Endothelin Receptor Antagonist Triterpenoid, Myriceric Acid A, Isolated from *Myrica cerifera*, and Structure Activity Relationships of Its Derivatives

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As the first non-peptide endothelin receptor antagonist from a higher plant, a new triterpenoid, myriceric acid A (50-235) (1) was isolated from the bayberry, *Myrica cerifera*. Myriceric acid A (1) inhibited not only an endothelin-1-induced increase in cytosolic free Ca^{2+} concentration ($IC_{50} = 11 \pm 2$ nM) but [^{125}I]endothelin-1 binding in rat aortic smooth muscle cells ($K_i = 66 \pm 15$ nM). Two new related triterpenoids, myriceric acid C (6), and myriceric acid D (8), were also isolated. Furthermore, the chemical modification of these natural products led to the synthesis of sulfated derivatives (13, 14, 15) which showed 1.5 to 20 times higher affinity for endothelin receptors. The structure activity relationships of myriceric acids and their derivatives are discussed.

Key words myriceric acid A; *Myrica cerifera*; endothelin receptor antagonist; triterpene; myriceric acid C; myriceric acid D

Endothelin-1 was discovered to be a potent vasoconstrictor peptide produced by vascular endothelial cells.[1] Analysis of the human genomic library resulted in the identification of two other isopeptides, endothelin-2 and -3.[2] These three endothelin isopeptides induce a variety of pharmacological effects, of which the potencies are not always the same.[3,4] Based on their binding profiles, two types of endothelin receptors (ET_A, ET_B) have been identified, cloned and characterized.[5] ET_A displays higher affinity for endothelin-1 and endothelin-2 than for endothelin-3, while ET_B shows the same affinity for all.

A line of evidence has suggested that endothelins may cause the pathogenesis of hypertension and pathological vascular spasms.[6] Therefore, many laboratories have been engaged in seeking endothelin receptor antagonists from natural sources. The first isolated was a cyclic peptide from the fermentation broth of *Streptomyces misakiensis*.[7] BQ-123, one of its potent synthetic analogs, is now widely used as a specific ET_A receptor antagonist.[8] Its further modification led to the development of a potent and selective ET_B receptor antagonist, BQ-788.[9] Recently, RES-701-1 has been isolated as another peptide ET_B receptor antagonist from the culture broth of *Streptomyces* sp. RE-70.[10]

In this paper, we report the isolation of a new triterpenoid (**1**), the first non-peptide ET_A receptor antagonist, from a higher plant, *Myrica cerifera*.[11]

We found that the methanol extract of fresh twigs of *Myrica cerifera*, which was cultivated in our laboratories, inhibited an endothelin-1-induced increase in cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) in rat aortic smooth muscle A7r5 cells ($IC_{50} = 36\,\mu g/ml$). This inhibitory effect appeared to be specific for endothelins, since it had no effect on the bradykinin- or bombesin-induced increase in $[Ca^{2+}]_i$. The methanol extract was partitioned between water and chloroform ($CHCl_3$). The $CHCl_3$–soluble fraction showed 1.8-fold higher endothelin receptor antagonist activity ($IC_{50} = 20\,\mu g/ml$) than the initial methanol extract. Silica gel chromatography of the $CHCl_3$–soluble fraction gave an active fraction ($IC_{50} = 0.11\,\mu g/ml$), which showed one major peak on reverse phase high performance liquid chromatography (HPLC) analysis. Semi-preparative reverse phase HPLC gave 50-235 (**1**, myriceric acid A), which potently inhibited not only endothelin-1-induced Ca^{2+} mobilization ($IC_{50} = 0.0075\,\mu g/ml$), but also the binding of [^{125}I]endothelin-1 to A7r5 cells with $IC_{50} = 0.04\,\mu g/ml$. Finally, it was confirmed that this compound was a specific ET_A receptor antagonist because it selectively antagonized the specific binding of [^{125}I]endothelin-1, but not of [^{125}I]endothelin-3, to rat cardiac membranes.[11]

High resolution liquid secondary ion mass spectrometry (HR-LSI-MS) of crystalline 50-235 (**1**, myriceric acid A) showed an ion peak at m/z 632.3715 for the molecular ion indicating the molecular formula $C_{39}H_{52}O_7$. Five signals on the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, δ_H 6.16 (1H, d, $J = 15.8$ Hz), 6.85 (1H, d, $J = 8.3$ Hz), 6.92 (1H, dd, $J = 1.5$, 8.3 Hz), 7.05 (1H, d, $J = 1.5$ Hz), and 7.49 (1H, d, $J = 15.8$ Hz), and nine signals on the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum, δ_C 114.16, 115.03, 115.51, 122.11, 126.86, 145.27, 145.55, 147.85, and 167.76, suggested a *trans*-caffeoyl group ($C_9H_7O_3$). As for the remaining moiety of the molecule, $C_{30}H_{45}O_4$, ^{1}H-NMR signals of six tertiary methyl groups at δ_H 0.84, 0.86, 0.93, 1.04, 1.04, and 1.08 and a CH_2OR group appeared as a pair of doublets at δ_H 4.16 (1H, d, $J = 12.7$ Hz) and δ_H 4.36 (1H, d, $J = 12.7$ Hz) indicated the existence of a triterpene moiety. A triplet centered at δ_H 5.64 (1H, t, $J = 3.2$ Hz) and a doublet of doublets centered at δ_H 2.93 (1H, dd, $J = 3.8$, 13.6 Hz) indicated H-12 and H-18 signals of an olean-12-ene derivative, respectively. The presence of ^{13}C-signals due to a carbonyl carbon (δ_C 219.27) and a carboxyl carbon (δ_C 181.27) indicated that 50-235 (**1**, myriceric acid A) seems to be a derivative of 3-oxoolean-12-en-28-oic acid with one of the seven methyl groups being substituted by a *trans*-caffeoyloxymethyl group.

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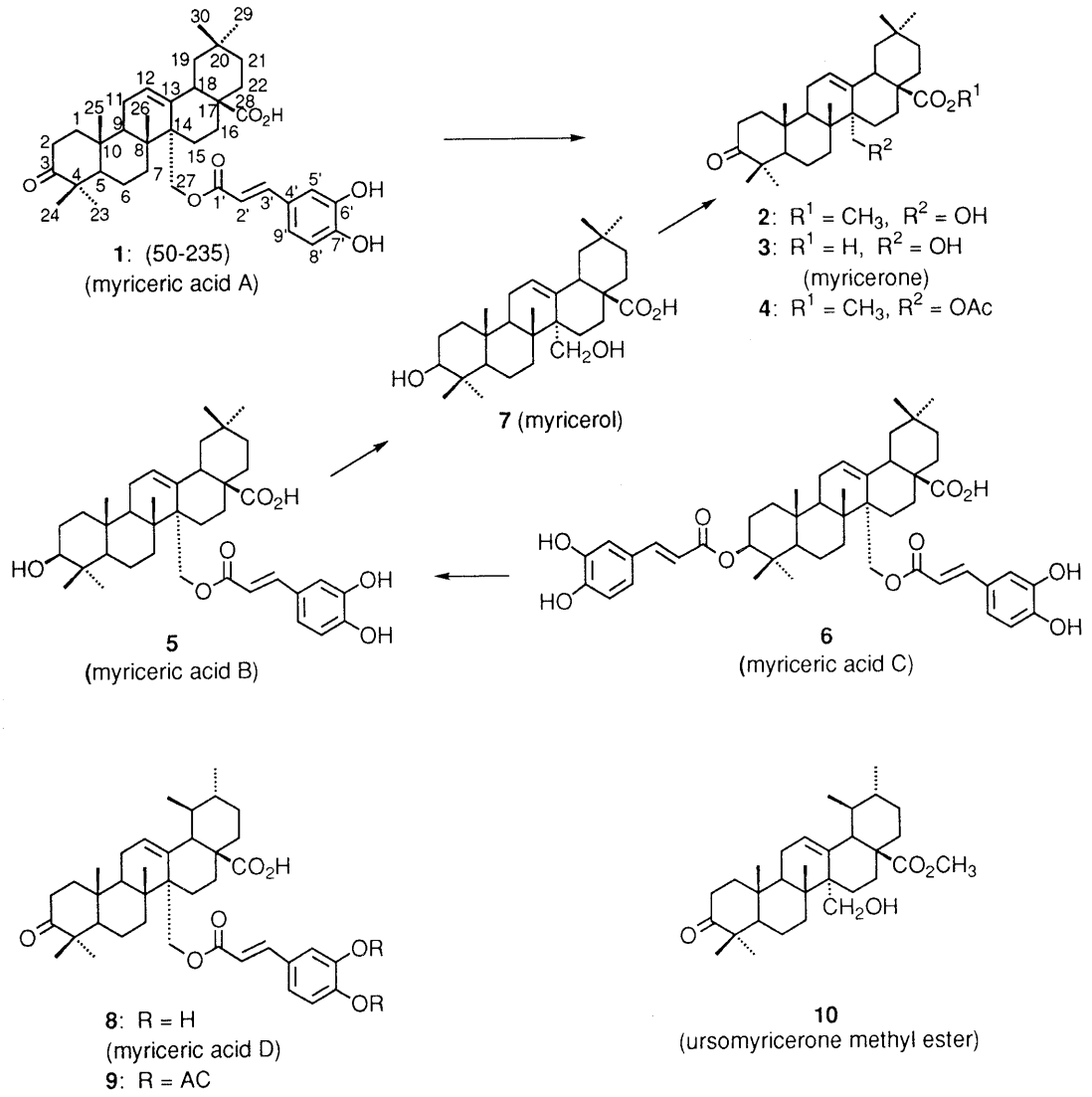


Chart 1

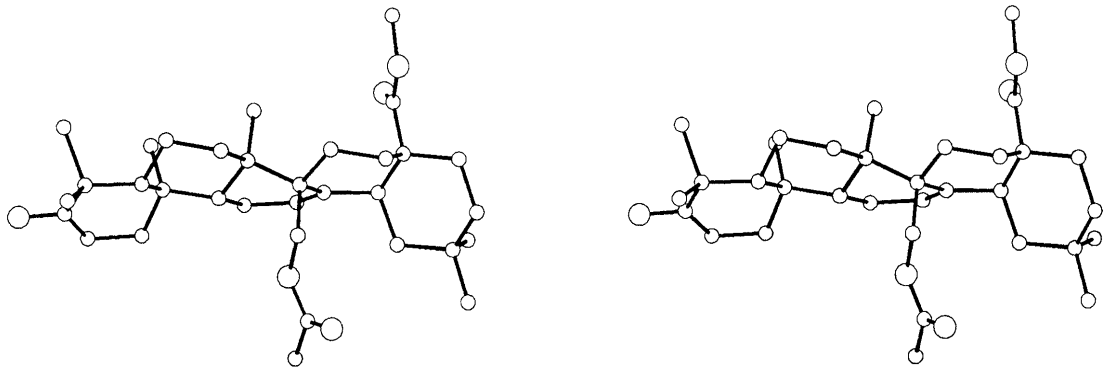


Fig. 1. Perspective View of 27-*O*-Acetylmyricerone Methyl Ester (**4**)

50-235 (**1**, myriceric acid A) was methylated and then hydrolyzed with potassium hydroxide to give a hydroxy methyl ester (**2**), which was alternatively obtained by the methylation of **3** (myricerone), an alkaline hydrolysis product of 50-235. Acetylation of the hydroxy methyl ester (**2**) afforded a crystalline acetate (**4**). X-ray analysis determined the structure of **4** to be the methyl ester of 27-acetoxy-3-oxoolean-12-en-28-oic acid, as shown in Fig. 1. The absolute configuration of **4** was based on circular dichroism (CD) of the hydroxy methyl ester (**2**), which showed a positive Cotton effect at 289 nm ($[\theta]_{289} = +2368$, $c = 0.291$ mM in MeOH). Together with the NMR data described above, the structure of 50-235 (myriceric acid A) was proposed to be 3-oxo-27-*trans*-caffeoyloxy-olean-12-en-28-oic acid (**1**).

Unambiguous assignments of all the carbon and proton NMR signals shown in Tables 1 and 2 were established by using distortionless enhancement by polarization transfer (DEPT), homonuclear Hartmann-Hahn spectroscopy (HOHAHA), double-quantum-filtered homo-

nuclear correlation spectroscopy (DQF-COSY), heteronuclear multiple-quantum coherence spectroscopy (HMQC), heteronuclear multiple-bond correlation spectroscopy (HMBC) and nuclear Overhauser effect (NOE) spectroscopy (NOESY).

Other triterpenoid constituents of *M. cerifera* were also examined and two new triterpenoids, **6** (myriceric acid C) and **8** (myriceric acid D), were isolated, together with a known triterpenoid **5** (myriceric acid B).

Myriceric acid B (**5**) showed NMR spectra very similar to that of myriceric acid A (**1**) except for C-3, which appeared at δ_C 78.76 instead of δ_C 219.27 in **1**, and at δ_H 3.18 (1H, dd, $J=7$, 10 Hz) which appeared in **5**. The molecular formula of **5**, $C_{39}H_{54}O_7$ based on HR-LSI-MS, suggested it to be 3β-hydroxy-27-*trans*-caffeoyloxyolean-12-en-28-oic acid, which was reported to be present in *Melianthus comosus*[12] and *Rhoiptelea chiliantha*[13] (As this compound has no name yet, we propose naming **5** myriceric acid B).

The NMR spectrum of myriceric acid C (**6**) resembled that of myriceric acid B (**5**) except for overlapped signals due to the two caffeoyl groups and a lower field shifted H-3 signal (δ_H 4.56, dd, $J=7$, 10 Hz). LSI-MS of **6** gave a molecular ion peak at m/z 796. These data indicated that the structure of **6** was 3β,27-di-*O*-*trans*-caffeoylmyricerol, which was confirmed by the chemical conversion of **6** to **5** and myricerone (**3**) as follows. Tetraacetate of **6** was treated with potassium hydroxide to give myriceric acid B (**5**) and also 3β,27-dihydroxyolean-12-en-28-oic acid (**7**, myricerol). Partial hydrolysis of diacetyl myricerol gave 27-*O*-acetylmyricerol, which was oxidized with Jones' reagent followed by deacetylation to afford myricerone (**3**). ^{1}H- and ^{13}C-NMR data are shown in Tables 1 and 2. The NMR data of the myricerol moiety agree well with those of asprellic acid A, B, and C, which are 27-*O*-*trans*- or *cis*-*p*-coumaloyl derivatives of myricerol isolated from *Ilex asprella*.[14]

Myriceric acid D (**8**) was initially isolated as a diacetate (**9**) from the crude 27-*O*-*trans*-caffeoylmyricerone (**1**). Later, we realized that the ratio of **8** against **1** in *M. cerifera* varied between 0.1% to 15% with collection sites based on HPLC analysis of methyl esters. On HR-LSI-MS of **8**, the elemental composition $C_{39}H_{52}O_7Na$ of the sodium adduct molecular ion $[M+Na]^+$ at m/z: 655.3628, showed the same molecular formula as that of myriceric acid A (**1**). ^{1}H-NMR spectra of **8** showed two doublet methyl signals at δ_H 0.87 (3H, d, $J=6.0$ Hz) and 0.91 (3H, d, $J=6.0$ Hz) and four singlet methyl signals (δ_H 0.87, 1.04, 1.04, 1.07), together with typical signals due to a caffeoyl group. These data suggested the structure of **8** to be 3-oxo-27-*trans*-caffeoyloxyurs-12-en-28-oic acid, which gave diacetate (**9**). Alkaline hydrolysis of **9** followed by methylation gave ursomyricerone methyl ester (**10**). The structure of **8** was confirmed by two dimensional (2D) NMR experiments and the data are shown in Tables 1 and 2.

Chemical Modification and Structure Activity Relationships We examined the effect of myriceric acid A (**1**) and its derivatives on endothelin-1-induced Ca^{2+} mobilization and on [^{125}I]endothelin-1 binding in rat aortic smooth muscle A7r5 cells (Table 3). Myriceric acid A (**1**) inhibited [^{125}I]endothelin-1 binding affinity in A7r5 cells with a K_i

Table 1. ^{1}H-NMR Data of Myriceric Acid A (**1**) and Its Related Compounds

	1[a]	**3**[a]	**8**[a]	**10**[a]	**2**[b]	**4**[b]	**5**[a]	**6**[a]
H-3	—	—	—	—	—	—	3.182 dd (7, 10)	4.561 dd (7, 10)
H-12	5.640 t (3.2)	5.857 br s	5.604 t (3.5)	5.720 t (3.4)	5.865 t (3.5)	5.596 t (3.6)	5.612 t (3.4)	5.623 t (3.5)
H-18	2.931 dd (3.8, 13.6)	2.956 dd (4.6, 13.8)	2.319 d (11.6)	2.365 d (11.1)	2.978 dd (4.6, 13.5)	2.923 dd (4.2, 13.8)	2.906 dd (4.3, 14.3)	2.914 dd (3.7, 13.8)
H-27a	4.158 d (12.7)	3.263 d (11.8)	4.185 d (12.9)	3.431 d (12.2)	3.236 d (12.0)	4.054 d (12.8)	4.157 d (12.6)	4.191 d (12.6)
H-27b	4.364 d (12.7)	3.775 d (11.8)	4.365 d (12.9)	3.761 d (12.2)	3.769 d (12.0)	4.206 d (12.8)	4.330 d (12.6)	4.340 d (12.6)
CH_3-23	1.076 s	1.088 s	1.074 s	1.086 s	1.083 s	1.089 s	0.962 s	0.898 s
CH_3-24	1.037 s	1.036 s	1.036 s	1.038 s	1.032 s	1.036 s	0.768 s	0.934 s
CH_3-25	1.037 s	1.007 s	1.036 s	1.016 s	1.003 s	1.044 s	0.916 s	0.974 s
CH_3-26	0.855 s	0.807 s	0.870 s	0.747 s	0.731 s	0.757 s	0.800 s	0.816 s
CH_3-29	0.843 s	0.912 s	0.868 d (6.0)	0.977 d (6.4)	0.908 s	0.873 s	0.832 s	0.844 s
CH_3-30	0.926 s	0.970 s	0.909 d (6.0)	0.961 d (6.4)	0.964 s	0.939 s	0.916 s	0.922 s
H-2′	6.161 d (15.8)	—	6.167 d (15.8)	—	—	—	6.168 d (16.0)	6.184 d (15.8)
H-3′	7.493 d (15.8)	—	7.498 d (15.8)	—	—	—	7.499 d (16.0)	7.513 d (15.8)
H-5′	7.052 d (1.5)	—	7.042 d (1.4)	—	—	—	7.058 d (1.8)	7.049 d (2.0)
H-8′	6.845 d (8.3)	—	6.840 d (8.2)	—	—	—	6.844 d (8.2)	6.848 d (8.2)
H-9′	6.917 dd (1.5, 8.3)	—	6.911 dd (1.4, 8.2)	—	—	—	6.924 dd (1.8, 8.3)	6.941 dd (2.0, 8.2)
H-2″	—	—	—	—	—	—	—	6.213 d (15.8)
H-3″	—	—	—	—	—	—	—	7.513 d (15.8)
H-5″	—	—	—	—	—	—	—	7.075 d (2.0)
H-8″	—	—	—	—	—	—	—	6.821 d (8.2)
H-9″	—	—	—	—	—	—	—	6.941 dd (2.0, 8.2)
OCH_3	—	—	—	3.639 s	3.657 s	3.657 s	—	—
OAc	—	—	—		—	2.010 s	—	—

a) Assignments are based on HMQC, DQF-COSY, ROESY, NOESY and HOHAHA experiments recorded on a Varian XL-400 instrument at 400 MHz in $CDCl_3+CD_3OD$ (10:1). *b*) Assignments are proposed by signal comparison of compound to compound in $CDCl_3$ recorded on a Varian Gemini-200 at 200 MHz. Chemical shifts (δ) are expressed in ppm, and coupling constants, J values, are in Hz in parenthesis; abbreviations are: s, singlet; d, doublet; dd, double doublet; m, multiplet; and br, broad.

Table 2. ^{13}C-NMR Data (100.6 MHz) of Myriceric Acid A (**1**) and Its Related Compounds

C-No.	**1**[a]	**3**[b]	**8**[b]	**10**[c]	**2**[c]	**4**[b]	**5**[b]	**6**[b]
C-1	39.11	38.68	39.30	39.05	38.76	39.20	38.59	38.25
C-2	34.14	[d]	34.09	33.90	34.04	34.18	26.79	23.62
C-3	219.27	219.28	219.38	219.21	217.64	218.89	78.76	81.17
C-4	47.52	47.50	47.43	47.48	47.34	47.57	38.76	38.01
C-5	55.13	54.94	55.09	54.99	54.85	55.29	55.28	55.46
C-6	19.68	19.65	19.63	19.70	19.16	19.65	18.36	18.30
C-7	32.54	32.40[e]	33.10	32.82	32.10	32.62	33.20	33.13
C-8	40.00	39.81	40.17	40.25	39.80	40.00	40.00	40.02
C-9	47.93	47.69	47.63	47.53	47.61	48.00	48.76	48.70
C-10	36.99	36.86	36.92	36.92	36.83	37.02	37.23	37.22
C-11	24.06	24.19	23.83	24.00	24.18	23.94	24.03	24.04
C-12	126.75	129.10	130.19	132.45	129.23	127.05	126.96	126.95
C-13	137.74	138.31	133.34	133.88	137.13	137.36	137.53	137.51
C-14	45.55	47.69	45.77	47.85[e]	47.73	45.21	45.36	45.45
C-15	23.62	24.42	23.60	23.84	24.46	23.45	23.61	23.71
C-16	22.90	22.65	24.19	24.21	22.64	22.90	22.87	22.88
C-17	46.36	46.04	47.63	47.82[e]	46.36	46.64	46.22	46.25
C-18	41.25	40.82	52.59	52.16	40.85	41.18	41.12	41.15
C-19	44.92	45.17	38.66	38.29	44.99	44.93	44.88	44.89
C-20	30.68	30.88	39.15	39.30	30.84	30.68	30.65	30.67
C-21	33.81	33.65	30.26	29.94	33.58	33.70	33.77	33.79
C-22	32.72	32.21[e]	36.78	36.70	32.22	32.40	32.53	32.55
C-23	26.65	26.58	26.73	26.69	26.62	26.57	28.01	28.13
C-24	21.49	21.37	21.42	21.40	21.41	21.51	15.66	16.91
C-25	15.34	15.58	15.59	15.79	15.53	15.27	15.60	15.71
C-26	17.93	18.02	17.84	17.99	18.08	17.91	18.05	18.08
C-27	65.63	63.05	65.85	64.34	63.21	66.08	65.87	65.78
C-28	181.27	180.41	180.93	178.35	177.93	178.64	180.84	180.80
C-29	32.98	33.07	17.48	18.36	33.09	33.03	32.96	32.96
C-30	23.62	23.85	21.04	21.21	23.93	23.66	23.61	23.62
C-1′	167.76		167.71				167.81	167.71
C-2′	115.03		114.95				115.13	115.20
C-3′	145.55		145.57				145.35	145.40
C-4′	126.86		126.80				126.88	126.95
C-5′	114.16		114.09				113.99	114.09
C-6′	145.27		145.05				145.04	144.97
C-7′	147.85		147.63				147.58	147.57
C-8′	115.51		115.41				115.37	115.39
C-9′	122.11		122.04				122.05	122.03
C-1″								168.09
C-2″								115.32
C-3″								145.24
C-4″								126.95
C-5″								114.09
C-6″								144.93
C-7″								147.44
C-8″								115.32
C-9″								122.03
OCH_3				51.82	51.76	51.87		
OAc						21.30		
						171.36		

Assignments of **1**, **5**, **6**, and **8** are based on DEPT, HMQC, and HMBC experiments and assignments of **2**, **3**, **4**, and **10** were made by signal comparison. *a*) $CDCl_3+CD_3OH$ (10:1). *b*) $CDCl_3+CD_3OD$ (10:1). *c*) $CDCl_3$. *d*) This signal collapsed in $CDCl_3+CD_3OD$ by deuteration, but appeared at δ_C 34.21 in $CDCl_3+CD_3OH$. *e*) Assignments may be reversed in each column.

value of 66 ± 15 nM. Myricerone (**3**), which has no caffeoyl group at C-27, completely lost its binding affinity. A photo-isomerization product of **1**, *cis*-caffeoyl isomer (**11**), also showed reduced receptor antagonist activity. 27-*O*-*trans*-Cinnamoylmyricerone (**12**), which was prepared from myricerone (**3**) by treatment with *trans*-cinnamoyl chloride, was 3-fold less potent than myriceric acid A (**1**) in blocking [^{125}I]endothelin-1 binding.

Although the 27-*trans*-caffeoyl ester group was indicated to play an important role in the antagonist activity, neither *trans*-caffeic acid nor *trans*-cinnamic acid itself had any effect on the [^{125}I]endothelin-1 binding (data not shown). In contrast, when the hydroxy groups on the *trans*-caffeoyl group were sulfated, the binding affinity increased by 20-, 4-, and 1.6-fold for mono sulfates (**13**, **14**), and the di-sulfate (**15**), respectively.

Table 3. Effects of Myriceric Acid A (**1**, 50-235) and Its Derivatives on [^{125}I]Endothelin-1 Binding and Endothelin-1-induced Ca^{2+} Mobilization in A7r5 Cells

Compound	Binding (K_i, nM)[a]	$[Ca^{2+}]_i$ (IC_{50}, nM)[a]
Myriceric acid A (**1**)[11]	66	11
Myricerone (**3**)[11]	>8500	>10000
27-*O*-*cis*-Caffeoylmyricerone (**11**)	ND	230
27-*O*-*trans*-Cinnamoylmyricerone (**12**)[11]	200	31
6′-Sulfonyl **1** (**13**)	15	26
7′-Sulfonyl **1** (**14**)	3.4	3.4
6′,7′-Disulfonyl **1** (**15**)	41	460
Myriceric acid B (**5**)[11]	4100	750
Myriceric acid C (**6**)[11]	>8500	5600
3α-Myriceric acid B (**17**)	ND	>10000
3-Deoxy **12** (**18**)[11]	>8500	>10000
3-Hydroxyimino **12** (**19**)	ND	>10000
Methyl ester of **12** (**20**)	ND	>10000
28-Decarboxylated **12** (**21**)[11]	>8500	>10000
Myriceric acid D (**8**)	ND	160
1,2-Dehydro **12** (**16**)	ND	240

a) Each value represents the average of two or three determinations from separate assays. ND, not determined.

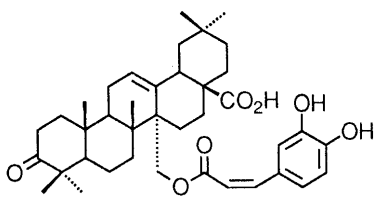
Replacement of the 3-carbonyl group by either a β-hydroxy group, a β-caffeoyloxy group or an α-hydroxy group such as **5**, **6** or **17**, caused a marked reduction in the affinity. The 3-hydroxyimino derivative (**19**) also showed reduced affinity. Replacement of it by two hydrogens such as **18** (the 27-*O*-*trans*-cinnnamoyl derivative) completely abolished the affinity. On *trans*-cinnamoyl derivatives, replacement of the carboxylic acid group at C-17 by a methyl ester (**20**) or a hydrogen (**21**) (preparation, see Experimental section) also abolished the activity.

Myriceric acid D (**8**), in which one of the methyl groups at C-20 of **1** had migrated to the next carbon, C-19, showed a 15-fold lower affinity than myriceric acid A, indicating the important role of the methyl groups in ring E for the affinity to the binding site of the receptor. The introduction of a double bond into the A ring (**16**) also caused a reduction of the binding affinity.

These results suggested that four functional groups, the carbonyl group at C-3, the carboxylic acid group at C-17, the *trans*-caffeoyloxy (or *trans*-cinnamoyloxy) group at C-27, and the dimethyl groups at C-20, are important for the endothelin receptor antagonist activity.

Experimental

General All melting points were determined using a Yanagimoto microscopic melting point apparatus and are corrected. Silica gel column chromatography was carried out using Merck Silica gel 60 (No. 9385) with the addition of 10% water by weight. In the usual manner, the reaction mixture was mixed with water, extracted with $CHCl_3$, then washed successively with dil. NaOH, water, dil. HCl, water, and 5% $NaHCO_3$. The solvent was dried with $MgSO_4$, and evaporated. Spectra reported herein were recorded on a Hitachi model 320 UV spectrometer, a JASCO A-202 infrared (IR) spectrometer, a Hitachi M-68 or a M-90



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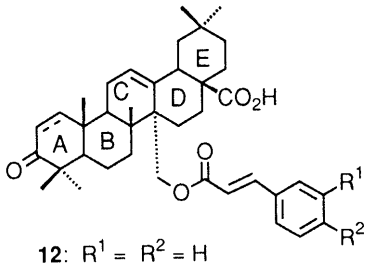
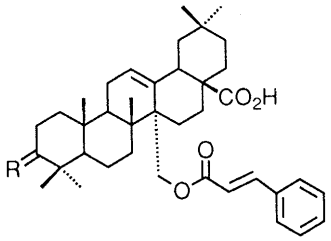
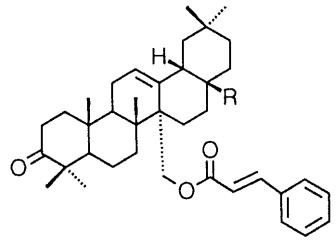


12: $R^1 = R^2 = H$
13: $R^1 = OSO_3H$, $R^2 = OH$
14: $R^1 = OH$, $R^2 = OSO_3H$
15: $R^1 = R^2 = OSO_3H$
16: $R^1 = R^2 = H$, $\Delta^{1(2)}$



17: R = α-OH, β-H
18: R = H_2
19: R = NOH



20: R = CO_2CH_3
21: R = H

Chart 2

mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 241 Polarimeter at 25 °C. NMR data were recorded on either a Varian XL-400, a Varian Gemini-200 or a Varian Gemini-300 NMR spectrometer, using the internal reference Me_4Si. The following abbreviations are used: s = singlet, d = doublet, dd = double doublet, m = multiplet, t = triplet and br = broad.

Cell Culture Rat aortic smooth muscle A7r5 cells were obtained from Dainippon Seiyaku (Osaka) and cultured in Dulbecco's modified Eagle's medium (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (GIBCO), 10 mM HEPES buffer (pH 7.4), 50 μg/ml of streptomycin, and 50 U/ml of penicillin G (GIBCO) in a 5% CO_2–95% air incubator at 37 °C.

Measurement of $[Ca^{2+}]_i$ $[Ca^{2+}]_i$ was measured fluorometrically using the Ca^{2+}-sensitive fluorescent dye fura-2. A7r5 cells were dispersed with 0.025% trypsin/1 mM EDTA. Cell suspensions were washed once with the growth medium. Single cells were counted and resuspended in HEPES (20 mM)-buffered Hanks' solution (pH 7.4) to a final concentration of 1×10^6 cells/ml. The cell suspensions were incubated with 2 μM fura-2-AM (Dojin, Kumamoto) at 37 °C for 30 min. The fura-2-loaded cells thus obtained were re-suspended in a cuvette (50 mm × 7 mm diameter) and stirred continuously. Peptides in 3 μl of phosphate-buffered saline containing 0.1% bovine serum albumin were added. A sample in 1 μl of methanol (MeOH) or dimethylsulfoxide (DMSO) was added 1 min before the addition of endothelin-1 (10^{-8} M). Fluoresence measurements were done with a spectrofluorometer (CAF-100) (Japan Spectroscopy, Inc., Tokyo) as described previously.[15]

Binding Studies A7r5 cells were cultured in 24-well culture plates. After 3 to 5 d, the culture medium was aspirated and the cells were washed twice with HEPES (20 mM)-buffered Hanks' solution (pH 7.4). Each well was incubated with 12.5 pM [^{125}I]endothelin-1 in 0.2 ml of HEPES-buffered Hanks' solution containing 0.1 mM phenylmethylsulfonylfluoride, 10 μg/ml of aprotinin, 10 μg/ml of leupeptin, 10 μg/ml of pepstatin A, 250 μg/ml of bacitracin and 10 μg/ml of soybean trypsin inhibitor in the absence and presence of various concentrations of samples. Equilibrium binding studies were performed at 37 °C for 60 min. The incubation was terminated by the rapid removal of the incubation medium and the addition of 0.25 ml of ice-cold HEPES-buffered Hanks' solution. Free ligand was removed by washing the intact attached cells four times with ice-cold HEPES-buffered Hanks' solution. The cells were then resolved in 0.1 N NaOH and transferred to a test tube, then the radioactivity was counted. Nonspecific binding was determined in the presence of 10^{-7} M endothelin-1, and it was about 5 to 10% of the total binding.

Isolation of Triterpenoids from *Myrica cerifera* Fresh twigs of *Myrica cerifera* (11.7 kg, a voucher specimen is kept in the herbarium of the Osaka Museum of Natural History: voucher No. 109215) cultivated at Aburahi Laboratories of Shionogi & Co., Ltd. were extracted with MeOH at room temperature, and then the MeOH extract (628 g) was partitioned between $CHCl_3$ and water. The $CHCl_3$–soluble fraction (152 g) showing an ET receptor antagonistic effect ($IC_{50} = 36$ μg/ml) was chromatographed on silica gel and eluted successively with $CHCl_3$, $CHCl_3$–MeOH and MeOH to afford fractions containing **1**, **5** and **6**. Each fraction was rechromatographed on silica gel and then reverse phase HPLC [column: Develosil ODS 10—20 (20 mm i.d. × 250 mm), solvent: MeOH–H_2O 9 : 1, detector: 330 nm] to afford **1** (1.358 g, 0.01%), **5** (1.24 g, 0.01%), and **6** (4.328 g, 0.04%).

Myriceric Acid A (**1**): Colorless plates, mp 211—213 °C (crystallized from MeOH–H_2O 8 : 2), $[\alpha]_D$ +149.4° ($c = 0.88$, MeOH). *Anal.* Calcd for $C_{39}H_{52}O_7 \cdot H_2O$: C, 71.97; H, 8.36. Found: C, 71.93; H, 8.09. IR ($CHCl_3$) cm^{-1}: 3528, 3194, 2946, 1694, 1631, 1604. UV λ_{max} (MeOH) nm (ε): 215 (15600), 245 (10300), 300 (13700), 330 (18560). HR-LSI-MS *m/z*: 632.3715 (Calcd for $C_{39}H_{52}O_7$ $[M]^{+\cdot}$ 632.3713).

Myriceric Acid B (**5**): Colorless plates, mp 242—244 °C (crystallized from EtOH), $[\alpha]_D$ +137.9° ($c = 1.02$, MeOH). *Anal.* Calcd for $C_{39}H_{54}O_7 \cdot 1/2H_2O$: C, 72.75; H, 8.61. Found: C, 72.88; H, 8.62. IR (Nujol) cm^{-1}: 3500—3200, 1692, 1630, 1604. UV λ_{max} (MeOH) nm (ε): 215 (18200), 235 (9800), 245 (10200), 300 (13300), 330 (18000). HR-LSI-MS *m/z*: 657.3774 (Calcd for $C_{39}H_{54}O_7Na$ $[M+Na]^+$ 657.3767).

Myriceric Acid C (**6**): Colorless needles, mp 241—243 °C (crystallized from MeOH), $[\alpha]_D$ +161.8° ($c = 0.97$, pyridine). *Anal.* Calcd for $C_{48}H_{60}O_{10}$: C, 72.34; H, 7.59. Found: C, 72.22; H, 7.54. IR (Nujol) cm^{-1}: 3246, 1695, 1679, 1660, 1625, 1603. UV λ_{max} (MeOH) nm (ε): 216 (33700), 225 (20400), 244 (20900), 300 (27500), 330 (36800). LSI-MS *m/z*: 796 $[M]^{+\cdot}$ for $C_{48}H_{60}O_{10}$.

Tetraacetate of **6**: Colorless needles, mp 209—211 °C (crystallized from MeOH). IR ($CHCl_3$) cm^{-1}: 1770, 1700, 1639. HR-LSI-MS *m/z*: 965.4675 (Calcd for $C_{56}H_{69}O_{14}$ $[M+H]^+$ 965.4687). ^{1}H-NMR ($CDCl_3$) δ_H: 0.79, 0.86, 0.90, 0.92, 0.92, 0.98, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.29, 2.29, 2.30, 2.30 (each 3H, s, $COCH_3$), 2.91 (1H, br d, $J = 12.0$ Hz, H-18), 4.16, 4.36 (each 1H, d, $J = 12.6$ Hz, H-27), 4.59 (1H, t, $J = 10$ Hz, H-3), 5.63 (1H, br s, H-12), 6.32, 6.37 (each 1H, d, $J = 16.0$ Hz, H-2′, 2″), 7.20 (2H, d, $J = 7.8$ Hz, H-8′, 8″), 7.37 (4H, m, H-5′, 5″, H-9′, 9″), 7.58 (2H, d, $J = 16.0$ Hz, H-3′, 3″).

Myricerone Methyl Ester (2) from 1 To a MeOH (1 ml) solution of **1** (29 mg) was added dropwise a solution of diazomethane in ethyl ether, and the reaction mixture was allowed to stand for 1 h at room temperature. The solvent was then removed to yield a yellow oily residue, which was chromatographed on silica gel and eluted with $CHCl_3$ to give a mixture of products. The product mixture was dissolved in 2% sodium hydroxide in MeOH (2 ml) and stirred for 1.5 h at 50 °C. The reaction mixture was diluted with water (5 ml) and MeOH was evaporated. The aqueous solution was acidified with dil. sulfuric acid and extracted with *n*-butanol. The *n*-butanol solution was washed with water and evaporated to yield an oily residue (15 mg), which was chromatographed on silica gel eluted with $CHCl_3$ to give myricerone methyl ester (**2**) (12 mg) as colorless needles, mp 223—225 °C. $[\alpha]_D$ +91.0° (c=0.50, $CHCl_3$). CD nm (θ): 216.3 (−127.6), 289.0 (+2368) (c=0.291 mM MeOH). *Anal.* Calcd for $C_{31}H_{48}O_4 \cdot 1/10H_2O$: C, 76.53; H, 9.99. Found: C, 76.40; H, 9.83. IR (Nujol) cm^{-1}: 3519, 1715, 1704. HR-LSI-MS m/z: 507.3451 (Calcd for $C_{31}H_{48}O_4Na$ $[M+Na]^+$ 507.3450).

27-*O*-Acetylmyricerone Methyl Ester (4) To a solution of **2** (12 mg) in pyridine (1 ml) was added acetic anhydride (1 ml) and the reaction mixture was allowed to stand overnight at room temperature. The reaction mixture was treated in the usual manner to yield a crystalline product, which was recrystallized from MeOH to yield 27-*O*-acetylmyricerone methyl ester (**4**) (7 mg) as colorless plates, mp 161 °C. $[\alpha]_D$ +119.6° (c=0.50, $CHCl_3$). *Anal.* Calcd for $C_{33}H_{50}O_5$: C, 74.96; H, 9.91. Found: C, 74.77; H, 9.63. IR (Nujol) cm^{-1}: 1736, 1728, 1705. HR-LSI-MS m/z: 549.3566 (Calcd. for $C_{33}H_{50}O_5Na$ $[M+Na]^+$ 549.3556).

X-Ray Analysis of 4 Crystal Data: $C_{33}H_{50}O_5$, Mr=526.8, orthorhombic, $P2_12_12_1$, a=15.264(1), b=21.666(2), c=9.113(1) Å, V=3013.6(6) Å^3, Z=4, D_c=1.161 g cm^{-3}, CuK_α radiation, λ= 1.54178 Å, μ=0.609 mm^{-1}, F(000) =1152.

A colorless plate crystal with the dimensions 0.10×0.20×0.25 mm, which was obtained from the MeOH solution, was used for X-ray measurements at 295 K on a Rigaku AFC5R diffractometer equipped with a graphite monochromator. Cell constants were determined from 24-well centered reflections in the range of $45<2\theta<55°$. Intensity data were collected to a maximum 2θ of 130° by the $\omega/2\theta$ scan technique. The total number of independent reflections measured was 2906, of which 2439 were considered to be observed [$F \geqq 4\sigma(F)$]. No absorption correction was applied. The structure was solved by direct methods. It was found that the carbonyl group of the A ring was statistically disordered between two orientations. All H atoms were located in difference Fourier maps except for those involved in the disorder. The positions of the latter were calculated geometrically. The structure was refined by full-matrix least-squares, with anisotropic temperature factors for non-H atoms and anisotropic temperature factors for H atoms. The weighting scheme employed was $\omega=1/\sigma(F)$. The refinement converged to R=0.070, wR=0.064. The residual densities were in the range of −0.45 to 0.54 eÅ^{-3}. All crystallographic calculations were done on a VAX3100 workstation using the program system *Xtal*3.2[16] with the scattering factors included in the program.

Alkaline Hydrolysis of 1 to Myricerone (3) A solution of 3% potassium hydroxide in MeOH (3 ml) was added to **1** (7.0 mg) and refluxed for 3 h. Water was added to the reaction mixture and MeOH was evaporated. The residual aq. solution was acidified with 1 N sulfuric acid and extracted with ethyl acetate (3×10 ml). The organic solution was washed with water and evaporated to afford a crystalline residue, which was recrystallized from MeOH to yield myricerone (**3**) (4.8 mg) as colorless needles, mp 226—227 °C (crystallized from MeOH), $[\alpha]_D$ +91.3° (c=1.01, $CHCl_3$). *Anal.* Calcd for $C_{30}H_{46}O_4 \cdot 1/5H_2O$: C, 75.97; H, 9.88. Found: C, 76.23; H, 10.18. IR (Nujol) cm^{-1}: 3403, 1719, 1689. HR-LSI-MS m/z: 493.3296 (Calcd for $C_{30}H_{46}O_4Na$ $[M+Na]^+$ 493.3294).

Alkaline Hydrolysis of Tetraacetate of 6 to Myricerol (7) and 27-*O*-*trans*-Caffeoylmyricerol (5) A solution of 5% potassium hydroxide in MeOH (7 ml) was added to the tetraacetate of **6** (29.6 mg) and refluxed for 2 h. The reaction mixture was diluted with water, and MeOH was removed to obtain an aq. solution, which was acidified with dil. sulfuric acid to pH 3 and extracted with ethyl acetate. The solvent was evaporated to give a residue which was chromatographed on preparative thin layer chromatography (silica gel plate: Merck No. 5715, $CHCl_3$–MeOH 93 : 7) to yield myricerol (**7**) (1.7 mg), myriceric acid B (**5**) (12.7 mg) and myriceric acid C (**6**) (4.2 mg).

Myricerol (**7**): Colorless needles, mp 250—251 °C (crystallized from MeOH), $[\alpha]_D$ +65.3° (c=1.02, $CHCl_3$). IR (Nujol) cm^{-1}: 3464, 1712, 1692. HR-LSI-MS m/z: 473.3623 (Calcd for $C_{30}H_{49}O_4$ $[M+H]^+$ 473.3631). ^{1}H-NMR (pyridine-d_5) δ_H: 0.88, 0.91, 1.02, 1.02, 1.05, 1.20 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 3.37 (2H, m, H-3, H-18), 3.82, 4.09 (each 1H, d, J=12.0 Hz, H-27), 5.88 (1H, br s, H-12). ^{13}C-NMR ($CDCl_3$) δ_C: 15.99 (C-25), 16.51 (C-24), 18.79 (C-6), 18.86 (C-26), 23.73 (C-11), 23.88 (C-30), 23.97 (C-16), 24.39 (C-15), 28.09 (C-2), 28.70 (C-23), 30.95 (C-20), 33.14 (C-22), 33.19 (C-29), 33.66 (C-7), 34.06 (C-21), 37.52 (C-10), 38.83 (C-1), 39.35 (C-4), 40.41 (C-8), 41.78 (C-18), 45.54 (C-19), 46.46 (C-17), 47.94 (C-14), 48.72 (C-9), 55.68 (C-5), 64.41 (C-27), 77.98 (C-3), 127.58 (C-12), 139.82 (C-13), 180.16 (C-28).

3,27-Diacetate of 7, 27-Acetate of 7, and 3-Acetate of 7 A solution of **7** (460 mg) in a mixture of pyridine (4 ml) and acetic anhydride (4 ml) was stirred for 30 min at room temperature. The reaction mixture was treated in the usual manner to yield a mixture of triterpenoid acetates (680 mg). The mixture was chromatographed on silica gel and then HPLC (column: Develosil ODS, MeOH–H_2O 85 : 15) to yield the 27-acetate of **7** (198 mg), 3,27-diacetate of **7** (181 mg), 3-acetate of **7** (23 mg) and starting material (**7**) (62 mg).

3,27-Diacetate of **7**: White powder, mp 185—186 °C. IR ($CHCl_3$) cm^{-1}: 1718, 1690 (sh). MS m/z: 556 ($M^{+\cdot}$, $C_{34}H_{52}O_6$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.72, 0.84, 0.86, 0.87, 0.93, 0.93, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.03, 2.05 (each 3H, s, $COCH_3$), 2.88 (1H, dd, J=4.6, 13.8 Hz, H-18), 4.03, 4.17 (each 1H, d, J=12.8 Hz, H-27), 4.47 (1H, dd, J=6.6, 9.4 Hz, H-3), 5.57 (1H, t, J=3.2 Hz, H-12).

27-*O*-Acetate of **7**: Colorless needles, mp 257—258 °C (crystallized from MeCN), $[\alpha]_D$ +103.5° (c=1.01, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{50}O_5 \cdot 1/4H_2O$: C, 74.02; H, 9.80. Found: C, 74.07; H, 9.69. IR (Nujol) cm^{-1}: 3517, 1712, 1691. MS m/z: 514 ($M^{+\cdot}$, $C_{32}H_{50}O_5$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.72, 0.76, 0.87, 0.90, 0.93, 0.98, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.02 (3H, s, $COCH_3$), 2.88 (1H, dd, J=4.2, 13.6 Hz, H-18), 3.22 (1H, dd, J=4.0, 9.8 Hz, H-3), 4.04, 4.18 (each 1H, d, J=12.4 Hz, H-27), 5.57 (1H, t, J=3.2 Hz, H-12).

3-Acetate of **7**: White powder, mp 211—212 °C. IR ($CHCl_3$) cm^{-1}: 3510, 1719, 1696. MS: m/z: 514 ($M^{+\cdot}$ $C_{32}H_{50}O_5$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.71, 0.84, 0.87, 0.92, 0.92, 0.96, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.04 (3H, s, $COCH_3$), 2.93 (1H, dd, J=4.4, 14.2 Hz, H-18), 3.21, 3.77 (each 1H, d, J=11.6 Hz, H-27), 4.50 (1H, dd, J=7.0, 9.4 Hz, H-3), 5.84 (1H, br s, H-12).

Conversion of Myricerol (7) to Myricerone (3) A solution of diacetate of **7** (1.482 g) in 5% potassium hydroxide in MeOH (500 ml) was stirred for 3 h at room temperature. The reaction mixture was neutralized with ammonium chloride (NH_4Cl) and extracted with ethyl acetate. Solvent evaporation gave a solid residue, which was chromatographed on silica gel and then HPLC as described above to yield 27-acetate of **7** (788 mg), myricerol (**7**) (324 mg), starting material (57 mg) and 3-acetate of **7** (39 mg). To a solution of 27-acetate of **7** (300 mg) in $CHCl_3$ (6 ml) was added Jones' reagent dropwise under ice-cooling. After 5 min, water was added and the reaction mixture was extracted with $CHCl_3$. Solvent evaporation gave a solid residue (291 mg), which was chromatographed on silica gel to give the acetate of myricerone (**3**) (270 mg). A solution of this acetate (258 mg) in 5% potassium hydroxide in MeOH (10 ml) was stirred for 24 h at room temperature, and then dil. hydrochloric acid was added to pH 3. The reaction mixture was extracted with $CHCl_3$. Solvent evaporation gave a residue (249 mg), which was chromatographed on silica gel and HPLC as described above to yield myricerone (**3**) (189 mg).

Acetate of Myricerone (**3**): White powder, $[\alpha]_D$ +107.5° (c=1.01, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 1725, 1696. MS m/z: 512 ($M^{+\cdot}$ $C_{32}H_{48}O_5$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.78, 0.88, 0.94, 1.02, 1.03, 1.08, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.01 (3H, s, $COCH_3$), 2.90 (1H, dd, J=4.4, 13.8 Hz, H-18), 4.05, 4.20 (each 1H, d, J=12.6 Hz, H-27), 5.59 (1H, t, J=3.2 Hz, H-12).

Myriceric Acid D (8) A methanol extract of *Myrica cerifera* twigs (1.0 kg, collected in Florida, U.S.A.) was treated as described above and gave crude **1** (210 mg) together with crude **5** (152 mg) and crude **6** (6.5 mg). Crude **1** (210 mg) was acetylated with pyridine (5 ml) and acetic anhydride (5 ml), then left overnight at room temperature to give a crude acetate (210 mg). Repeated preparative HPLC (Develosil ODS, MeOH–H_2O 9 : 1) afforded acetates of **1** (63 mg, 0.006%) and **9** (14 mg, 0.0012%). To a solution of acetate **9** (10 mg) in CH_2Cl_2 (1 ml) was added 2% potassium hydroxide in MeOH (1 ml), and this was stirred for 15 min at room temperature under argon. The reaction mixture was acidified with 0.5 N HCl to pH 3.0 after treatment with ammonium chloride and extracted

with ethyl acetate (2 × 25 ml). Solvent evaporation gave a residue (9.1 mg), which was purified with HPLC (LiChrosorb RP-18, MeOH–H_2O 85 : 15) to give myriceric acid D (**8**) (6.6 mg) as colorless plates, mp 220 °C (from MeOH), $[\alpha]_D$ +121.5° (c=0.50, MeOH). IR (Nujol) cm^{-1}: 3324, 1694, 1631, 1603. UV λ_{max} (MeOH) nm (ε): 246 (9200), 301 (12000), 331 (16400). HR-LSI-MS m/z: 655.3628 (Calcd for $C_{39}H_{52}O_7Na$ $[M+Na]^+$ 655.3611).

Myriceric Acid D Diacetate (**9**): White plates, mp 147—149 °C (crystallized from MeOH–H_2O 9 : 1), $[\alpha]_D$ +93.6° (c=0.52, MeOH). IR ($CHCl_3$) cm^{-1}: 1773, 1698, 1639. UV λ_{max} (MeOH) nm (ε): 216 (16800), 278 (22700). HR-LSI-MS m/z: 739.3827 (Calcd for $C_{43}H_{56}O_9Na$ $[M+Na]^+$ 739.3822). ^{1}H-NMR ($CDCl_3$) δ_H: 0.87 (3H, d, J=6.4 Hz, H-29 or H-30), 0.84 (3H, s), 0.91 (3H, d, J=9.6 Hz, H-30 or H-29), 1.02, 1.04, 1.08, (each 3H, s), 2.31, 2.32 (each 3H, s, $COCH_3$), 4.17, 4.39 (each 1H, d, J=12.8 Hz, H-27), 5.60 (1H, t, J=3 Hz, H-12), 6.30 (1H, d, J=16 Hz, H-2′), 7.23 (1H, d, J=8.2 Hz, H-8′), 7.33 (H, d, J=1.8 Hz, H-5′), 7.38 (1H, dd, J=1.8, 8.2 Hz, H-9′), 7.57 (1H, d, J=16 Hz, H-3′). ^{13}C-NMR ($CDCl_3$) δ_C: 15.57 (C-25), 17.48 (C-26), 17.89 (C-30), 19.54 (C-6), 20.65, 20.65 (2 × $COCH_3$), 21.06 (C-24), 21.41 (C-29), 23.41 (C-11), 23.81 (C-15), 23.95 (C-16), 26.77 (C-23), 30.12 (C-21), 32.94 (C-7), 33.97 (C-2), 36.64 (C-10), 36.87 (C-22), 38.51 (C-20), 39.02 (C-19), 39.21 (C-8), 40.16 (C-1), 45.58 (C-14), 47.23 (C-4), 47.61 (C-9), 47.71 (C-17), 52.16 (C-18), 54.91 (C-5), 65.82 (C-27), 119.28 (C-2′), 122.63 (C-8′), 124.01 (C-9′), 126.48 (C-12), 130.40 (C-5′), 132.88 (C-4′), 133.13 (C-13), 142.47 (C-6′), 142.99 (C-3′), 143.58 (C-7′), 166.23 (C-1′), 167.97, 168.12 (2 × $COCH_3$), 183.63 (C-28), 217.54 (C-3).

Ursomyricerone Methyl Ester (10) A solution of myriceric acid D diacetate (**9**), (71 mg) in 10% KOH in 90% aq. MeOH (10 ml) was refluxed for 7 h under argon. To the reaction mixture was added sat. NH_4Cl (20 ml), and this was extracted with AcOEt. Solvent evaporation gave a residue (71 mg), which was dissolved in MeOH (1 ml), and an ether solution of diazomethane was added. The reaction mixture was stirred for 30 min at room temperature. Evaporation of the solvent gave a residue (67 mg), which was purified by HPLC (LiChrosorb RP-18, MeOH) to afford ursomyricerone methyl ester (**10**) (42 mg, 88%) as a white powder. IR ($CHCl_3$) cm^{-1}: 3536, 1716, 1699. ^{1}H- and ^{13}C-NMR data are shown in Tables 1 and 2.

HPLC Analysis of Myriceric Acid D (8) To crude myriceric acid A (**1**) (1 mg) dissolved in a mixture of CH_2Cl_2 (100 μl) and MeOH (20 μl) was added 10% trimethylsilyldiazomethane in hexane (400 μl), and this was left overnight at room temperature. Removal of the solvent under N_2 afforded a residue. Myriceric acid A (**1**) and myriceric acid D (**8**) had retention times of 12.4 and 13.6 min as their methyl esters, respectively, when analyzed by HPLC (column: Develosil ODS (4.6 mm i.d. × 150 mm); mobile phase: MeOH–H_2O, 9 : 1, flow rate, 1.0 ml/min, detector: 330 nm).

27-*O*-*cis*-Caffeoylmyricerone (11) A solution of myriceric acid A (**1**) (31 mg) in CH_3CN (50 ml) was irradiated with a daylight lamp (National PRF 500W) for 6 h. Solvent evaporation gave a residue, which was chromatographed on HPLC (LiChrosorb RP-18, MeOH–H_2O, 9 : 1) to give 27-*O*-*cis*-caffeoylmyricerone (**11**) (11 mg, 35%) and a starting material (**1**) (12 mg, 39%).

27-*O*-*cis*-Caffeoylmyricerone (**11**): White powder. IR (Nujol) cm^{-1}: 3342, 1694. UV λ_{max} (MeOH) nm (ε): 245 (7000), 324 (8800). ^{1}H-NMR (CD_3OD) δ_H: 0.82, 0.87, 0.94, 0.97, 1.00, 1.04 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30). 2.92 (1H, dd, J=3.0, 12.8 Hz, H-18), 4.17, 4.39 (each 1H, d, J=12.8 Hz, H-27), 5.54 (1H, t, J=3.0 Hz, H-12), 5.68 (1H, d, J=12.8 Hz, H-2′), 6.71 (1H, d, J=8.2 Hz, H-8′), 6.74 (1H, d, J=12.8 Hz, H-3′), 7.01 (1H, dd, J=2.2, 8.2 Hz, H-9′), 7.27 (1H, d, J=2.2 Hz, H-5′).

27-*O*-*trans*-Cinnamoylmyricerone (12) To a solution of myricerone (**3**) (23.7 mg, 0.05 mmol) in CH_2Cl_2 (1.2 ml) was added dimethylaminopyridine (30.8 mg, 0.25 mmol) and cinnamoyl chloride at room temperature. The mixture was stirred overnight and concentrated under reduced pressure. Next, 0.1 N NaOH (2 ml) and tetrahydrofuran (THF) (6 ml) were added, and stirring was continued for 10 min. The mixture was acidified with dil. HCl and extracted with ethyl acetate. The organic layer was separated and washed with aqueous NaCl, then dried and concentrated to give an oily residue. Purification by silica gel chromatography (hexane–ethyl acetate, 2 : 1) gave 27-*O*-*trans*-cinnamoylmyricerone (**12**) (26 mg, 86%) as a white foam. HR-LSI-MS m/z: 623.3704 (Calcd for $C_{39}H_{52}O_5Na$ $[M+Na]^+$ 623.3709). ^{1}H-NMR ($CDCl_3$) δ_H: 0.83 (3H, s), 0.86 (3H, s), 0.93 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.07 (3H, s), 2.2—2.4 (2H, m), 2.92 (1H, br d, J=12.6 Hz), 4.17 (1H, d, J=12.6 Hz), 4.38 (1H, d, J=12.6 Hz), 5.65 (1H, br s), 6.37 (1H, d, J=15.6 Hz), 7.40—7.53 (5H, m), 7.63 (1H, d, J=15.6 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: 15.27, 18.09, 21.41, 22.65, 23.45, 23.99, 26.56, 30.60, 32.35, 32.55, 32.91, 33.61, 33.99, 36.93, 38.96, 39.96, 40.86, 44.71, 45.30, 46.36, 47.31, 47.85, 54.99, 65.61, 118.25, 126.95, 128.08, 128.24, 128.97, 130.44, 134.32, 137.32, 144.78, 166.59, 183.87, 217.35.

6′,7′-Disulfonylmyriceric Acid A (15) To a dimethylformamide (DMF) (0.22 ml) solution of myriceric acid A (**1**) (20 mg, 0.0316 mmol) was added 4 N NaOH (0.016 ml) and sulfur trioxide–trimethylamine (8.8 mg) at 0 °C, and then the mixture was stirred for 2 h at room temperature. The addition of 4 N NaOH (0.016 ml) and sulfurtrioxide–trimethylamine (8.8 mg) was repeated 4 times to complete the reaction. The resulting mixture was condensed under reduced pressure, dissolved in H_2O and chromatographed on HP-20 resin (H_2O then aq. MeOH) to give **15** (14 mg, 59%) as a white powder. ^{1}H-NMR (D_2O, HOD at δ_H 4.47) δ_H: 0.45 (3H, s), 0.50 (3H, s), 0.55 (3H, s), 0.65 (9H, s), 0.8—2.3 (23H, m), 3.85 (1H, d, J=12.8 Hz), 4.06 (1H, d, J=12.8 Hz), 5.31 (1H, br s), 6.12 (1H, d, J=16.0 Hz), 7.1—7.3 (2H, m), 7.26 (1H, d, J=16.0 Hz), 7.38 (1H, d, J=1.6 Hz).

6′-Sulfonylmyriceric Acid A (13) and 7′-Sulfonylmyriceric Acid A (14) To a solution of **15** (50 mg) in ethyl acetate–2-butanone–H_2O (0.6 ml, 0.6 ml, 1.2 ml) was added 1 N H_2SO_4 (2 ml), and the mixture was stirred for 2 h at room temperature. The resulting mixture was neutralized with 1 N NaOH and condensed under reduced pressure. The residue (36 mg) was dissolved in ethanol (0.36 ml) and purified by silica gel chromatography (ethyl acetate–acetic acid–H_2O, 40 : 1 : 1) to give **13** (7 mg), **14** (20 mg) and their mixture. Each fraction was rechromatographed on HPLC (Hibar LiChrosorb Si 60, ethyl acetate–acetic acid–H_2O, 40 : 1 : 1) to give pure 6′-sulfonylmyriceric acid A (**13**) and 7′-sulfonylmyriceric acid A (**14**).

6′-Sulfonylmyriceric Acid A (**13**): Colorless foam. ^{1}H-NMR (D_2O, HOD at δ_H 4.79) δ_H: 0.76 (3H, s), 0.80 (3H, s), 0.87 (3H, s), 0.98 (9H, s), 1.0—2.8 (23H, m), 4.12 (1H, d, J=13.2 Hz), 4.34 (1H, d, J=13.2 Hz), 5.60 (1H, br s), 6.24 (1H, d, J=16.0 Hz), 6.95 (1H, d, J=8.4 Hz), 7.33 (1H, dd, J=2.0, 8.4 Hz), 7.54 (1H, d, J=2.0 Hz), 7.50 (1H, d, J=16 Hz).

7′-Sulfonylmyriceric Acid A (**14**): Colorless foam. ^{1}H-NMR (D_2O, HOD at δ_H 4.77) δ_H: 0.75 (3H, s), 0.79 (3H, s), 0.86 (3H, s), 0.96 (9H, s), 1.0—2.8 (23H, m), 4.12 (1H, d, J=12.6 Hz), 4.35 (1H, d, J=12.6 Hz), 5.60 (1H, br s), 6.30 (1H, d, J=16.0 Hz), 7.02 (1H, dd, J=1.2, 8.2 Hz), 7.12 (1H, d, J=1.2 Hz), 7.37 (1H, d, J=8.2 Hz), 7.48 (1H, d, J=16 Hz).

3-Oxo-27-*trans*-cinnamoyloxyolean-1,12-dien-28-oic Acid (16) To a benzene (6 ml) solution of 27-*O*-*trans*-cinnamoylmyricerone (**12**) (120 mg, 0.2 mmol) was added benzeneselenenic anhydride (70% purity, 113 mg, 0.22 mmol), and the solution was refluxed for 1.5 h. Ethyl acetate (20 ml) and H_2O (5 ml) were added to the mixture and the organic layer was separated. The organic layer was dried, concentrated and purified by silica gel chromatography to give 3-oxo-27-*trans*-cinnamoyloxyolean-1,12-dien-28-oic acid (**16**) (93 mg, 78% yield) as a white foam. HR-LSI-MS m/z: 621.3560 (Calcd for $C_{39}H_{50}O_5Na$ $[M+Na]^+$ 621.3554). ^{1}H-NMR δ_H: 0.87 (6H, s), 0.94 (3H, s), 1.07 (3H, s), 1.14 (3H, s), 1.18 (3H, s), 2.92 (1H, br d, J=13.6 Hz), 4.21 (1H, d, J=12.8 Hz), 4.37 (1H, d, J=12.8 Hz), 5.70 (1H, br s), 5.78 (1H, d, J=10.2 Hz), 6.35 (1H, d, J=16.0 Hz), 7.00 (1H, d, J=10.2 Hz), 7.39—7.53 (5H, m), 7.59 (1H, d, J=16.0 Hz). ^{13}C-NMR δ_C: 18.65, 18.83, 19.05, 21.59, 22.68, 23.44, 23.55, 23.74, 27.77, 30.61, 32.32, 32.81, 32.89, 33.61, 39.60, 40.65, 41.00, 42.70, 44.54, 44.60, 45.53, 46.41, 53.29, 65.77, 118.19, 125.39, 125.52, 126.48, 128.05, 128.97, 130.48, 134.29, 137.90, 144.83, 158.28, 166.56, 183.86, 204.94.

3α-Myriceric Acid B (17) To a solution of diacetate of myriceric acid A (**1**) (199 mg, 0.28 mmol) in dry THF (10 ml) was added 1.0 M K-selectride in THF (1.4 ml) under nitrogen, and the mixture was stirred for 4 h at −78 °C. To the reaction mixture were added 10% KOH (1.2 ml) and 30% hydrogen peroxide (1.2 ml). The solution was acidified with 0.5 N HCl to pH 4 at room temperature and extracted with ethyl acetate. Solvent evaporation gave a residue, which was chromatographed on HPLC (LiChrosorb RP-18, MeOH–H_2O, 85 : 15) to give 3α-myriceric acid B (**17**) (60 mg, 34.1%) and a small amount of myriceric acid B (**5**) (4 mg, 2.3%) together with myriceric acid A (**1**) (84 mg).

3α-Myriceric Acid B (**17**): White powder, $[\alpha]_D$ +115.4° (c=0.50, MeOH). IR (Nujol) cm^{-1}: 3482, 3348, 1689, 1670. UV λ_{max} (MeOH) nm (ε): 217 (14300), 245 (9400), 300 (12700), 330 (17300). HR-LSI-MS m/z: 657.3773 (Calcd for $C_{39}H_{54}O_7Na$ $[M+Na]^+$ 657.3767). ^{1}H-NMR ($CDCl_3$) δ_H: 0.79, 0.84, 0.85, 0.92, 0.94, 0.95 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30). 2.90 (1H, dd, J=5.0, 13.6 Hz, H-18), 3.42 (1H,

br s, H-3), 4.22, 4.35 (each 1H, d, J=13.0 Hz, H-27), 5.63 (1H, t, J=2.6 Hz, H-12), 6.18 (1H, d, J=15.8 Hz, H-2′), 6.86 (1H, d, J=8.0 Hz, H-8′), 6.97 (1H, dd, J=2.0, 8.0 Hz, H-9′), 7.07 (1H, d, J=2.0 Hz, H-5′), 7.50 (1H, d, J=15.8 Hz, H-3′).

3-Deoxy-27-*trans*-Cinnamoyloxyoleanolic Acid (18) 27-*O*-Acetyl Myricerol Diphenylmethyl Ester: To a solution of 27-*O*-acetylmyricerol (48 mg) in dry benzene (5 ml) was added diphenyldiazomethane (30 mg, 1.5 eq), and the mixture was refluxed for 2 h. After removal of the solvent, the residue was chromatographed on HPLC (LiChrosorb RP-18, MeOH–CH_2Cl_2 95 : 5) to give 27-*O*-acetylmyricerol diphenylmethyl ester (44 mg, 69%). IR ($CHCl_3$) cm^{-1}: 3478, 1722. ^{1}H-NMR ($CDCl_3$) δ_H: 0.22, 0.75, 0.79, 0.87, 0.95, 0.96 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.01 (3H, s, $COCH_3$), 3.01 (1H, dd, J=5.0, 12.5 Hz, H-18), 3.18 (1H, m, H-3), 4.01, 4.16 (each 1H, d, J=12.5 Hz, H-27), 5.53 (1H, t, J=3.5 Hz, H-12), 6.84 (1H, s, C<u>H</u>=Ph_2), 7.12—7.45 (10H, m, Arm. H).

3-*O*-Phenoxythiocarbonyl-27-*O*-acetylmyricerol Diphenylmethyl Ester: To a solution of 27-*O*-acetylmyricerol diphenylmethyl ester (44 mg) in dry CH_2Cl_2 (2 ml) were added pyridine (40 μl, 7.7 eq) and phenyl chlorothionocarbonate (20 μl, 1.7 eq), and the mixture was stirred for 2 h at room temperature. The reaction mixture was flash chromatographed on silica gel (1 g, active II, $CHCl_3$–MeOH, 9 : 1), and the residue was chromatographed on HPLC (LiChrosorb RP-18, MeOH–CH_2Cl_2 95 : 5) to give 3-*O*-phenoxythiocarbonyl-27-*O*-acetylmyricerol diphenylmethyl ester (40 mg, 76%). IR ($CHCl_3$) cm^{-1}: 1724. ^{1}H-NMR ($CDCl_3$) δ_H: 0.22, 0.84, 0.87, 0.87, 0.94, 0.96 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.02 (3H, s, $COCH_3$), 3.01 (1H, dd, J=4.4, 13.6 Hz, H-18), 4.02, 4.14 (each 1H, d, J=12.6 Hz, H-27), 4.94 (1H, dd, J=4.8, 11.6 Hz, H-3), 5.53 (1H, t, J=3.5 Hz, H-12), 6.84 (1H, s, C<u>H</u>=Ph_2), 7.12—7.45 (15H, m, Arm. H).

3-Deoxy-27-acetoxyoleanolic Acid Diphenylmethyl Ester: To a solution of 3-*O*-phenoxythiocarbonyl-27-*O*-acetylmyricerol diphenylmethyl ester (40 mg) in dry toluene (2 ml) were added tributyltin hydride (40 μl, 3.0 eq) and di-*tert*-butylperoxide (4 μl), and the mixture was refluxed for 2 h under argon. The reaction mixture was evaporated and the residue was crystallized from MeOH to give 3-deoxy-27-acetoxyoleanolic acid diphenylmethyl ester (33 mg, quantitative). IR ($CHCl_3$) cm^{-1}: 1722. ^{1}H-NMR ($CDCl_3$) δ_H: 0.22, 0.78, 0.78, 0.83, 0.87, 0.94 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.01 (3H, s, $COCH_3$), 3.01 (1H, dd, J=3.8, 14.6 Hz, H-18), 4.01, 4.17 (each 1H, d, J=12.5 Hz, H-27), 5.53 (1H, t, J=3.5 Hz, H-12), 6.84 (1H, s, C<u>H</u>=Ph_2), 7.2—7.4 (10H, m, Arm. H).

3-Deoxy-27-acetoxyoleanolic Acid: To a solution of 3-deoxy-27-acetoxyoleanolic acid diphenylmethyl ester (32 mg) in THF (3 ml) was added palladium carbon (5%, 10 mg), and the mixture was stirred for 3 h under hydrogen. The reaction mixture was flash chromatographed on silica gel, and the residue was purified by HPLC (LiChrosorb RP-18, MeOH–CH_2Cl_2, 95 : 5) to give 3-deoxy-27-acetoxyoleanolic acid (22 mg, 92%). IR ($CHCl_3$) cm^{-1}: 1726, 1695. ^{1}H-NMR ($CDCl_3$) δ_H: 0.72, 0.79, 0.85, 0.87, 0.90, 0.93 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.02 (3H, s, $COCH_3$), 2.88 (1H, dd, J=3.8, 12.6 Hz, H-18), 4.04, 4.19 (each 1H, d, J=12.6 Hz, H-27), 5.56 (1H, t, J=3.2 Hz, H-12).

3-Deoxy-27-hydroxyoleanolic Acid: A solution of 3-deoxy-27-acetoxyoleanolic acid (22 mg) in 5% potassium hydroxide in 10% aqueous MeOH (3 ml) was refluxed for 1 h. The reaction mixture was diluted with water and extracted with ethyl acetate after acidification with 5 N HCl. Solvent evaporation gave a residue, which was crystallized from MeOH to give 3-deoxy-27-hydroxyoleanolic acid (20 mg, quantitative) as white needles. mp 221—222 °C (from MeOH). IR ($CHCl_3$) cm^{-1}: 3504, 1695. MS m/z: 456 ($M^{+\cdot}$, $C_{30}H_{48}O_3$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.70, 0.78, 0.85, 0.88, 0.91, 0.96 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.91 (1H, dd, J=4.2, 13.0 Hz, H-18), 3.19, 3.79 (each 1H, d, J=11.6 Hz, H-27), 5.84 (1H, t, J=3 Hz, H-12).

3-Deoxy-27-*trans*-cinnamoyloxyoleanolic Acid (**18**): To a solution of 3-deoxy-27-hydroxyoleanolic acid (19 mg) in pyridine (5 ml) was added *trans*-cinnamic anhydride (15 mg), and the mixture was stirred for 3 d at 80 °C. Evaporation of the solvent gave a residue, which was flash chromatographed on silica gel (*n*-hexane–$CHCl_3$ 1 : 1) and then purified by HPLC (LiChrosorb RP-18, MeOH) to give 3-deoxy-27-*trans*-cinnamoyloxyoleanolic acid (**18**) (7.8 mg, 32%) as white needles, mp 223—225 °C (from MeOH). IR ($CHCl_3$) cm^{-1}: 1697, 1635. HR-LSI-MS m/z: 609.3925 (Calcd for $C_{39}H_{54}O_4Na$ $[M+Na]^+$ 609.3920). ^{1}H-NMR ($CDCl_3$) δ_H: 0.77, 0.79, 0.84, 0.84, 0.91, 0.92 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.91 (1H, br d, J=12.4 Hz, H-18), 4.18, 4.35 (each 1H, d, J=12.8 Hz, H-27), 5.63 (1H, br s, H-12), 6.38 (1H, d, J=16.2 Hz, H-2′), 7.40 (3H, m, H-6′, 7′, 8′), 7.52 (2H, m, H-5′, 9′), 7.63 (1H, d, J=16.2 Hz, H-3′).

3-Hydroxyimino-27-*trans*-cinnamoyloxyoleanolic Acid (19) An ethanol solution (9 ml) of 27-*O*-*trans*-cinnamoylmyricerone (**12**) (180 mg, 0.3 mmol) and hydoxylamine hydrochloride (104 mg, 11.5 mmol) was refluxed for 5 h and concentrated under reduced pressure. The residue was partitioned with CH_2Cl_2 (25 ml) and H_2O (5 ml), and the organic layer was separated. The organic layer was washed, dried and concentrated to give a white residue. Ethanol (3 ml) was added to the residue and the resulting precipitate was collected by filtration to yield 3-hydroxyimino-27-*trans*-cinnamoyloxyoleanolic acid (**19**) as a white powder (93 mg, 50%). HR-LSI-MS m/z: 638.3820 (Calcd for $C_{39}H_{53}NO_5$-Na $[M+Na]^+$ 638.3819). ^{1}H-NMR ($CDCl_3$) δ_H: 0.81 (3H, s), 0.84 (3H, s), 0.91 (3H, s), 1.01 (3H, s), 1.04 (3H, s), 1.14 (3H, s), 2.88—3.05 (2H, m), 4.16 (1H, d, J=12.8 Hz), 4.33 (1H, d, J=12.8 Hz), 5.64 (1H, br s), 6.35 (1H, d, J=16.0 Hz), 7.38—7.53 (5H, m), 7.61 (1H, d, J=16.0 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: 15.15, 17.40, 18.18, 19.05, 22.74, 23.50, 23.60, 24.02, 27.47, 30.62, 32.39, 32.77, 32.93, 33.64, 37.21, 38.04, 40.01, 40.13, 40.95, 44.74, 45.30, 46.24, 48.11, 55.35, 65.68, 118.39, 126.98, 128.10, 128.97, 130.38, 134.39, 137.35, 144.69, 166.61, 167.10, 183.19.

27-*O*-*trans*-Cinnamoylmyricerone Methyl Ester (20) To a dry pyridine solution of myricerone methyl ester (**2**) (92 mg, 0.19 mmol) were added cinnamoyl chloride (73 mg, 0.44 mmol) and dimethylaminopyridine (10 mg), and the mixture was stirred for 16 h at room temperature. The solvent was evaporated to give a residue, which was chromatographed on HPLC (Intersil-PREP-ODS-s, MeOH) to give 27-*O*-*trans*-cinnamoylmyricerone methyl ester (**20**) (80 mg, 68%) as a white powder, $[\alpha]_D$ +144.9° (c=0.50, MeOH). IR ($CHCl_3$) cm^{-1}: 1699, 1636. HR-LSI-MS m/z: 637.3876 (Calcd for $C_{40}H_{54}O_5Na$ $[M+Na]^+$ 637.3869). ^{1}H-NMR ($CDCl_3$) δ_H: 0.79, 0.84, 0.92, 1.04, 1.04, 1.07 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.96 (1H, dd, J=4.8, 14.4 Hz, H-18), 3.66 (3H, s, $COOCH_3$), 4.15, 4.38 (each 1H, d, J=13.0 Hz, H-27), 5.65 (1H, t, J=3.6 Hz, H-12), 6.36 (1H, d, J=16.0 Hz, H-2′), 7.40—7.52 (5H, m, Arm. H), 7.62 (1H, d, J=16.0 Hz, H-3′). ^{13}C-NMR ($CDCl_3$) δ_C: 15.26 (C-25), 17.86 (C-26), 19.56 (C-6), 21.44 (C-24), 22.79 (C-16), 23.47 (C-15), 23.62 (C-30), 23.96 (C-11), 26.57 (C-23), 30.59 (C-20), 32.24 (C-7), 32.56 (C-22), 32.94 (C-29), 33.64 (C-21), 34.00 (C-2), 36.87 (C-10), 38.98 (C-1), 39.90 (C-8), 41.17 (C-18), 44.71 (C-19), 45.30 (C-14), 46.49 (C-17), 47.31 (C-4), 47.85 (C-9), 51.69 ($COOCH_3$), 54.99 (C-5), 65.60 (C-27), 118.30 (C-2′), 126.66 (C-12), 128.05 (C-6′), 128.05 (C-8′), 128.94 (C-5′), 128.94 (C-9′), 130.38 (C-7′), 134.31 (C-4′), 137.52 (C-13), 144.67 (C-3′), 166.56 (C-1′), 178.08 (C-28), 217.47 (C-3).

3-Oxo-27-*trans*-cinnamoyloxy-28-desmethylolean-12-ene (21)[17] 3,27-Diacetoxy-28-desmethylolean-12-ene: To a solution of phenyl chlorothionocarbonate (19 mg, 0.107 mmol) in pyridine (50 μl) was added a solution of 3,27-*O*-diacetylmyricerol (46 mg, 0.082 mmol) in CH_2Cl_2 (2 ml) for 2 h at room temperature under argon. Solvent evaporation gave a residue, which was purified by HPLC (LiChrosorb RP-18, MeOH) to afford a mixed anhydride (40 mg, 77%) as a white foam. IR ($CHCl_3$) cm^{-1}: 1773, 1723. ^{1}H-NMR ($CDCl_3$) δ_H: 0.80, 0.85, 0.86, 0.90, 0.93, 0.96, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.04, 2.05 (each 3H, s, $COCH_3$), 2.88 (1H, dd, J=3.0, 12.4 Hz, H-18), 4.04, 4.20 (each 1H, d, J=12.6 Hz, H-27), 4.47 (1H, dd, J=7.4, 8.6 Hz, H-3), 5.66 (1H, t, J=3.5 Hz, H-12), 7.19 (2H, dt, J=2, 8 Hz, H-2′, 6′), 7.27 (1H, tt, J=2, 8 Hz, H-4′), 7.39 (2H, tt, J=2, 8 Hz, H-3′, 5′).

To a solution of the mixed anhydride (44 mg, 0.063 mmol) in toluene (2 ml) were added tributyltin hydride (43 mg, 0.15 mmol) and di-*tert*-butylperoxide (4 μl), and the mixture was stirred for 2.5 h at room temperature under argon. Next, water (3 ml) was added to the reaction mixture, and it was extracted with CH_2Cl_2 (2 × 5 ml). Solvent evaporation gave a residue, which was purified by HPLC (LiChrosorb RP-18, MeOH) to afford 3,27-diacetoxy-28-desmethylolean-12-ene (22 mg, 67%) as colorless needles, mp 179—182 °C (crystallized from MeOH). IR ($CHCl_3$) cm^{-1}: 1723. HR-LSI-MS m/z: 535.3760 (Calcd for $C_{33}H_{52}O_4Na$ $[M+Na]^+$ 535.3763). ^{1}H-NMR ($CDCl_3$) δ_H: 0.84, 0.84, 0.86, 0.87, 0.89, 0.94 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.03, 2.05 (each 3H, s, $COCH_3$), 2.40 (1H, td, J=4.0, 13.8 Hz, H-18), 4.04, 4.19 (each 1H, d, J=12.6 Hz, H-27), 4.48 (1H, dd, J=5.6, 10.2 Hz, H-3), 5.46 (1H, t, J=3.5 Hz, H-12). ^{13}C-NMR ($CDCl_3$) δ_C: 15.56 (CH_3), 16.73 (CH_3), 18.27 (CH_2), 18.70 (CH_3), 21.33 (2 × CH_3), 22.01 (CH_2), 23.45 (CH_2), 23.71 (CH_2), 23.85 (CH_3), 26.50 (CH_2), 27.78 (CH_2), 28.07 (CH_2), 31.08 (C), 33.37 (2 × CH_2), 33.54 (CH_3), 35.05 (CH), 37.17 (C), 37.65 (C), 38.08 (CH_2), 39.70 (C), 40.38 (CH), 44.04 (C), 45.72 (CH_2), 48.70 (CH), 55.33 (CH), 65.77 (CH_2), 80.85 (CH), 125.59 (CH), 139.68 (C), 170.89

(C), 171.02 (C).

3β-Hydroxy-27-acetoxy-28-desmethylolean-12-ene: A solution of 3β,27-diacetoxy-28-desmethylolean-12-ene (22 mg) in 5% potassium hydroxide in MeOH (3 ml) was stirred for 4 h at room temperature. The reaction mixture was diluted with water (3 ml) and extracted with CH_2Cl_2 (2 × 5 ml). Solvent evaporation gave a residue, which was purified by HPLC (LiChrosorb RP-18, MeOH) to afford 3β-hydroxy-27-acetoxy-28-desmethylolean-12-ene (8 mg, 40%) together with a small amount of 3β-acetoxy-27-hydroxy-28-desmethylolean-12-ene and 3β,27-dihydroxy-28-desmethylolean-12-ene.

3β-Hydroxy-27-acetoxy-28-desmethylolean-12-ene: White needles, mp 174—175 °C (crystallized from MeOH). IR ($CHCl_3$) cm^{-1}: 3604, 3458, 1725. ^{1}H-NMR ($CDCl_3$) δ_H: 0.78, 0.84, 0.84, 0.89, 0.92, 0.99, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.02 (3H, s, $COCH_3$), 2.40 (1H, td, J = 4.0, 14.0 Hz, H-18), 3.22 (1H, dd, J = 6.6, 10.2 Hz, H-3), 4.06, 4.20 (each 1H, d, J = 12.6 Hz, H-27), 5.46 (1H, t, J = 3.5 Hz, H-12).

3-Oxo-27-acetoxy-28-desmethylolean-12-ene: To a solution of 3β-hydroxy-27-acetoxy-28-desmethylolean-12-ene (14 mg) in acetone (2 ml) was added Jones' reagent, and the mixture was stirred for 0.5 h at 0 °C. The reaction mixture was neutralized with 5% $NaHCO_3$ (5 drops). Solvent evaporation gave a residue, which was chromatographed on silica gel (*n*-hexane–AcOEt, 95 : 5) to afford 3-oxo-27-acetoxy-28-desmethylolean-12-ene (13 mg, 93%) as a white powder. IR ($CHCl_3$) cm^{-1}: 1727, 1699. ^{1}H-NMR ($CDCl_3$) δ_H: 0.85, 0.90, 0.90, 1.05, 1.05, 1.09, (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.02 (3H, s, $COCH_3$), 2.30—2.65 (3H, m, H-2, H-18), 4.07, 4.22 (each 1H, d, J = 12.6 Hz, H-27), 5.48 (1H, t, J = 3.6 Hz, H-12).

3-Oxo-27-hydroxy-28-desmethylolean-12-ene: A solution of 3-oxo-27-acetoxy-28-desmethylolean-12-ene (13 mg) in 5% potassium hydroxide in MeOH (3 ml) was refluxed for 1 h. The reaction mixture was diluted with water and then extracted with ethyl acetate. Solvent evaporation gave a residue, which was chromatographed on HPLC (LiChrosorb RP-18, MeOH) to afford 3-oxo-27-hydroxy-28-desmethylolean-12-ene (10 mg, 85%) as colorless plates, mp 200—202 °C (crystallized from MeOH). IR ($CHCl_3$) cm^{-1}: 3514, 1697. MS *m/z*: 426 ($M^{+\cdot}$ $C_{29}H_{46}O_2$). ^{1}H-NMR ($CDCl_3$) δ_H: 0.87, 0.89, 0.93, 1.02, 1.04, 1.09 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.30—2.60 (3H, m, H-2, H-18), 3.27, 3.78 (each 1H, d, J = 11.5 Hz, H-27), 5.76 (1H, t, J = 3.5 Hz, H-12).

3-Oxo-27-*trans*-cinnamoyloxy-28-desmethylolean-12-ene (21) A solution of 3-oxo-27-hydroxy-28-desmethylolean-12-ene (9 mg) and cinnamic anhydride (10 mg) in pyridine (1 ml) was stirred for 3 d at 70 °C under argon. The reaction mixture was diluted with water and then extracted with $CHCl_3$. Solvent evaporation gave a residue, which was chromatographed on HPLC (LiChrosorb RP-18, MeOH–$CHCl_3$, 95 : 5) to afford 3-oxo-27-*trans*-cinnamoyloxy-28-desmethylolean-12-ene (**21**) (8 mg, 66%) as an amorphous powder. IR ($CHCl_3$) cm^{-1}: 1696, 1636. HR-LSI-MS *m/z*: 579.3816 (Calcd for $C_{38}H_{52}O_3Na$ $[M+Na]^+$ 579.3814). ^{1}H-NMR ($CDCl_3$) δ_H: 0.83, 0.89, 0.94, 1.04, 1.04, 1.08 (each 3H, s, H-23, H-24, H-25, H-26, H-29, H-30), 2.43 (3H, m, H-2, H-18), 4.18, 4.40 (each 1H, d, J = 12.8 Hz, H-27), 5.54 (1H, t, J = 3.4 Hz, H-12), 6.38 (1H, d, J = 16.1 Hz, H-2′), 7.40 (3H, m, H-6′, 7′, 8′), 7.52 (2H, m, H-5′, 9′), 7.63 (1H, d, J = 16.1 Hz, H-3′).

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