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Dorsamin-A's, Glycerolipids Carrying a Dehydrophenylalanine Ester Moiety from the Seed-Eating Larvae of the Bruchid Beetle *Bruchidius dorsalis*

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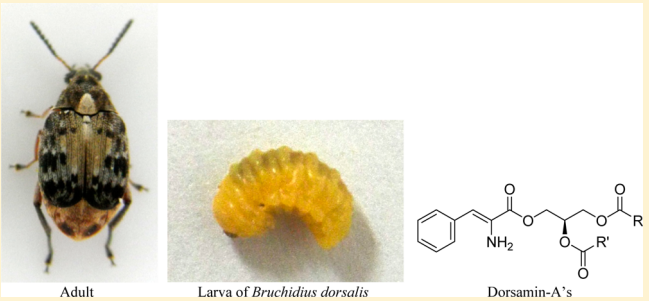
Supporting Information

ABSTRACT: Using a TLC autographic assay for radical-scavenging activity with the ABTS radical, the presence of lipophilic antioxidants in the larvae of the wild bruchid seed beetle *Bruchidius dorsalis* was detected. Assay-guided fractionation of the $CHCl_3$-soluble fraction of the larvae resulted in the isolation of new glycerolipids, designated dorsamin-A763, -A737, -A765, -A739, and -A767, comprising 1,2-diacyl-*sn*-glycero-3-dehydrophenylalanine ester structural units. The ABTS radical scavenging activity of the dorsamin-A's was comparable with or stronger than that of Trolox.



Excessive generation of reactive oxygen species (ROS) causes damage to proteins, nucleic acids, and lipids. Living organisms have some antioxidant defense systems for protection against ROS-induced oxidative damage. Phytophagous insect species are subject to oxidative stress caused by dietary pro-oxidants, extreme temperatures, and microbial infection as well as by endogenously generated ROS during metabolism.[1] For protection against oxidative stress, insect larvae possess antioxidant enzymes, such as superoxide dismutase and thioredoxin,[2−5] and also low-molecular-weight antioxidants such as ascorbic acid, tocopherols, and plasmalogens.[6,7]

Larvae of the wild bruchid seed beetle *Bruchidius dorsalis* infest dry mature seeds of the Japanese honey locust, *Gleditsia japonica*.[8,9] During our ongoing search for biologically active compounds from natural sources,[10−12] we observed that the $CHCl_3$ extract of *B. dorsalis* larvae exhibited radical-scavenging activity. Activity-directed fractionation of the $CHCl_3$ extract resulted in the isolation of new antioxidant lipids, designated dorsamin-A763 (**1**), -A737 (**2**), -A765 (**3**), -A739 (**4**), and -A767 (**5**). Here we describe the isolation and structure elucidation of the dorsamin-A's.

RESULTS AND DISCUSSION

The $CHCl_3$ extract of fourth instar *B. dorsalis* larvae was analyzed using silica gel TLC (Merck Kieselgel 60 F_{254}). After development and drying, the TLC plate was sprayed with an ABTS radical solution. Free radical scavenging constituents with an R_f value of 0.29 appeared as a pale yellow spot (spot "d") against a dark green background (Figure 1A). The constituents were more polar than triacylglycerols (spot "tg") (Figure 1B). The constituents gave a violet spot under UV light

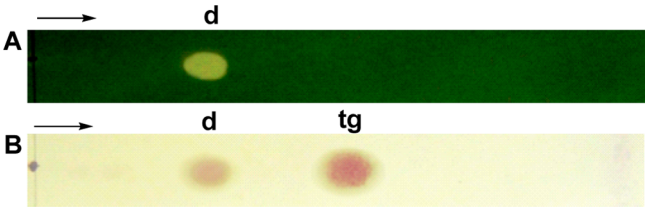


Figure 1. Analysis by silica gel TLC (EtOAc−*n*-hexane = 1:9 v/v) of the $CHCl_3$ extract of *Bruchidius dorsalis* after spraying with an ABTS radical solution (A) and treatment with vanillin−H_2SO_4 (B). The major spots were dorsamin-A's (d) and triacylglycerols (tg).

of 302 nm and a positive response to ninhydrin spray, suggesting the presence of conjugated double bonds and an amino group. The $CHCl_3$ extract was subjected to silica gel column chromatography, to afford ABTS radical scavenging compounds, designated dorsamin-A's. The 50% scavenging concentration (SC_{50}) value of the dorsamin-A's was 9.6 μg/mL. The IR spectrum showed the presence of NH_2, carbonyl, and phenyl groups. The ^1H and ^{13}C NMR spectra indicated that dorsamin-A's are glycerol derivatives with long-chain fatty acyl residues and a conjugated system containing a phenyl group. The COSY, HMQC, and HMBC spectra suggested that the conjugated system of dorsamin-A's constitutes a dehydrophenylalanine ester unit (Figure 2). This finding was supported by the fact that the ^1H and ^{13}C NMR chemical shifts of the dehydrophenylalanine ester unit were similar to those of synthetic dehydrophenylalanine methyl and ethyl esters.[13,14] The EIMS and CIMS spectra indicated that dorsamin-A's are

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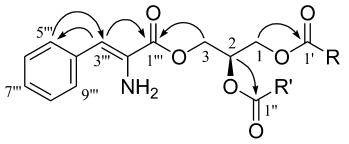

1: R, R' = $C_{17}H_{33}$, $C_{17}H_{31}$
2: R, R' = $C_{15}H_{31}$, $C_{17}H_{31}$
3: R = R' = $C_{17}H_{33}$
4: R, R' = $C_{15}H_{31}$, $C_{17}H_{33}$
5: R, R' = $C_{17}H_{35}$, $C_{17}H_{33}$

Figure 2. Structures of dorsamin-A's (**1**–**5**) and key HMBC correlations (arrows).

glycerol derivatives consisting of two long-chain fatty acyl residues and a single dehydrophenylalanine ester unit and have a range of molecular weights from 737 to 767. The dorsamin-A's were separated by ODS-HPLC to yield five compounds, **1**–**5** (Figure 3). The molecular weights of **1**–**5** were determined

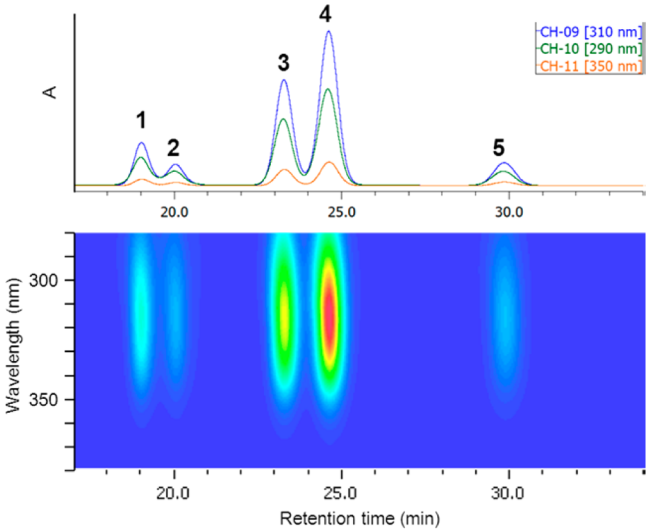

Figure 3. ODS-HPLC-PDA analysis (Inertsil PREP-ODS, CH_3CN–$CHCl_3$, 7:3 v/v) of dorsamin-A's.

to be 763, 737, 765, 739, and 767, respectively, on the basis of EIMS and (+)-CIMS data, and the compounds were therefore designated dorsamin-A763 (**1**), -A737 (**2**), -A765 (**3**), -A739 (**4**), and -A767 (**5**).

Using high-resolution APCITOFMS measurement of the $[M + H]^+$ ion at *m/z* 766.5986 (Δ +0.6 mmu), the molecular formula of dorsamin-A765 (**3**), $[\alpha]^{25}_D$ +61 (*c* 0.2, $CHCl_3$), was established as $C_{48}H_{79}NO_6$. The prominent EIMS fragment ion at *m/z* 265 indicated the presence of an oleoyl residue (Figure 4). The geometry of the olefinic bond in the oleoyl residue was established to be *cis* on the basis of the ^{13}C chemical shift of the allylic carbons (δ_C 27.3).[15] Consequently, the structure of **3** was

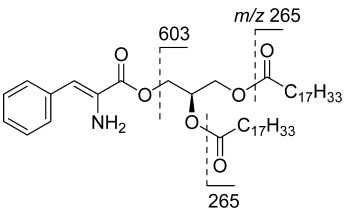

Figure 4. Structure of dorsamin-A765 (**3**) and EIMS fragmentations.

determined to be 1,2-dioleoylglycero-3-dehydrophenylalanine ester.

The UV and ^1H NMR spectra of dorsamin-A763 (**1**), -A737 (**2**), -A739 (**4**), and -A767 (**5**) were similar to those of **3**, indicating that the structures of **1**, **2**, **4**, and **5** were 1,2-diacylglycero-3-dehydrophenylalanine esters. The fatty acid composition was determined based on analysis of the fragment ions observed in the EIMS. The retention of dorsamin-A's on the ODS-HPLC column depended on the acyl carbon number and the number of double bonds, similar to that of triacylglycerols.[16,17] The fatty acid composition of **1**–**5** is summarized in Table 1.

Table 1. Fatty Acid Composition of 1–5

compound	MW	EIMS fragment ions	fatty acid composition
1	763	*m/z* 263, 265	C18:1–C18:2
2	737	*m/z* 239, 263	C16:0–C18:2
3	765	*m/z* 265	C18:1–C18:1
4	739	*m/z* 239, 265	C16:0–C18:1
5	767	*m/z* 265, 267	C18:0–C18:1

The similarity of the electronic circular dichroism (ECD) spectra of **1**–**5** indicated that they have the same absolute configuration, and therefore we used a mixture of the compounds for determination of the absolute configuration. The mixture was gradually decomposed in a $CHCl_3$ solution to yield 1,2-diacylglycerols. Such a selective hydrolysis of the dehydrophenylalanine ester bond in dorsamin-A's might be attributed to inductive effects of the α-amino substituent and the unsaturated bond.[18−21] The 1,2-diacylglycerols were converted into 1,2-diacylglycerol-3-*O*-TBDMS derivatives. The ECD spectrum of these derivatives ($\Delta\varepsilon_{220}$ −0.18) was in agreement with that of the 3-*O*-TBDMS derivative ($\Delta\varepsilon_{220}$ −0.14) derived from authentic 1,2-dioleoyl-*sn*-glycerol (Cayman Chemical), confirming the absolute configuration of dorsamin-A's to be *R*. Consequently, the structures of dorsamin-A's indicated that they were new 1,2-diacyl-*sn*-glycero-3-dehydrophenylalanine esters **1**–**5**.

Purified dorsamin-A763 (**1**), -A737 (**2**), -A765 (**3**), -A739 (**4**), and -A767 (**5**) exhibited ABTS radical scavenging activity, with 50% scavenging concentration (SC_{50}) values of 9.7–13.8 μM (Table 2). These values were comparable with or stronger than that of a vitamin E analogue, Trolox (SC_{50} = 19 μM). The SC_{50} values of dehydrophenylalanine methyl ester and methyl phenylpyruvate were 9.6 and 8.8 μM, respectively. On the contrary, methyl cinnamate showed no activity. Our findings indicate that the α-amino or α-hydroxy functionality attached

Table 2. ABTS Radical Scavenging Activity of 1–5 and Related Compounds

compound	SC_{50}[a] (μM)
1	12.1 ± 0.9
2	13.8 ± 0.4
3	11.0 ± 0.4
4	9.7 ± 0.6
5	10.5 ± 0.8
dehydrophenylalanine methyl ester	9.6 ± 0.3
methyl phenylpyruvate	8.8 ± 0.4
methyl cinnamate	>1000

[a]50% scavenging concentration.

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to an α,β-unsaturated ester skeleton plays an important role in ABTS radical scavenging activity.

A special class of ether lipids, plasmalogens (1-*O*-alk-1′-enyl 2-acyl glycerophospholipids), serves as endogenous antioxidants and have been found in several insect larvae, e.g., the tobacco budworm, *Heliothis virescens*,[22] mealworm beetle, *Tenebrio molitor*,[23] and silkworm, *Bombyx mori*.[7] Another type of glycerolipids, 1,2-diacyl-3-*O*-lysylglycerols, which contain an amino acid ester unit, have been isolated from *Mycobacterium phlei*, but their biological properties have not been described.[24] Several glycerolipidic cationic surfactants such as 1,2-diacyl-3-*O*-arginylglycerols have been widely used for DNA transfections into cells;[25,26] however, the antioxidant activity of these surfactants has not been reported.

We have shown that dorsamin-A's isolated from *B. dorsalis* larvae constitute a new type of endogenous antioxidant lipids. The structures were determined to be 1,2-diacylglycerol derivatives, carrying a 3-dehydrophenylalanine ester functionality. Several naturally occurring compounds with a dehydroamino acid unit have been reported; however, most of these compounds are dehydropeptides.[27−30] Dehydroamino acid residues of dehydropeptides have been shown to contribute to conformational stability and antimicrobial activity.[31] This is the first report of the isolation of glycerol derivatives carrying a dehydroamino acid ester unit. The ABTS radical scavenging activity of the dorsamin-A's was comparable with or stronger than that of Trolox. Similar to tocopherols and carotenoids, dorsamin-A's are lipophilic antioxidants. In contrast to the larvae of *B. dorsalis*, the adults contained minute quantities of dorsamin-A's (data not shown). Further experiments using biological systems are required to demonstrate the physiological role of dorsamin-A's in *B. dorsalis* larvae. However, we propose that they may play an important role in protecting cell membranes against oxidative stress caused by toxic oxygen radicals.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Horiba SEPA-300 polarimeter. UV and IR spectra were recorded on JASCO V-630 and Horiba FT720 spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on JEOL ECX500 (500 MHz for ^{1}H, 125 MHz for ^{13}C) and JEOL AL400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) at 25 °C. APCITOFMS and ESITOFMS were measured on a Shimadzu LCMS-IT-TOF mass spectrometer. EIMS and CIMS were measured on a Shimadzu GCMS-QP2010 Plus mass spectrometer. Electronic circular dichroism spectra were recorded on a JASCO J-820 spectropolarimeter. Column chromatography was carried out using silica gel (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) was carried out on precoated Kieselgel 60 F_{254} (Merck) and RP_{18} F_{254} plates (Merck). High-performance liquid chromatography (HPLC)-photodiode array (PDA) analyses were performed with an Inertsil PREP-ODS column (CH_3CN−$CHCl_3$, 7:3 v/v) on a JASCO LC-2000 instrument equipped with a JASCO MD-2015 multiwavelength detector. Phenylpyruvic acid, methyl cinnamate, and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Tokyo Kasei Industry Co. (Tokyo, Japan).

Insects. Fourth instar larvae of the wild bruchid seed beetle *Bruchidius dorsalis* were collected in Shiga Prefecture, Japan, in March 2010. Voucher specimens are kept at the Laboratory of Ecological Biochemistry, Graduate School of Biosphere Science, Hiroshima University.

Extraction, Isolation, and Characterization. The larvae (0.6 g) of *B. dorsalis* were homogenized and extracted with MeOH. After filtration, the residue was extracted with $CHCl_3$. The $CHCl_3$ extract (123 mg) was separated on a silica gel column (1 cm i.d. × 25 cm) employing EtOAc in *n*-hexane gradient mixtures [0:1, 1:49, 1:19, 1:9, 1:4, 1:2, 1:1, and 1:0 (10 mL of each solvent mixture)] to give eight fractions (A−H). Fraction D, which eluted with EtOAc−*n*-hexane (1:9), was a mixture of dorsamin-A's (9 mg).

Mixture of Dorsamin-A's: colorless oil; UV (*n*-hexane) λ_{max} ($E_{1\ cm}^{1\%}$) 309 (113) nm; IR (KBr) ν_{max} 3456, 3373 (N−H), 1743, 1716 (C=O), 1635 (C=C), 1593, 1508, and 1494 cm^{-1} (aromatic ring); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.86 (t, J = 6.4 Hz, CH_3), 1.19−1.35 (m, CH_2), 1.51−1.64 (m, H_2-3′ and H_2-3″, 1.95−2.12 (m, =C−CH_2−), 2.26−2.35 (m, H_2-2′ and H_2-2″), 2.73−2.77 (m, =C−CH_2−C=), 4.20 (dd, J = 12.1, 5.9 Hz, H-1a), 4.34 (dd, J = 12.1, 6.2 Hz, H-3a), 4.35 (dd, J = 12.1, 4.4 Hz, H-1b), 4.43 (dd, J = 12.1, 4.0 Hz, H-3b), 5.31 (m, olefinic H), 5.37 (m, H-2), 6.43 (s, H-3‴), 7.21 (t, J = 7.7 Hz, H-7‴), 7.36 (t, J = 7.7 Hz, H-6‴ and H-8‴), 7.42 (d, J = 7.7 Hz, H-5‴ and H-9‴); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.1 and 172.6 (C-1′ and C-1″), 165.1 (C-1‴), 135.8 (C-4‴), 131.5 (C-2‴), 129.9 and 129.6 (CH=CH), 128.7 (C-6‴ and C-8‴), 128.2 (C-5‴ and C-9‴), 126.9 (C-7‴), 109.8 (C-3‴), 68.8 (C-2), 63.5 (C-3), 62.1 (C-1), 34.3−22.8 (CH_2), 14.3 (CH_3).

Preparative HPLC of Dorsamin-A's. The dorsamin-A's (7 mg) were separated using ODS-HPLC (CH_3CN−$CHCl_3$, 7:3 v/v) to afford dorsamin-A763 (**1**; 0.3 mg), -A737 (**2**; 0.2 mg), -A765 (**3**; 2 mg), -A739 (**4**; 3 mg), and -A767 (**5**; 0.3 mg).

Dorsamin-A763 (1): colorless oil; UV (*n*-hexane) λ_{max} (log ε) 309 (4.24) nm; ECD (*n*-hexane) $\Delta\varepsilon_{260}$ −0.50, $\Delta\varepsilon_{310}$ +0.10; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.86 (t, J = 6.4 Hz, CH_3), 1.20−1.35 (m, CH_2), 1.51−1.63 (m, H_2-3′ and H_2-3″), 1.95−2.05 (m, =C−CH_2−), 2.29−2.34 (m, H_2-2′ and H_2-2″), 2.72−2.76 (m, =C−CH_2−C=), 4.20 (dd, J = 12.1, 5.9 Hz, H-1a), 4.34 (dd, J = 12.1, 6.2 Hz, H-3a), 4.35 (dd, J = 12.1, 4.4 Hz, H-1b), 4.42 (dd, J = 12.1, 4.0 Hz, H-3b), 5.29−5.35 (m, =CH), 5.37 (m, H-2), 6.43 (s, H-3‴), 7.21 (t, J = 7.7 Hz, H-7‴), 7.36 (t, J = 7.7 Hz, H-6‴ and H-8‴), 7.42 (d, J = 7.7 Hz, H-5‴ and H-9‴); EIMS (70 eV) m/z (rel int) 763 (4, M$^+$), 601 (24), 265 (17), 263 (20), 55 (100); CIMS (isobutane) m/z (rel int) 764 (39, [M + H]$^+$), 601 (100); HRAPCITOFMS m/z 764.5825 [M + H]$^+$ (calcd for $C_{48}H_{78}NO_6$, 764.5829).

Dorsamin-A737 (2): colorless oil; UV (*n*-hexane) λ_{max} (log ε) 308 (3.98) nm; ECD (*n*-hexane) $\Delta\varepsilon_{260}$ −0.35, $\Delta\varepsilon_{310}$ +0.15; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.86 (t, J = 6.4 Hz, CH_3), 1.19−1.35 (m, CH_2), 1.51−1.64 (m, H_2-3′ and H_2-3″), 1.96−2.04 (m, =C−CH_2−), 2.27−2.34 (m, H_2-2′ and H_2-2″), 2.72−2.77 (m, =C−CH_2−C=), 4.20 (dd, J = 12.1, 5.9 Hz, H-1a), 4.33 (dd, J = 12.1, 6.2 Hz, H-3a), 4.35 (dd, J = 12.1, 4.4 Hz, H-1b), 4.42 (dd, J = 12.1, 4.0 Hz, H-3b), 5.29−5.34 (m, =CH), 5.37 (m, H-2), 6.43 (s, H-3‴), 7.20 (t, J = 7.7 Hz, H-7‴), 7.36 (t, J = 7.7 Hz, H-6‴ and H-8‴), 7.42 (d, J = 7.7 Hz, H-5‴ and H-9‴); EIMS (70 eV) m/z (rel int) 737 (4, M$^+$), 575 (32), 263 (22), 239 (13), 55 (100); CIMS (isobutane) m/z (rel int) 738 (34, [M + H]$^+$), 575 (100); HRAPCITOFMS m/z 738.5670 [M + H]$^+$ (calcd for $C_{46}H_{76}NO_6$, 738.5673).

Dorsamin-A765 (3): colorless oil; $[\alpha]^{25}_D$ +61 (c 0.2, $CHCl_3$); UV (*n*-hexane) λ_{max} (log ε) 310 (4.02) nm; ECD (*n*-hexane) $\Delta\varepsilon_{260}$ −0.30, $\Delta\varepsilon_{310}$ +0.25; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.85 (t, J = 6.4 Hz, CH_3), 1.20−1.32 (m, CH_2), 1.53−1.64 (m, H_2-3′ and H_2-3″), 1.94−2.01 (m, =C−CH_2−), 2.24−2.34 (m, H_2-2′ and H_2-2″), 4.19 (dd, J = 12.1, 5.9 Hz, H-1a), 4.33 (dd, J = 12.1, 6.2 Hz, H-3a), 4.35 (dd, J = 12.1, 4.4 Hz, H-1b), 4.42 (dd, J = 12.1, 4.0 Hz, H-3b), 5.29−5.34 (m, =CH), 5.36 (m, H-2), 6.43 (s, H-3‴), 7.21 (t, J = 7.7 Hz, H-7‴), 7.36 (t, J = 7.7 Hz, H-6‴ and H-8‴), 7.42 (d, J = 7.7 Hz, H-5‴ and H-9‴); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 173.2 and 173.1 (C-1′ and C-1″), 165.1 (C-1‴), 135.8 (C-4‴), 131.8 (C-2‴), 129.9 and 129.6 (CH=CH), 128.7 (C-6‴ and C-8‴), 128.2 (C-5‴ and C-9‴), 126.9 (C-7‴), 109.8 (C-3‴), 68.8 (C-2), 63.5 (C-3), 62.1 (C-1), 34.3 and 34.2 (C-2′ and C-2″), 32.0 (C-16′ and C-16″), 29.9−29.2 (CH_2), 27.3 (allylic CH_2), 25.1 (C-3′ and C-3″),, 22.8 (C-17′ and C-17″), 14.3 (C-18′ and C-18″); EIMS (70 eV) m/z (rel int) 765 (5, M$^+$), 603 (100), 265 (20), 55 (80); CIMS (isobutane) m/z (rel int) 766 (57, [M + H]$^+$), 603 (100); HRAPCITOFMS m/z 766.5986 [M + H]$^+$ (calcd for $C_{48}H_{80}NO_6$, 766.5980).

Dorsamin-A739 (4): colorless oil; $[\alpha]^{25}_D$ +38 (c 0.3, $CHCl_3$); UV (*n*-hexane) λ_{max} (log ε) 310 (4.24) nm; ECD (*n*-hexane) $\Delta\varepsilon_{260}$ −0.45,

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$\Delta\varepsilon_{310}$ +0.30; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.86 (t, J = 6.4 Hz, CH_3), 1.18−1.35 (m, CH_2), 1.50−1.63 (m, H_2-3′ and H_2-3″), 1.94−2.02 (m, =C−CH_2−), 2.26−2.35 (m, H_2-2′ and H_2-2″), 4.20 (dd, J = 12.1, 5.9 Hz, H-1a), 4.34 (dd, J = 12.1, 6.2 Hz, H-3a), 4.35 (dd, J = 12.1, 4.4 Hz, H-1b), 4.43 (dd, J = 12.1, 4.0 Hz, H-3b), 5.29−5.34 (m, =CH), 5.36 (m, H-2), 6.43 (s, H-3‴), 7.21 (t, J = 7.7 Hz, H-7‴), 7.36 (t, J = 7.7 Hz, H-6‴ and H-8‴), 7.42 (d, J = 7.7 Hz, H-5‴ and H-9‴); EIMS (70 eV) m/z (rel int) 739 (6, M^+), 577 (100), 265 (8), 239 (10), 55 (60); CIMS (isobutane) m/z (rel int) 740 (67, $[M + H]^+$), 577 (100); HRAPCITOFMS m/z 740.5829 $[M + H]^+$ (calcd for $C_{46}H_{78}NO_6$, 740.5829).

Dorsamin-A767 (5): colorless oil; UV (*n*-hexane) λ_{max} (log ε) 309 (3.81) nm; ECD (*n*-hexane) $\Delta\varepsilon_{260}$ −0.10, $\Delta\varepsilon_{310}$ +0.15; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 0.86 (t, J = 6.4 Hz, CH_3), 1.19−1.35 (m, CH_2), 1.51−1.64 (m, H_2-3′ and H_2-3″), 1.95−2.02 (m, =C−CH_2−), 2.26−2.35 (m, H_2-2′ and H_2-2″), 4.20 (dd, J = 12.1, 5.9 Hz, H-1a), 4.34 (dd, J = 12.1, 6.2 Hz, H-3a), 4.35 (dd, J = 12.1, 4.4 Hz, H-1b), 4.43 (dd, J = 12.1, 4.0 Hz, H-3b), 5.29−5.34 (m, =CH), 5.37 (m, H-2), 6.43 (s, H-3‴), 7.21 (t, J = 7.7 Hz, H-7‴), 7.36 (t, J = 7.7 Hz, H-6‴ and H-8‴), 7.42 (d, J = 7.7 Hz, H-5‴ and H-9‴); EIMS (70 eV) m/z (rel int) 767 (4, M^+), 605 (100), 267 (10), 265 (10), 55 (90); CIMS (isobutane) m/z (rel int) 768 (45, $[M + H]^+$), 605 (100); HRAPCITOFMS m/z 768.6142 $[M + H]^+$ (calcd for $C_{48}H_{82}NO_6$, 768.6142).

Conversion of Dorsamin-A's into TBDMS Derivatives of 1,2-Diacylglycerol. Treatment of the dorsamin-A's (2 mg) with $CHCl_3$ (0.6 mL) at room temperature for 1 month gave a degradation mixture. The mixture was subjected to silica gel column chromatography (0.6 cm i.d. × 10 cm) using EtOAc in *n*-hexane gradient mixtures [0:1, 1:9, 1:4, 1:2, and 1:1 (3 mL of each solvent mixture)] to give five fractions. The fraction that eluted with EtOAc−*n*-hexane (1:2) was a mixture of 1,2-diacylglycerols. After drying, the fraction was dissolved in Et_3N (0.5 mL), and DMAP (10 mg) and TBDMS chloride (10 mg) were added. The mixture was stirred at 25 °C for 1 h to afford a crude product, which was purified by silica gel column chromatography (0.6 cm i.d. × 10 cm) using EtOAc in *n*-hexane gradient mixtures [0:1, 1:49, 1:19, 1:9, and 1:2 (3 mL of each solvent mixture)] to give five fractions. The fraction that eluted with EtOAc−*n*-hexane (1:19) was TBDMS derivatives of 1,2-diacylglycerol (1 mg). The structure was confirmed by NMR and MS. The ECD spectra of the derivative showed a negative Cotton effect ($\Delta\varepsilon_{220}$ −0.18). The TBDMS derivative of the authentic 1,2-dioleoyl-*sn*-glycerol (Catalog No. 62230, Cayman Chemical, Ann Arbor, MI, USA) was prepared in a similar manner and also showed a negative Cotton effect ($\Delta\varepsilon_{220}$ −0.14).

Preparation of Dehydrophenylalanine Methyl Ester. A solution of phenylpyruvic acid (1.6 g), benzyl carbamate (1.5 g), and *p*-toluenesulfonic acid monohydrate (1.0 g) in dry benzene (100 mL) was refluxed for ca. 4 h, after which the theoretical amount of water produced was separated out by trapping over anhydrous Na_2SO_4.[29] The reaction mixture was reduced to 20 mL by evaporating the benzene and was allowed to stand at room temperature overnight. The precipitate was filtered to yield *N*-benzyloxycarbonyldehydrophenylalanine as colorless needles (1.8 g). MeOH (1 mL) was added to a solution of *N*-benzyloxycarbonyldehydrophenylalanine (0.5 g), DMAP (0.3 g), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (0.5 g) in anhydrous CH_2Cl_2 (2 mL). The mixture was stirred at room temperature for 15 h and subjected to silica gel column chromatography (1.5 cm i.d. × 25 cm) using 10% EtOAc in *n*-hexane, to yield dehydrophenylalanine methyl ester (7 mg).

Dehydrophenylalanine methyl ester: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.85 (3H, s, OCH_3), 4.20 (2H, br s, NH_2), 6.46 (1H, s, H-3), 7.20 (1H, t, J = 7.7 Hz, H-7), 7.36 (2H, t, J = 7.7 Hz, H-6 and H-8), 7.43 (2H, d, J = 7.7 Hz, H-5 and H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.2 (C-1), 136.1 (C-4), 132.1 (C-2), 128.7 (C-6 and C-8), 128.3 (C-5 and C-9), 126.7 (C-7), 109.2 (C-3), 52.6 (OCH_3); HRESITOFMS m/z 178.0864 $[M + H]^+$ (calcd for $C_{10}H_{12}NO_2$, 178.0863).

Preparation of Methyl Phenylpyruvate. To a solution of phenylpyruvic acid (5 mg) in MeOH (1 mL) was added a solution of trimethylsilyldiazomethane in *n*-hexane (1 mL) at 0 °C. After standing for 5 min, the mixture was concentrated under reduced pressure to afford a crude product. The crude product was purified by silica gel column chromatography (EtOAc−*n*-hexane, 1:9 v/v), to afford methyl phenylpyruvate (3 mg).

Methyl phenylpyruvate: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 3.91 (3H, s, OCH_3), 6.40 (1H, d, J = 1.8 Hz, OH), 6.51 (1H, br d, J = 1.8 Hz, H-3), 7.26 (1H, t, J = 7.7 Hz, H-7), 7.36 (2H, t, J = 7.7 Hz, H-6 and H-8), 7.74 (2H, d, J = 7.7 Hz, H-5 and H-9); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.6 (C-1), 138.9 (C-4), 134.1 (C-2), 129.8 (C-6 and C-8), 128.4 (C-5 and C-9), 127.9 (C-7), 111.1 (C-3), 53.3 (OCH_3); HRESITOFMS m/z 177.0551 $[M - H]^-$ (calcd for $C_{10}H_9O_3$, 177.0552).

ABTS Radical Scavenging Activity Assay. The assay was based on the method of Re et al.,[32] with slight modification. A stable stock solution of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical was prepared by mixing equal volumes of aqueous solutions of 14 mM ABTS and 4.9 mM potassium persulfate for 16 h in the dark at room temperature. The ABTS radical solution was diluted with EtOH to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at room temperature. After addition of 1.0 mL of diluted ABTS radical solution to 10 μL of sample in EtOH, the absorbance at 734 nm was recorded in the dark at room temperature for 6 min. Trolox (SC_{50} = 19 ± 2 μM) was used as a standard.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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Notes

The authors declare no competing financial interest.

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