Structures of New Flavonoids, Erycibenins D, E, and F, and NO Production Inhibitors from *Erycibe expansa* Originating in Thailand

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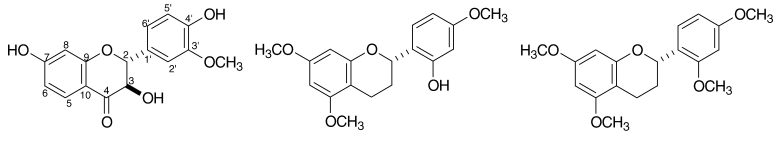
A new flavanol, erycibenin D, and two new flavans, erycibenins E and F, were isolated from the stems of *Erycibe expansa* originating in Thailand. The structures of new flavonoids were elucidated on the basis of chemical and physicochemical evidence. In addition, the inhibitory activities of the isolated constituents from *E. expansa* on lipopolysaccharide-activated nitric oxide production in mouse peritoneal macrophages were examined. Among the principal constituents, two isoflavones, clycosin (IC_{50}=13 μM) and erythrinin B (18 μM), and two rotenoids, deguelin (26 μM) and rotenone (27 μM), were found to show potent inhibitory activity.

Key words *Erycibe expansa*; erycibenin; flavanol; flavan; nitric oxide inhibitor; rotenoid

The Convolvulaceae plant, *Erycibe expansa*, is widely distributed in the Southeast Asian countries and the stems of *E. expansa* have been used for the treatment of hepatitis, hepatic dysfunction, and hepatosis in Thailand traditional medicine.[1)] We have reported bioactive constituents from several natural medicines originating in Thailand such as *Albizia myriophylla*,[2)] *Alpinia galanga*,[3—6)] *Salacia chinensis*,[7—9)] *Piper chaba*,[10)] and Thai Zedoary.[11)] In the course of our characterization studies on the bioactive constituents from Thai natural medicines, we previously reported three new flavonoids, erycibenins A (**4**), B (**5**), and C (**6**), from the methanolic extract of the dried stems of *E. expansa* together with 17 constituents (**7**—**23**).[1)] As a continuing study of this natural medicine, we additionally isolated a new flavanol, erycibenin D (**1**), and two new flavans, erycibenins E (**2**) and F (**3**), together with 14 compounds. This paper deals with the isolation and stereostructure elucidation of three new flavonoids and the effects of principal constituents on lipopolysaccharide (LPS)-activated nitric oxide (NO) production in mouse peritoneal macrophages.

The methanolic extract from the dried stems of *E. expansa* was partitioned into a mixture of ethyl acetate (EtOAc) and water to provide the EtOAc-soluble portion and H_2O-soluble portion as previously described.[1)] The EtOAc-soluble portion was further subjected to silica gel and ODS column chromatographies and finally HPLC to give erycibenins D (**1**, 0.00009% from the natural medicine), E (**2**, 0.00026%), and F (**3**, 0.00027%) together with two flavans, 5,7-dimethoxy-4′-hydroxyflavan[12)] (**24**, 0.00028%), 5,7,4′-trimethoxyflavan[13)] (**25**, 0.00018%), a flavone, luteolin[14)] (**26**, 0.00048%), a flavanone, eriodictyol[15)] (**27**, 0.00055%), four rotenoids, deguelin[16)] (**28**, 0.0027%), tephrosin[16)] (**29**, 0.0020%), rotenone[17)] (**30**, 0.0062%), and 12a-hydroxyrotenone[18)] (**31**, 0.0019%), an aromatic compound, lasiodiplodin[19)] (**32**, 0.00014%), three sterols, β-sitosterol[20)] (0.0051%), β-sitosterol 3-*O*-β-D-glucopyranoside[21)] (0.012%), and 5α,8α-epidioxyergosta-6,22-diene-3β-ol[22)] (0.00066%), and two fatty acids, oleic acid[23)] (0.0040%) and linoleic acid[23)] (0.026%).

Stereostructures of Erycibenins D (1), E (2), and F (3) Erycibenin D (**1**) was isolated as a pale yellow powder with negative optical rotation ($[\alpha]_D^{27}$ −11.0°). In the UV spectrum of **1**, the absorption maxima were observed at 234 (log ε 4.19), 278 (4.11), and 311 (3.78) nm. The IR spectrum of **1** showed absorption bands at 3630, 3420, 1676, 1597, and 1034 cm^{-1} ascribable to hydroxyl, carbonyl, and ether functions and aromatic ring. In the electron impact (EI)-MS of **1**, molecular and fragment ion peaks were observed at *m*/*z* 302 (M^+) and 137 (base peak). The molecular formula $C_{16}H_{14}O_6$ of **1** was determined by high-resolution EI-MS measurement. The ^{1}H- (acetone-d_6) and ^{13}C-NMR (Table 1) spectra[24)] of **1** showed signals assignable to a methoxyl group [δ 3.89 (3H, s, 3′-OCH_3)], two methines bearing an oxygen function [δ 4.61, 5.05 (1H each, both d, *J*=11.9 Hz, 3,2-H)], and six aromatic protons [δ 6.41 (1H, d, *J*=2.2 Hz, 8-H), 6.63 (1H, dd, *J*=2.2, 8.6 Hz, 6-H), 6.88 (1H, d, *J*=8.3 Hz, 5′-H), 7.05 (1H, dd, *J*=1.9, 8.3 Hz, 6′-H), 7.24 (1H, d, *J*=1.9 Hz, 2′-H), 7.73 (1H, d, *J*=8.6 Hz, 5-H)]. The ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) experiment on **1** indicated the presence of three partial structures written in the bold lines (C-2–C-3, C-5–C-6, C-5′–C-6′) (Fig. 1). In the heteronuclear multiple bond correlations (HMBC) experiment of **1**, long-range correlations were observed between the following proton and carbon pairs (H-2 and C-3, 4, 1′, 2′, 6′; H-3 and C-4; H-5 and C-4, 9; H-6 and C-7, 10; H-8 and C-7, 9, 10; H-2′ and C-2, 4′; H-5′ and C-3′; H-6′ and C-2, 4′; 3′-O$\underline{C}H_3$ and C-3′). Next, the relative stereostructure of **1** was elucidated using nuclear Overhauser enhancement spectroscopy (NOESY), which showed NOE correlations between the fol-



Erycibenin D (**1**) Erycibenin E (**2**) Erycibenin F (**3**)

Chart 1

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lowing proton pairs (H-3 and H-2′, 6′; 3′-OC$\underline{H}_3$ and H-2′). Finally, the absolute configuration of **1** was determined on the basis of circular dicronic (CD) spectroscopic analysis. Thus, the CD spectra of **1** showed negative Cotton effect [303 ($\Delta\varepsilon$ −5.23) nm], which indicated the absolute configuration of the 2 and 3-positions to be 2*R* and 3*R* orientation, respectively.[25)]

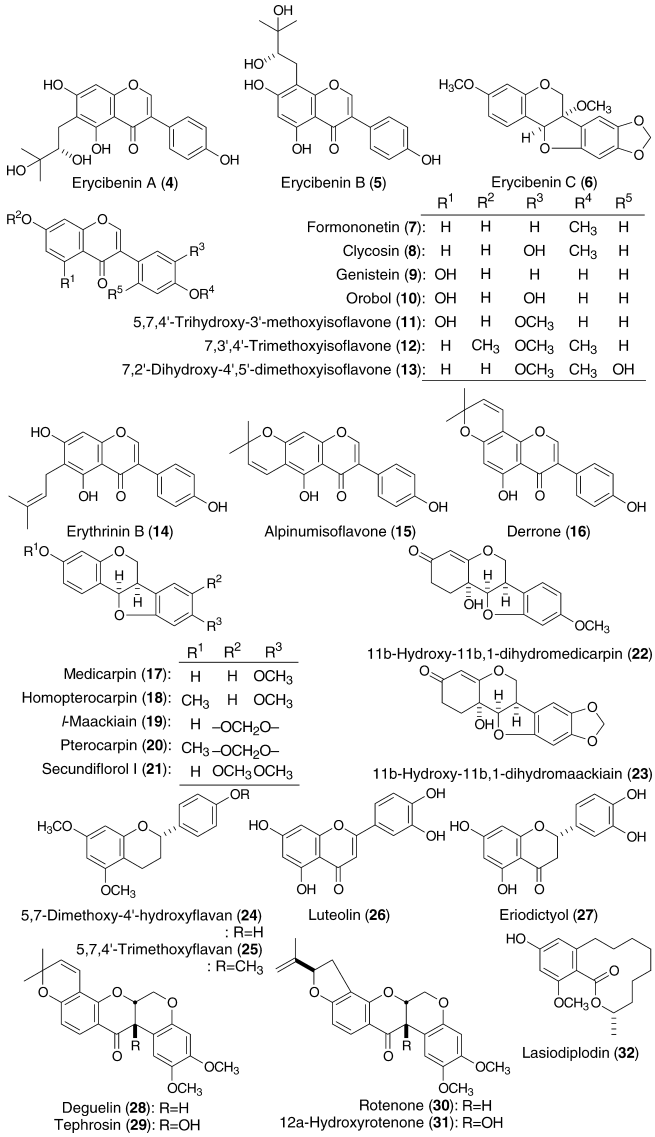

Chart 2

Erycibenins E (**2**) and F (**3**) were also obtained as a pale yellow powder with negative optical rotation (**2**: $[\alpha]_D^{26}$ −26.2°; **3**: $[\alpha]_D^{29}$ −21.2°). The molecular formulas of **2** and **3** were determined from the EI-MS and by high-resolution EI-MS analysis to be $C_{18}H_{20}O_5$ and $C_{19}H_{22}O_5$, respectively. The UV spectra of **2** and **3** showed similar absorption maxima [**2**: 256 ($\log\varepsilon$ 3.49), 278 (3.64); **3**: 255 (3.29), 277 (3.38) nm]. The IR spectrum of **2** showed absorption bands at 3450, 1620, 1593, 1497, and 1073 cm^{-1} assignable to hydroxyl and ether functions and aromatic ring, while the IR spectrum of **3** showed at 1590, 1509, 1210, and 1073 cm^{-1} assignable to aromatic ring and ether function. The ^{1}H- (acetone-d_6) and ^{13}C-NMR (Table 1) spectra[24)] of **2** showed signals assignable to two methylenes [δ 1.89, 2.18 (1H each, both m, 3-H_2), 2.63 (2H, m, 4-H_2)], a methine [δ 5.25 (1H, dd, J=2.1, 10.1 Hz, 2-H)], and five aromatic protons [δ 6.05 (1H, d, J=2.4 Hz, 8-H), 6.11 (1H, d, J=2.4 Hz, 6-H), 6.46 (1H, dd, J=2.4, 8.3 Hz, 5′-H), 6.48 (1H, d, J=2.4 Hz, 3′-H), 7.26 (1H, d, J=8.3 Hz, 6′-H)] together with three methoxyl groups [δ 3.74, 3.75, 3.79 (3H each, all s, 7, 4′, 5-OCH_3)]. The planar structure of **2** was confirmed by ^{1}H–^{1}H COSY and HMBC experiments. As shown in Fig. 1, the ^{1}H–^{1}H

Table 1. ^{13}C-NMR Data for Erycibenins D—F (**1**—**3**)

	1	**2**	**3**
C-2	85.2	73.5	72.9
C-3	73.9	28.9	29.1
C-4	193.2	20.1	20.1
C-5	129.8	158.4	159.5
C-6	111.8	91.7	91.7
C-7	166.0	160.4	160.5
C-8	103.7	94.4	94.4
C-9	164.5	157.7	157.7
C-10	113.0	104.0	104.0
C-1′	129.9	121.6	123.3
C-2′	112.4	155.8	158.1
C-3′	148.2	102.3	99.0
C-4′	148.1	161.1	161.5
C-5′	115.5	105.7	105.5
C-6′	122.2	128.3	128.0
5-OCH_3		55.8	55.8
7-OCH_3		55.5	55.5
2′-OCH_3			55.9
3′-OCH_3	56.4		
4′-OCH_3		55.4	55.6

Measured in acetone-d_6 at 125 MHz.

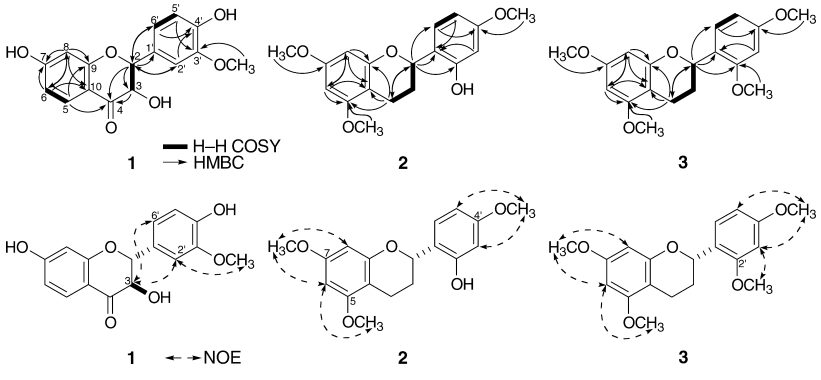

Fig. 1

Table 2. Inhibitory Effects of Constituents from *E. expansa* on NO Production in LPS-Activated Mouse Peritoneal Macrophages

	Inhibition (%)						IC_{50} (μM)
	0 μM	1 μM	3 μM	10 μM	30 μM	100 μM	
Formononetin (**7**)	0.0±3.7	−11.0±6.6	2.0±7.0	−4.7±8.2	22.9±3.9*	64.7±0.9*,#	
Clycosin (**8**)	0.0±7.0	7.0±1.8	12.3±4.0	38.0±2.1**	75.0±0.9**	96.6±0.4**	13
Genistein (**9**)	0.0±1.1	9.8±3.7*	4.7±1.0	14.2±1.9**	44.5±2.2**	82.4±1.2**	49
Orobol (**10**)	0.0±3.2	1.8±4.1	4.1±3.3	15.0±2.7*	42.7±3.4**	88.6±0.9**	44
11	0.0±3.3	−11.0±1.8	−4.8±4.5	2.4±2.4	44.3±0.9**	85.0±1.5**	37
Erythrinin B (**14**)	0.0±2.6	0.8±2.2	8.1±2.7*	13.3±0.6**	91.7±0.4**	100.6±0.3**,#	18
Medicarpin (**17**)	0.0±2.3	−0.6±2.0	−1.0±1.8	−1.7±3.1	20.7±2.3**	91.7±2.1**	49
Homopterocarpin (**18**)	0.0±3.0	−3.1±4.4	1.0±1.0	−6.9±2.9	−5.7±1.0	2.5±1.0	
l-Maackiain (**19**)	0.0±3.1	12.2±2.4	10.6±4.8*	13.5±2.6*	22.0±1.6**	53.2±1.4**	89
Secundiflorol I (**21**)	0.0±1.6	3.8±2.1	−0.8±1.5	0.9±2.3	22.6±3.9**	93.1±2.5**	48
24	0.0±3.6	−3.7±2.4	−0.8±3.3	9.5±3.2	30.8±2.4**	101.9±0.6**,#	
5,7,4′-Trimethoxyflavan (**25**)	0.0±2.9	0.5±1.1	4.5±2.6	3.4±1.8	14.6±3.0**	68.4±1.6**	66
Eriodictyol (**27**)	0.0±0.3	−2.8±1.5	−4.4±0.9	−6.0±1.1	5.4±0.8	78.8±0.6**	62
Deguelin (**28**)	0.0±6.4	5.8±1.9	−4.1±6.7	14.3±7.2	55.8±1.1**	100.8±0.3**,#	26
Tephrosin (**29**)	0.0±4.4	−5.1±1.0	−2.2±2.0	3.3±3.0	46.5±1.9**	100.5±0.3**,#	
Rotenone (**30**)	0.0±1.1	−2.8±1.1	2.3±1.6	13.8±1.5**	46.4±2.6**	99.5±0.1**	27
12a-Hydroxyrotenone (**31**)	0.0±6.1	−28.2±3.7	−27.1±1.1	−18.1±0.8	−7.6±4.4	99.9±0.1**,#	
Lasiodiplodin (**32**)	0.0±2.4	2.3±1.6	1.3±1.5	5.8±3.5	30.8±2.6**	100.4±0.1**,#	
β-Sitosterol	0.0±1.2	1.3±1.0	1.3±1.1	1.3±0.7	4.5±0.7	5.9±1.2	
β-Sitosterol 3-*O*-Glc	0.0±1.4	0.2±1.3	−0.4±0.5	0.4±0.3	1.1±0.9	2.2±0.7	
5α,8α-Epoxyergosta-6,22-diene-3β-ol	0.0±2.7	3.3±1.3	1.2±0.8	−1.5±0.3	0.9±1.2	74.0±0.9**,#	
Oleic acid	0.0±5.2	−2.8±0.5	−4.4±1.7	−2.8±0.6	3.2±1.3	12.7±0.9**	
Linoleic acid	0.0±3.8	0.1±5.0	−0.6±9.0	5.0±3.7	3.3±2.4	17.1±6.4	
L-NMMA[56]	0.0±4.0	5.9±0.9	10.3±3.7	15.0±1.6**	34.1±3.2**	63.1±1.2**	57
CAPE[56]	0.0±0.7	3.8±0.1	1.4±0.1	68.2±0.0**	93.7±0.2**	99.6±0.0**,#	15
GED[56]	0.0±0.0	6.2±0.1	24.4±0.1**	57.9±0.1**	89.7±0.2**	97.9±0.0**	7.4

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, * $p<0.05$, ** $p<0.01$. # Cytotoxic effect was observed.

COSY experiment on **2** indicated the presence of two partial structures written in the bold lines (C-2–C-4, C-5′–C-6′), while long-range correlations in the HMBC experiment on **2** were observed between the following proton and carbon pairs (H-2 and C-4, 1′, 2′, 6′; H-4 and C-2, 5, 9, 10; H-6 and C-5, 8, 10; H-8 and C-6, 9, 10; H-3′ and C-1′; H-5′ and C-1′; H-6′ and C-4′; 5-OC$\underline{H}_3$ and C-5; 7-OC$\underline{H}_3$ and C-7; 4′-OC$\underline{H}_3$ and C-4′). Furthermore, the positions of methoxyl groups in **2** were also clarified by NOESY, in which NOE correlations were observed as shown in Fig. 1. Consequently, the planar structure of **2** was determined to be 2′-hydroxy-5,7,4′-trimethoxyflavan. On the other hand, the proton and carbon signals in the ^{1}H- (acetone-d_6) and ^{13}C-NMR (Table 1) spectra[24] of **3** were found to be superimposable on those of **2**, except for the 2′-position {two methylenes [δ 1.83, 2.12 (1H each, both m, 3-H_2), 2.60 (2H, m, 4-H_2)], four methoxyl groups [δ 3.74, 3.79, 3.81, 3.86 (3H each, all s, 7, 5, 4′, 2′-OCH_3)], a methine [δ 5.22 (1H, dd, J=2.1, 10.1 Hz, 2-H)], and five aromatic protons [δ 6.04 (1H, d, J=2.5 Hz, 8-H), 6.11 (1H, d, J=2.5 Hz, 6-H), 6.55 (1H, dd, J=2.5, 8.6 Hz, 5′-H), 6.59 (1H, d, J=2.5 Hz, 3′-H), 7.32 (1H, d, J=8.6 Hz, 6′-H)]}. Thus, the planar structure of **3** was elucidated to be 2′-*O*-methylerycibenin E. Finally, the absolute configurations of **2** and **3** were determined on the basis of CD spectroscopic analysis. Thus, the CD spectra of **2** and **3** showed negative Cotton effect [**2**: 273 ($\Delta\varepsilon$ −0.56); **3**: 272 ($\Delta\varepsilon$ −0.39) nm], which indicated the absolute configuration of the 2-position to be *S* orientation, respectively.[26]

Inhibitory Effects on NO Production in LPS-Activated Mouse Peritoneal Macrophages The inorganic free radical NO has been implicated in physiologic and pathologic processes, such as vasodilation, nonspecific host defense, ischemia-reperfusion injury, and chronic or acute inflammation. NO is produced by the oxidation of L-arginine by NO synthase (NOS). In the family of NOS, inducible NOS (iNOS) is specifically involved in pathologic aspects with the overproduction of NO and can be expressed in response to proinflammatory agents such as interleukin-1β, tumor necrosis factor-α, and LPS in various cell types including macrophages. As a part of our studies to characterize the bioactive components of natural medicines, we have investigated various NO production inhibitors, *i.e.*, higher unsaturated fatty acids,[27] polyacetylenes,[28—30] coumarins,[28,30,31] flavonoids,[29,32] stilbenes,[33,34] lignans,[35—37] sesquiterpenes,[38—45] diterpenes,[46,47] triterpenes,[48—51] diarylheptanoids,[52—54] cyclic peptides,[50] alkaloids,[55,56] and phenylpropanoids.[5,6,37] Continuing of these studies, the effects of the principal constituents from *E. expansa* on NO production from LPS-activated macrophages were examined, and the results were summarized in Table 2. Among them, two isoflavones, clycosin (**8**, IC_{50}=13 μM) and erythrinin B (**14**, 18 μM), and two rotenoids, deguelin (**28**, 26 μM) and rotenone (**30**, 27 μM), were found to show inhibitory activity, whose activities were nearly equivalent to that of caffeic acid phenetyl ester (CAPE), an inhibitor of nuclear factor-κB activation (IC_{50}=15 μM).[6]

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); CD spectra, JASCO J-720WI spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS, CI-MS and high-resolution CI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high-

resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors. HPLC column, YMC-Pack ODS-A (250×4.6 mm i.d.) and (250×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., Aichi, Japan, 150—350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., Aichi, Japan, 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The stems of *E. expansa* were purchased in Thailand in July 2000, and identified by Dr. Yutana Pongpiriyadacha (Lecturer of Faculty of Agriculture Nakhon si thammarat, Rajamangala Institute of Technology). A voucher specimen (No. T-14) is on file in our laboratory.

Extraction and Isolation Fractions 2 (10.9 g), 3-8 (103 mg), 3-11 (111 mg), 4-7 (666 mg), 4-10 (247 mg), 5-3 (102 mg), 6-5 (172 mg), 6-12 (1156 mg), 7-6 (806 mg), and 7-9 (296 mg) were obtained from the EtOAc-soluble farction of the MeOH extract of dried stems of *E. expansa* as reported previously.[1)] Fraction 2 (10.9 g) was subjected to ordinary-phase silica gel column chromatography [330 g, *n*-hexane–EtOAc (20 : 1→10 : 1→5 : 1→2 : 1, v/v)→MeOH] to give nine fractions [Fr. 2-1 (196 mg), Fr. 2-2 (203 mg), Fr. 2-3 (2234 mg), Fr. 2-4 (3886 mg), Fr. 2-5 (718 mg), Fr. 2-6 (278 mg), Fr. 2-7 (1246 mg), Fr. 2-8 (326 mg), and Fr.2-9 (610 mg)]. Fraction 2-4 (500 mg) was purified by HPLC [YMC-Pack ODS-A, 20×250 mm, i.d., MeOH–H_2O (90 : 10, v/v)] to give oleic acid (18.0 mg, 0.0040%) and linoleic acid (114.0 mg, 0.026%). Fraction 2-6 (278 mg) was purified by HPLC [MeOH–H_2O (95 : 5, v/v)] to give β-sitosterol (175.9 mg, 0.0051%). Fraction 3-8 (103 mg) was separated by HPLC [MeOH–H_2O (70 : 30, v/v)] to give erycibenin E (**2**, 9.1 mg, 0.00026%) and erycibenin F (**3**, 9.4 mg, 0.00027%). Fraction 3-11 (111 mg) was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give 5,7-dimethoxy-4′-hydroxyflavan (**24**, 9.6 mg, 0.00028%) and 5,7,4′-trimethoxyflavan (**25**, 6.3 mg, 0.00018%). Fraction 4-7 (666 mg) was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give deguelin (**28**, 93.1 mg, 0.0027%), tephrosin (**29**, 76.7 mg, 0.0020%), rotenone (**30**, 214.3 mg, 0.0062%), and 12a-hydroxyrotenone (**31**, 64.4 mg, 0.0019%) together with erythrinin B (**14**, 50.0 mg, 0.0014%), which was described previously.[1)] Fraction 4-10 (247 mg) was purified by HPLC [MeOH–H_2O (95 : 5, v/v)] to give 5α,8α-epidioxy-ergosta-6,22-diene-3β-ol (22.9 mg, 0.00066%). Fraction 5-3 (102 mg) was separated by HPLC [MeOH–H_2O (35 : 65, v/v)] to give erycibenin D (**1**, 3.1 mg, 0.00009%). Fraction 6-5 (172 mg) was further subjected to ordinary-phase silica gel column chromatography [6.0 g, *n*-hexane–EtOAc (1 : 1→1 : 2, v/v)→MeOH] to give eriodictyol (**27**, 19.0 mg, 0.00055%). Fraction 6-12 (1156 mg) was purified by HPLC [MeOH–H_2O (95 : 5, v/v)] to give β-sitosterol 3-*O*-β-D-glucopyranoside (419.9 mg, 0.012%). Fraction 7-6 (806 mg) was purified by HPLC [MeOH–H_2O (55 : 45, v/v)] to give luteolin (**26**, 16.5 mg, 0.00048%) together with 7,2′-dihydroxy-4′,5′-dimethoxyisoflavone (**13**, 9.2 mg, 0.00027%), which was described previously.[1)] Fraction 7-9 (296 mg) was purified by HPLC [MeOH–H_2O (70 : 30, v/v)] to give lasiodiplodin (**32**, 4.7 mg, 0.00014%).

The known compounds were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values[12—22)] or those of commercial samples.[23)]

Erycibenin D (**1**): A pale yellow powder, $[\alpha]_D^{27}$ −11.0° (c=1.00, MeOH). CD [MeOH, nm, $\Delta\varepsilon$]: 239 (+3.15), 270 (+1.68), 303 (−5.23), 330 (+1.43). High-resolution EI-MS: Calcd for $C_{16}H_{14}O_6$ (M^+) 302.0790; Found 302.0796. UV [MeOH, nm, (log ε)]: 234 (4.19), 278 (4.11), 311 (3.78). IR (KBr, cm^{-1}): 3630, 3420, 1676, 1597, 1509, 1460, 1169, 1034. ^{1}H-NMR (500 MHz, acetone-d_6) δ: 3.89 (3H, s, OCH_3), 4.61 (1H, d, J=11.9 Hz, 3-H), 5.05 (1H, d, J=11.9 Hz, 2-H), 6.41 (1H, d, J=2.2 Hz, 8-H), 6.63 (1H, dd, J=2.2, 8.6 Hz, 6-H), 6.88 (1H, d, J=8.3 Hz, 5′-H), 7.05 (1H, dd, J=1.9, 8.3 Hz, 6′-H), 7.24 (1H, d, J=1.9 Hz, 2′-H), 7.73 (1H, d, J=8.6 Hz, 5-H). ^{13}C-NMR (125 MHz, acetone-d_6) δ_C: given in Table 1. EI-MS *m*/*z* (%): 302 (M^+, 17), 137 (100).

Erycibenin E (**2**): A pale yellow powder, $[\alpha]_D^{26}$ −26.2° (c=0.90, MeOH). CD [CH_3CN, nm, $\Delta\varepsilon$]: 273 (−0.56). High-resolution EI-MS: Calcd for $C_{18}H_{20}O_5$ (M^+) 316.1311; Found 316.1309. UV [MeOH, nm, (log ε)]: 256 (3.49), 278 (3.64). IR (KBr, cm^{-1}): 3450, 1593, 1497, 1198, 1073. ^{1}H-NMR (500 MHz, acetone-d_6) δ: 1.89, 2.18 (1H each, both m, 3-H_2), 2.63 (2H, m, 4-H_2), 3.74, 3.75, 3.79 (3H each, all s, 7, 4′, 5-OCH_3), 5.25 (1H, dd, J=2.1, 10.1 Hz, 2-H), 6.05 (1H, d, J=2.4 Hz, 8-H), 6.11 (1H, d, J=2.4 Hz, 6-H), 6.46 (1H, dd, J=2.4, 8.3 Hz, 5′-H), 6.48 (1H, d, J=2.4 Hz, 3′-H), 7.26 (1H, d, J=8.3 Hz, 6′-H). ^{13}C-NMR (125 MHz, acetone-d_6) δ_C: given in Table 1. EI-MS (*m*/*z*, %): 316 (M^+, 42), 150 (17), 167 (100).

Erycibenin F (**3**): A pale yellow powder, $[\alpha]_D^{29}$ −21.2° (c=0.40, MeOH). CD [CH_3CN, nm, $\Delta\varepsilon$]: 272 (−0.39). High-resolution EI-MS: Calcd for $C_{19}H_{22}O_5$ (M^+) 330.1467; Found 330.1465. UV [MeOH, nm, (log ε)]: 255 (3.29), 277 (3.38). IR (KBr, cm^{-1}): 1590, 1509, 1210, 1073. ^{1}H-NMR (500 MHz, acetone-d_6) δ: 1.83, 2.12 (1H each, both m, 3-H_2), 2.60 (2H, m, 4-H_2), 3.74, 3.79, 3.81, 3.86 (3H each, all s, 7, 5, 4′, 2′-OCH_3), 5.22 (1H, dd, J=2.1, 10.1 Hz, 2-H), 6.04 (1H, d, J=2.5 Hz, 8-H), 6.11 (1H, d, J=2.5 Hz, 6-H), 6.55 (1H, dd, J=2.5, 8.6 Hz, 5′-H), 6.59 (1H, d, J=2.5 Hz, 3′-H), 7.32 (1H, d, J=8.6 Hz, 6′-H). ^{13}C-NMR (125 MHz, acetone-d_6) δ_C: given in Table 1. EI-MS (*m*/*z*, %): 330 (M^+, 43), 164 (100).

Bioassay. NO Production from LPS-Stimulated Macrophages Inhibitory effects on the NO production by mouse macrophages were evaluated using the method reported previously.[5,6)] Briefly, TGC-induced peritoneal exudate cells (5×10^5 cells/well) were collected from the peritoneal cavities of male ddY mice and were suspended in 100 μl of RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 μg/ml), and pre-cultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing with PBS, and the adherent cells were cultured in 200 μl of fresh medium containing 10 μg/ml LPS and various concentrations of test compound for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent. Cytotoxicity was determined by the MTT colorimetric assay, after 20 h incubation with test compounds. Each test compound was dissolved in DMSO, and the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated using the following formula and IC_{50} was determined graphically (n=4).

$$\text{inhibition (\%)} = [(A-B)/(A-C)]\times 100$$

A—*C*: NO_2^- concentration (μM) [*A*: LPS (+), sample (−); *B*: LPS (+), sample (+); *C*: LPS (−), sample (−)]

Statistics Values are expressed as mean±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

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