-Glc-2), 3.47 (overlapping, H-Glc-3), 3.47 (overlapping, H-Glc-4), 3.38 (1H, t, *J* = 9.5 Hz, H-Rha-4), 3.24 (1H, m, H-Glc-5), 1.06 (3H, d, *J* = 6.5 Hz, H-Rha-6); ^{13}C NMR (methanol-d_4, 100 MHz) *δ* 180.0 (C-4), 159.6 (C-7), 159.5 (C-2), 159.0 (C-5), 150.1 (C-8a), 146.8 (C-4′), 138.2 (C-3′ and 5′), 136.5 (C-3), 126.6 (C-8), 121.8 (C-1′), 110.0 (C-2′ and 6′), 106.0 (C-4a), 105.8 (C-Glc-1), 104.1 (C-Rha-1), 96.5 (C-6), 78.2 (C-Glc-5), 77.8 (C-Glc-3), 75.7 (C-Glc-2), 73.4 (C-Rha-4), 72.1 (C-Rha-5), 72.0 (C-Rha-3), 71.9 (C-Rha-2), 71.2 (C-Glc-4), 62.4 (C-Glc-6), 57.1 (7-OMe), 17.7 (Rha-6); HRFABMS (positive) *m*/*z* 657.1682 $[M + H]^+$ (calcd for $C_{28}H_{33}O_{18}$, 657.1666).

8-Acetoxy-3′,4′,5,5′-tetrahydroxy-7-methoxy-3-α-L-rhamnopyranosyloxyflavone (**2**): yellowish, amorphous solid; $[\alpha]^{22}_D$ −85 [*c* 0.9, DMSO−MeOH (1:1)]; ^{1}H NMR (DMSO-d_6, 400 MHz) *δ* 12.56 (1H, s, 5-OH), 9.39 (2H, br s, 3′-OH and 5′-OH), 8.95 (1H, br s, 4′−OH), 6.86 (2H, s, H-2′ and 6′), 6.69 (1H, s, H-6), 5.21 (1H, s, H-Rha-1), 4.00 (1H, br s, H-Rha-2), 3.91 (3H, s, 7-OMe), 3.57 (1H, dd, *J* = 9.5, 3.0 Hz, H-Rha-3), 3.37 (overlapping, H-Rha-5), 3.17 (1H, m, H-Rha-4), 2.45 (3H, s, 8-OAc), 0.84 (3H, d, *J* = 6.5 Hz, H-Rha-6); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 177.8 (C-4), 168.4 [8-OAc (C═O)], 158.3 (C-5), 157.5 (C-2), 156.8 (C-7), 146.7 (C-8a), 145.8 (C-3′ and 5′), 136.8 (C-4′), 134.3 (C-3), 119.1 (C-1′), 118.4 (C-8), 107.6 (C-2′ and 6′), 103.9 (C-Rha-1), 101.9 (C-4a), 95.6 (C-6), 71.1 (C-Rha-4), 70.6 (C-Rha-5), 70.3 (C-Rha-3), 69.9 (C-Rha-2), 56.8 (7-OMe), 19.9 [8-OAc (CH_3)], 17.4 (C-Rha-6); HRFABMS (positive) *m*/*z* 537.1249 $[M + H]^+$ (calcd for $C_{24}H_{25}O_{14}$, 537.1244).

3′,4′,5,5′,8-Pentahydroxy-7-methoxy-3-α-L-rhamnopyranosyloxyflavone (**3**): yellowish, amorphous solid; $[\alpha]^{22}_D$ −126 [*c* 0.3, DMSO−MeOH (1:1)]; ^{1}H NMR (DMSO-d_6, 400 MHz) *δ* 12.22 (1H, s, 5-OH), 9.30 (2H, br s, 3′-OH and 5′-OH), 8.89 (1H, s, 4′-OH), 8.84 (1H, s, 8-OH), 6.98 (2H, s, H-2′ and 6′), 6.56 (1H, s, H-6), 5.22 (1H, d, *J* = 1.0 Hz, H-Rha-1), 4.00 (1H, dd, *J* = 3.0, 1.0 Hz, H-Rha-2), 3.91 (3H, s, 7-OMe), 3.57 (1H, m, H-Rha-3), 3.35 (overlapping, H-Rha-5), 3.16 (1H, m, H-Rha-4), 0.84 (3H, d, *J* = 6.5 Hz, H-Rha-6); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 178.2 (C-4), 157.7 (C-2), 153.8 (C-7), 152.6 (C-5), 145.6 (C-3′ and 5′), 143.6 (C-8a), 136.5 (C-4′), 133.8 (C-3), 126.0 (C-8), 119.6 (C-1′), 108.0 (C-2′ and 6′), 104.2 (C-4a), 104.2 (C-Rha-1), 95.3 (C-6), 71.2 (C-Rha-4), 70.5 (C-Rha-5), 70.3 (C-Rha-3), 69.9 (C-Rha-2), 56.3 (7-OMe), 17.4 (C-Rha-6); HRFABMS (positive) *m*/*z* 495.1159 $[M + H]^+$ (calcd for $C_{22}H_{23}O_{13}$, 495.1138).

8-Acetoxy-3,3′,4′,5,5′-pentahydroxy-7-methoxyflavone (**4**): yellowish, amorphous solid; ^{1}H NMR (DMSO-d_6, 400 MHz) *δ* 12.42 (1H, s, 5-OH), 9.60 (1H, s, 3-OH), 9.31 (2H, br s, 3′-OH and 5′-OH), 8.87 (1H, br s, 4′-OH), 7.18 (2H, s, H-2′ and 6′), 6.67 (1H, s, H-6), 3.90 (3H, s, 7-OMe), 2.45 (3H, s, 8-OAc); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 175.8 (C-4), 168.4 [8-OAc (C═O)], 157.7 (C-5), 156.4 (C-7), 147.1 (C-2), 146.4 (C-8a), 145.6 (C-3′ and 5′), 136.2 (C-4′), 136.0 (C-3), 120.4 (C-1′), 118.4 (C-8), 107.0 (C-2′ and 6′), 102.9 (C-4a), 94.9 (C-6), 56.7 (7-OMe), 20.0 [8-OAc (CH_3)]; HRFABMS (positive) *m*/*z* 391.0653 $[M + H]^+$ (calcd for $C_{18}H_{15}O_{10}$, 391.0665).

3,3′,4′,5,5′,8-Hexahydroxy-7-methoxyflavone (**5**): yellowish, amorphous solid; ^{1}H NMR (DMSO-d_6, 400 MHz) *δ* 12.04 (1H, s, 5-OH), 9.34 (1H, s, 3-OH), 9.26 (2H, s, 3′-OH and 5′), 8.83 (1H, br s, 4′-OH), 8.77 (1H, s, 8-OH), 7.35 (2H, s, H-2′ and 6′), 6.55 (1H, s, H-6), 3.91 (3H, s, 7-OMe); ^{13}C NMR (DMSO-d_6, 100 MHz) *δ* 176.2 (C-4), 153.2 (C-7), 152.0 (C-5), 147.1 (C-2), 145.5 (C-4′), 143.6 (C-8a), 135.9 (C3′ and 5′), 135.5 (C-3), 125.9 (C-8), 120.9 (C-1′), 107.3 (C-2′ and 6′), 103.2 (C-4a), 94.7 (C-6), 56.2 (7-OMe); HRFABMS (positive) *m*/*z* 349.0562 $[M + H]^+$ (calcd for $C_{16}H_{13}O_9$, 349.0559).

Fisetinidol 3′-O-β-D-glucopyranoside (**13**): colorless, amorphous solid; $[\alpha]^{22}_D$ −89 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log *ε*) 281 (4.06), 254 (3.68); ECD (*c* 0.000 17, MeOH) nm ([*θ*]) 216 (−25 000), 243 (+3700), 285 (−6000), 301 (+2300), 320 (+1400), 397 (+2600) nm; ^{1}H NMR (methanol-d_4, 400 MHz) *δ* 7.20 (1H, d, *J* = 2.0 Hz, H-2′), 6.96 (1H, dd, *J* = 8.5, 2.0 Hz, H-6′), 6.87 (1H, d, *J* = 8.0 Hz, H-5), 6.84 (1H, d, *J* = 8.5 Hz, H-5′), 6.35 (1H, dd, *J* = 8.0, 2.5 Hz, H-6), 6.27 (1H, d, *J* = 2.5 Hz, H-8), 4.79 (1H, d, *J* = 7.5 Hz, H-Glc-1), 4.67 (1H, d, *J* = 7.5 Hz, H-2), 4.04 (1H, m, H-3), 3.78 (1H, dd,

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J = 12.5, 2.5 Hz, H-Glc-6), 3.68 (1H, dd, J = 12.5, 5.0 Hz, H-Glc-6), 3.47 (1H, dd, J = 8.5, 7.5 Hz, H-Glc-2), 3.45 (overlapping, H-Glc-3), 3.41 (overlapping, H-Glc-4), 3.34 (overlapping, H-Glc-5), 2.89 (1H, dd, J = 16.0, 5.5 Hz, H-4α), 2.71 (1H, dd, J = 16.0, 8.5 Hz, H-4β); ^{13}C NMR (methanol-d_4, 100 MHz) δ 158.0 (C-7), 156.2 (C-8a), 148.2 (C-4′), 146.6 (C-3′), 132.4 (C-1′), 131.4 (C-5), 123.7 (C-6′), 117.3 (C-2′), 116.9 (C-5′), 112.7 (C-4a), 109.6 (C-6), 103.9 (C-Glc-1), 103.7 (C-8), 83.1 (C-2), 78.3 (C-Glc-5), 77.7 (C-Glc-3), 74.9 (C-Glc-2), 71.2 (C-Glc-4), 68.7 (C-3), 62.4 (C-Glc-6), 33.4 (C-4); HRFABMS (positive) m/z 459.1272 [M + Na]$^+$ (calcd for $C_{21}H_{24}O_{10}Na$, 459.1266).

3,4,5-Trimethoxybenzyl α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (18): yellowish, amorphous solid; $[\alpha]^{22}_D$ −28 (c 0.1, MeOH); ^{1}H NMR (methanol-d_4, 400 MHz) δ 6.75 (2H, s, H-2 and 6), 4.82 (1H, d, J = 12.0 Hz, H-7), 4.79 (1H, d, J = 1.5 Hz, H-Rha-1), 4.61 (1H, d, J = 12.0 Hz, H-7), 4.32 (1H, d, J = 7.5 Hz, H-Glc-1), 4.00 (1H, dd, J = 11.5, 1.5 Hz, H-Glc-6), 3.86 (1H, dd, J = 3.5, 1.5 Hz, H-Rha-2), 3.84 (6H, s, 3-OMe and 5-OMe), 3.74 (3H, s, 4-OMe), 3.69 (1H, dd, J = 9.5, 6.5 Hz, H-Rha-5), 3.66 (overlapping, H-Rha-3), 3.65 (1H, dd, J = 11.5, 6.5 Hz, H-Glc-6), 3.39 (1H, m, H-Rha-4), 3.37 (1H, m, H-Glc-5), 3.34 (1H, t, J = 9.0 Hz, H-Glc-3), 3.30 (overlapping, H-Glc-4), 3.25 (1H, dd, J = 9.0, 7.5 Hz, H-Glc-2), 1.27 (3H, d, J = 6.5 Hz, H-Rha-6); ^{13}C NMR (methanol-d_4, 100 MHz) δ 154.5 (C-3 and 5), 138.5 (C-4), 135.1 (C-1), 106.3 (C-2 and 6), 103.1 (C-Rha-1), 102.4 (C-Glc-1), 78.1 (C-Glc-3), 77.1 (C-Glc-5), 75.1 (C-Glc-2), 74.1 (C-Rha-4), 72.5 (C-Rha-3), 72.3 (C-Rha-2), 71.8 (C-7), 71.6 (C-Glc-4), 69.9 (C-Rha-5), 68.3 (C-Glc-6), 61.1 (4-OMe), 56.7 (3-OMe and 5-OMe), 18.1 (C-Rha-6); HRFABMS (positive) m/z 507.2090 [M + H]$^+$ (calcd for $C_{22}H_{35}O_{13}$, 507.2078).

Sugar Identification. Identification of glycosidic moieties was carried out as previously described.[41] Compounds **1** (4.3 mg), **2** (0.7 mg), **3** (0.9 mg), **13** (1.2 mg), and **18** (0.7 mg) were separately hydrolyzed with 7% HCl (1 mL) at 60 °C for 2 h. Each residue was stirred with *l*-cysteine methyl ester (5 mg) and *o*-tolyl isothiacyanate (10 μL) in pyridine (0.5 mL). The reaction mixtures were analyzed by HPLC [column, Cosmosil 5C$_{18}$-AR-II, 1.0 × 250 mm; mobile phase, CH_3CN−H_2O containing 0.2% TFA (1:4) at 1.0 mL/min; detector, 250 nm]. D-Glucose (t_R = 17.2 min) of **1**, **13**, and **18** and L-rhamnose (t_R = 28.5 min) of **1**−**3** and **18** were identified as their glycosidic moieties based on comparisons with authentic samples of D-glucose derivative (t_R = 17.2 min), L-glucose derivative (t_R = 15.6 min), and L-rhamnose derivative (t_R = 28.5 min).

Measurement of DPPH Radical Scavenging. The DPPH radical scavenging activity of the compounds was measured as described previously.[47] Briefly, solutions of each compound (0.1−200 μg/mL) were prepared in MeOH. These sample solutions (100 μL) were added to each well of a 96-well microplate containing 100 μL of 0.06 mM DPPH in MeOH and gently mixed for 30 min at room temperature. Optical density was measured at 510 nm using an ImmunoMini NJ-2300 microplate reader (Cosmo Bio., Ltd., USA), and the percentage inhibition was calculated. The IC$_{50}$ value expresses the concentration of sample required to scavenge 50% of the DPPH free radicals. All samples were analyzed in triplicate. Trolox (Wako Pure Chemical Ind., Ltd., Japan) was used as a positive control.

Phenoloxidase-Containing Crude Enzyme Solution from *A. pisum*. One pea aphid, *A. pisum*, was obtained from Tohoku Medical and Pharmaceutical University, Sendai, Miyagi, Japan. The aphid and its clone offspring were reared on pea sprouts of *Pisum sativum* (Murakami Farm, Hiroshima, Japan) cultured in a plant incubator (20 ± 2 °C, 16 h light:8 h dark photoperiod). Adults and the last instars of *A. pisum* (total 475 heads, 925.3 mg) were collected into microtubes; H_2O (23.1 mL) was added, and they were frozen and stored at −20 °C for 12 h. After thawing, pestle homogenates were prepared prior to centrifugation (7000 rpm, 5 min). The supernatant was filtered (0.22 μm, 25 mm, hydrophilic nylon, Starlab Scientific, China). To prevent phenoloxidase induction, the aphid innate immune system cascade was blocked using a selective Ca^{2+} chelating reagent, tetrapotassium *O*,*O*′-bis(2-aminophenyl)ethylene glycol-*N*,*N*,*N*′,*N*′-tetraacetate hydrate (Dojindo, Kumamoto, Japan), at a final concentration of 0.1 mM.

***A. pisum* Phenoloxidase Assay.** The phenoloxidase activity was assayed spectrophotometrically using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as the substrate. The assay medium, consisting of 40 μL of the above crude enzyme solution, 80 μL of 5 mM bis-Tris HCl buffer solution, and 40 μL of each sample solution containing 5% DMSO (final concentration of each compound: 1, 0.3, 0.1, 0.03, and 0.01 mM), was mixed in a well of a 96-well microplate and preincubated at 25 °C for 5 min. The reaction was initiated by the addition of 40 μL of L-DOPA solution (1 mM final concentration). Control wells included 5% DMSO, instead of the test sample. The plate was incubated at 25 °C, and the absorbance was measured at 510 nm after 120 and 240 min. The percentage inhibition of insect phenoloxidase was calculated as follows: Inhibition percent (%) = 1 − [(Sample Abs$_{510nm,240min}$ − Sample Abs$_{510nm,120min}$)/ (Control Abs$_{510nm,240min}$ − Control Abs$_{510nm,120min}$)] × 100. *N*-Phenylthiourea (final concentration: 0.3, 0.1, and 0.03 μM) was used as a positive control.

Enzymatic Assay of Tyrosinase. This assay was performed according to the procedure described by Mason et al.,[48] with slight modification. Tyrosinase activity was assayed spectrophotometrically using L-DOPA as the substrate. The assay medium, consisting of 40 μL of mushroom tyrosinase (Sigma-Aldrich Co. LLC, St. Louis, MO, USA) solution (150 units/mL), 80 μL of phosphate-buffered saline (1/15 mol/L, pH 6.8), and 40 μL of sample solution containing 5 mM DMSO (final concentration of each compound: 5, 1, 0.3, 0.1, 0.03, and 0.01 mM), was mixed in a 96-well microplate and preincubated at 25 °C for 5 min. The reaction was then initiated by the addition of 40 μL of L-DOPA solution (1 mM final concentration). Control wells (A) did not contain any test sample, and blank wells (B) contained heat-inactivated mushroom tyrosinase. The absorbance was measured at 510 nm after incubation for 10 min, giving the sample values (C). The percentage tyrosinase inhibition was calculated as follows: Inhibition percent (%) = 100 − [(A − C)/(A − B) × 100]. Kojic acid and *N*-phenylthiourea were used as positive controls.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.6b00720.

Tables S1 and S2 and the NMR spectroscopy data for **1**−**5**, **8**−**12**, **13**, and **18**, including ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}COSY, HMQC, and HMBC (PDF)

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Notes

The authors declare no competing financial interest.

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