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Coumaroyl triterpenes from *Casuarina equisetifolia*

Hironobu Takahashi, Miho Iuchi, Yoshimi Fujita, Hiroyuki Minami, Yoshiyasu Fukuyama*

Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima, 770-8514, Japan

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Abstract

Seven new β-amyrin derived oleanane-type triterpene coumaroyl esters were isolated from the twigs and leaves of *Casuarina equisetifolia*, together with two known triterpenoids, erythrodiol and oleanolic acid, and a number of benzoic acid derivatives. The structures of the seven new compounds have been elucidated as 3-*O*-(*E*)-coumaroyl β-amyrin, 3-*O*-(*Z*)-coumaroyl β-amyrin, 3-*O*-dihydrocoumaroyl β-amyrin, 3-*O*-(*E*)-coumaroyl erythrodiol, 3-*O*-(*Z*)-coumaroyl erythrodiol, 3-*O*-(*E*)-coumaroyl oleanolic acid and 3-*O*-(*Z*)-coumaroyl oleanolic acid by spectroscopic analyses and chemical degradation. Evaluation of the antioxidant activities of the isolated compounds indicates that gallic acid is one of the antioxidant substances present in *C. equisetifolia*.

Keywords: *Casuarina equisetifolia*; Casuarinaceae; Coumaroyl triterpenes

1. Introduction

Overgeneration of reactive superoxide radicals has been known to induce lipid peroxidation and to damage membranes in biological systems, thus resulting in the initiation and/or progression of a number of diseases such as cancer, senescence and inflammation (Fridorich, 1978). It is therefore important to search for natural products which can protect membranes against oxidative damage by inhibiting or quenching free radicals and reactive species. In our antioxidant screening program of natural products using three in vitro assay systems, such as anti-lipid peroxidation, free radical scavenging and superoxide anion scavenging activities (Stocks et al., 1974; Blois, 1958; McCord and Fridovich, 1969), we have previously reported the isolation and structures of several antioxidant xanthones from *Garcinia subelliptica* (Minami, Kinoshita, Fukuyama, Kodama, Yoshizawa, Sugiura, Nakagawa & Tago, 1994; Fukuyama et al., 1991). The methanol extract of *Casuarina equisetifolia* (Casuarinaceae) exhibited promising antioxidant activity in a three in vitro assay system. *C. equisetifolia* indigenous to north Australia, has been planted for hedges, as a windbreak and for street trees in Okinawa and Ogasawara islands, Japan, and a literature survey indicates that no chemical study of this plant has been done. Therefore, we decided to investigate the chemical components of the methanol extract of *C. equisetifolia* aimed at the isolation of the antioxidant substances. As a result, seven new oleanane-type triterpene coumaroyl esters **1**–**7** were isolated, these being derived from β-amyrin (**8**). Two known oleanane-type triterpenoids **9**–**10** and a number of known aromatic compounds **11**–**16** were also obtained. This paper describes the structural elucidation of the seven new oleanane-type triterpene coumaroyl esters and their antioxidant properties.

* Corresponding author. Tel: +81 886 22 9611; fax: +81 886 55 3051.

E-mail address: fukuyama@kongou.mandala.co.jp (Y. Fukuyama)

2. Results and discussion

The leaves of *C. equisetifolia* were extracted with

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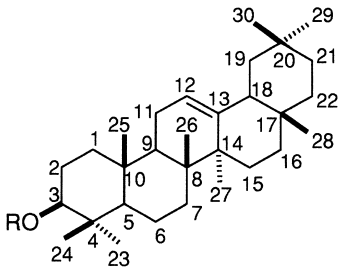
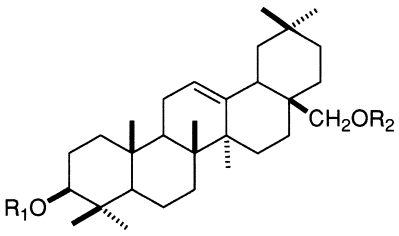
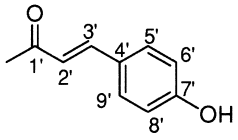
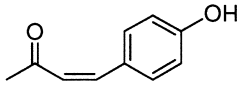
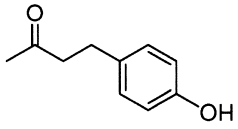




1 R = 

2 R = 

3 R = 

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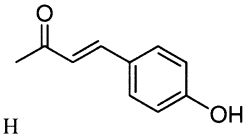
4 R_1 = 

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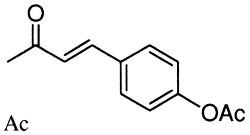
4a R_1 = 

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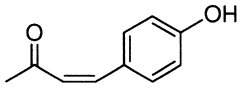
5 R_1 = 

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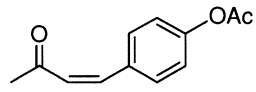
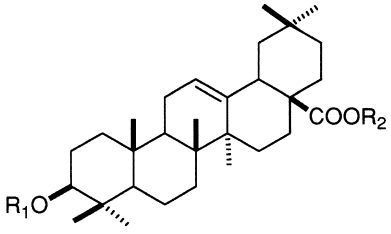
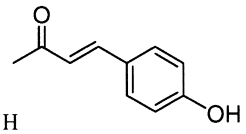
5a R_1 = 

R_2 = Ac

9 R_1 = H, R_2 = H



6 R_1 = 

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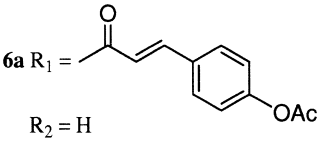
6a R_1 = 

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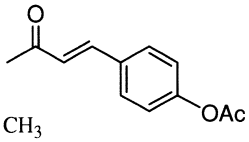
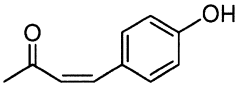
6b R_1 = 

R_2 = CH_3

7 R_1 = 

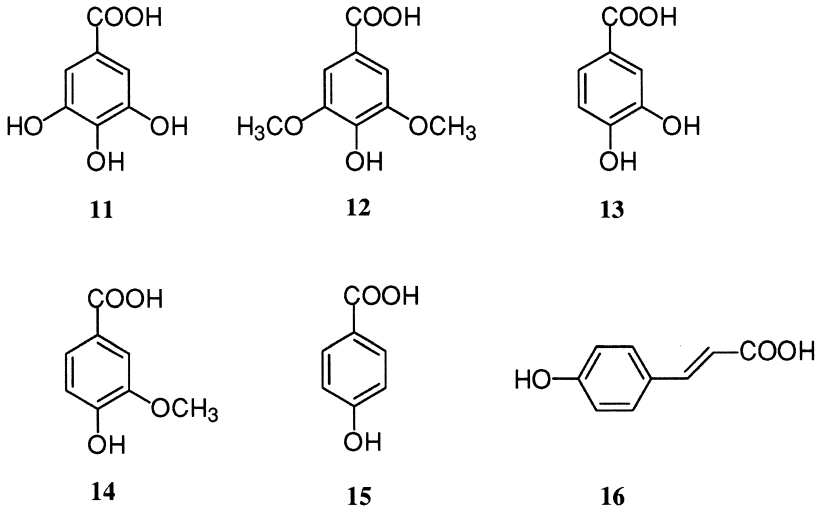
R_2 = H

10 R_1 = R_2 = H

Table 1
Antioxidant activity of compounds **11**–**16**

Concentration (μg/ml)	ALP[a] (%)			DPPH[b] (%)			O_2^-[c] (%)		
	5	2.5	1	10	5	1	10	5	1
MeOH extract	16.1	5.2			26.7	85.7	60.7	67.9	
EtOAc extract	21.5	6.9			60.4	38.6	100.0	85.7	21.4
11			5.6	92.6		20.8	92.0		100.0
12	6.1				82.4		20.8	22.2	
13	25.2				86.4	23.7	[e]		
14	[d]			[d]			[d]		
15	[d]			[d]			[d]		
16	[d]			[d]			[d]		
Catechin	45.2		88.5	54.5	16.4	57.1		14.3	

[a] Anti-lipid peroxidation activity (percent inhibition in rat homogenates).
[b] Chemically stable radical scavenging activity (percent inhibition of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical).
[c] Superoxidation anion scavenging activity (percent inhibition in xanthine and xanthine oxidase system).
[d] No inhibition at 50 μg/ml.
[e] 15% inhibition at 15 μg/ml..



MeOH. The MeOH extract, which exhibited antioxidant activity (Table 1), was fractionated successively over celite eluting with *n*-hexane, CH_2Cl_2, EtOAc and MeOH. The EtOAc-soluble portion, in which the antioxidant activity was concentrated, was further chromatographed on Si gel and Sephadex LH-20 to give seven known aromatic compounds (**11**–**16**). On the other hand, the *n*-hexane and CH_2Cl_2 soluble portions, which showed much less antioxidant activity than the MeOH extract, were fractioned by a combination of Si gel and Sephadex LH-20 chromatographies to give the new coumaroyl triterpenes **1**–**3** and **4**–**7**, respectively.

Compound **1** had the molecular formula $C_{39}H_{56}O_3$ (HR-EIMS, *m*/*z* 572.4229 $[M]^+$). The IR spectrum of **1** displayed absorption bands attributable to a hydroxyl group (3351 cm^{-1}) and a carbonyl group (1682 cm^{-1}). The presence of a *p*-coumaroyl moiety was suggested from the ^1H NMR resonances at δ_H 6.30 (1H, *d*, $J=16.0$ Hz), 6.85 (2H, *d*, $J=8.8$ Hz), 7.43 (2H, *d*, $J=8.8$ Hz) and 7.61 (1H, *d*, $J=16.0$ Hz) and a prominent fragment ion peak at *m*/*z* 147 in the EI-MS. The ^1H NMR spectrum of **1** contained the signals due to eight tertiary methyl groups (δ_H 0.83, 0.86, 0.88, 0.90, 0.94, 0.98, 0.99 and 1.14) and one olefinic proton (δ_H 5.19), which are typical of β-amyrin (**8**). This was also supported by the observation of a base ion peak at *m*/*z* 218 (Budzikiewicz, Wilson & Djerassi, 1963; Karliner and Djarassi, 1966). Additionally, ^{13}C NMR data for this compound were very similar to those of an oleanane-type triterpene related to β-amyrin (Knight, 1974), except for C-2, 3 and 4 (Table 2). These results indicate that **1** is β-amyrin bearing a *p*-coumaroyl ester moiety at the C-3 position. In fact, hydrolysis of **1** with 10% KOH yielded β-amyrin (**8**) (Knight, 1974; Boar and Allen, 1973) and *p*-coumaric acid (**16**), identical in all respects with commercially

Table 2
^{13}C NMR spectral data for compounds **1**–**6**, **9** and **10**

C	**1**[a]	**2**[b]	**3**[a]	**4**[c]	**5**[d]	**6**[d]	**9**[a]	**10**[a]
1	38.3	38.3	38.2	37.7	38.3	37.4	38.6	38.4
2	23.7	23.6	23.6	23.5	23.9	23.1	27.2	27.2
3	81.0	81.1	81.0	79.9	80.4	79.8	79.9	79.0
4	38.0	37.8	37.7	37.5	37.9	37.5	38.8	38.8
5	55.3	55.3	55.3	54.9	55.4	54.9	55.2	55.3
6	18.3	18.3	18.3	17.8	18.5	17.8	18.4	18.3
7	32.6	32.6	32.6	32.1	31.8	32.5	32.6	32.7
8	41.7	41.7	41.7	39.4	40.0	39.0	39.8	39.3
9	47.6	47.6	47.6	47.1	47.7	47.2	47.6	47.7
10	36.9	36.9	36.9	36.4	37.0	37.5	36.9	37.1
11	23.7	23.6	23.7	23.2	23.8	23.4	23.6	23.1
12	121.7	121.7	121.7	121.6	122.0	121.6	122.3	122.4
13	145.2	145.2	145.2	144.5	144.7	144.1	144.2	143.9
14	39.8	39.8	39.8	41.3	41.9	41.4	41.7	41.6
15	26.2	26.2	26.1	25.4	26.0	27.6	25.6	27.7
16	26.9	27.0	26.9	22.2	22.8	23.0	22.0	23.4
17	32.5	32.5	32.5	37.0	37.5	45.9	36.9	46.7
18	47.3	47.3	47.2	42.0	42.6	41.3	42.3	41.3
19	46.8	46.8	46.8	46.4	47.0	45.7	46.5	45.9
20	31.1	31.1	31.1	30.6	30.5	30.3	31.0	30.7
21	34.8	34.8	34.8	34.0	34.6	36.5	34.1	33.9
22	37.2	37.2	37.2	31.2	32.7	32.3	31.0	32.4
23	28.1	28.1	28.0	27.6	28.2	27.6	28.1	28.1
24	16.8	16.7	16.8	16.2	16.9	14.7	15.5	15.6
25	15.6	15.6	15.6	15.0	15.6	16.5	15.5	15.2
26	16.9	16.8	16.8	16.5	17.1	16.7	16.7	16.8
27	26.0	26.0	26.0	25.5	26.1	25.5	25.9	25.9
28	28.4	28.4	28.4	68.0	68.5	179.3	69.7	178.4
29	33.4	33.3	33.3	32.8	33.4	33.5	33.2	33.1
30	23.6	23.7	23.6	23.2	23.8	23.1	23.6	23.6
Coumaroyl								
1′	167.5	166.6	172.9	166.6	166.3	166.4		
2′	116.2	117.8	31.1	116.2	116.6	115.0		
3′	144.2	144.3	29.7	144.4	143.5	144.0		
4′	127.3	127.5	132.8	125.5	126.4	125.4		
5′	130.0	132.2	129.4	130.1	133.4	129.9		
6′	115.9	115.0	115.3	115.1	115.7	116.0		
7′	157.7	156.7	154.0	160.8	160.2	160.6		
8′	115.9	115.0	115.3	115.1	115.7	116.0		
9′	130.0	132.3	129.4	130.1	133.4	129.9		

[a] 100 MHz in $CDCl_3$.
[b] 150 MHz in $CDCl_3$.
[c] 150 MHz in py-d_5.
[d] 100 MHz in py-d_5.

available samples. Thus, compound **1** was determined to be 3-*O*-(*E*)-coumaroyl *β*-amyrin.

The EI mass spectrum for compound **2** showed the same molecular ion and base ion peaks at *m*/*z* 572 and 218 as **1**. The ^{1}H and ^{13}C NMR spectral data (Table 2) were closely related to those of **1**, except for the olefin proton signals at δ_H 5.84 (*d*, J=12.7 Hz) and 6.83 (*d*, J=12.7 Hz) assignable to H-3 and H-4 on the coumaroyl group. Judging from the small J value (12.7 Hz), the double bond involving the coumaroyl group should have a *Z* geometry. These spectral data implies that **2** was *β*-amyrin-type triterpene bearing a *Z*-coumaroyl group. On the basis of the HMBC, the triterpene part was determined to be *β*-amyrin, the *Z*-coumaroyl moiety was located at C-3 by correlation of the H-3 signal (δ_H 4.56) with the C-1 carbonyl signal (δ_C 166.6). Additionally, the 2D NOESY verified the stereochemistry for the triterpene part of **2** to be identical with that of *β*-amyrin. These spectral data confirmed that the structure of **2** was 3-*O*-(*Z*)-coumaroyl *β*-amyrin.

Compound **3** has the molecular formula $C_{39}H_{58}O_3$ (HR-EIMS, *m*/*z* 574.4365 $[M]^+$), two mass units higher than those of **1** and **2**. The ^{1}H and ^{13}C NMR spectra of **3** were very similar to those of **1** and **2**, except for the presence of two methylenes [δ_H 2.59 (2H, *t*, J=7.1 Hz) and δ_C 31.1; δ_H 2.88 (2H, *t*, J=7.1 Hz) and δ_C 29.7] instead of the double bond involving the coumaroyl group of **1** and **2**. This suggested the presence of a 2,3-dihydro-*p*-coumaroyl moiety in **3**. Further, the ^{13}C NMR spectral data (Table 2) and the EI-MS fragment ions of **3** corresponding to the triterpene part were almost identical with those of **1** and **2**. Thus, the structure of **3** was assigned as 3-*O*-2,3-dihydrocoumaroyl *β*-amyrin.

Compounds **4** and **5** had the same molecular formula $C_{39}H_{56}O_4$, as established by HR-EIMS at *m*/*z* 588 $[M]^+$. The ^{1}H and ^{13}C NMR spectral data (Table 2) for the aliphatic region of **4** and **5**, which were very similar, indicated the presence of seven tertiary methyl groups (**4**: δ_H 0.88, 0.92, 0.94, 0.96, 0.97, 0.98, 1.27; **5**: δ_H 0.87, 0.91, 0.93, 0.95, 0.96, 0.99, 1.27), a trisubstituted double bond [**4**: δ_H 5.22 (*dd*, J=3.9, 3.3 Hz) and δ_C 121.6, 144.5; **5**: δ_H 5.23 (*br s*) and δ_C 122.0, 144.7], an oxymethylene [**4**: δ_H 3.56 (*d*, J=10.4 Hz), 3.83 (*d*, J=10.4 Hz) and δ_C 68.0; **5**: δ_H 3.57 (*d*, J=10.2 Hz), 3.84 (*d*, J=10.2 Hz) and δ_C 68.5] as well as of an oxymethine [**4**: δ_H 4.87 (*dd*, J=11.5, 4.7 Hz) and δ_C 81.0; **5**: δ_H 4.80 (*dd*, J=11.7, 4.9 Hz) and δ_C 79.9]. Acetylation of **4** gave the diacetate **4a**, indicating the presence of a hydroxyl methyl group [δ_H 2.96, 3.71 (*d*, J=11.0 Hz) and 4.04 (*d*, J=11.0 Hz)] and a phenolic hydroxyl group (δ_H 2.31). The presence of a *p*-coumaroyl group was evident from spectral data of **4**, and the HMBC correlations from the seven methyl and the hydroxyl methyl signals indicated an erythrodiol-type triterpene in **4**. This was supported by a fragment ion peak at *m*/*z* 234 in the EI-MS, and treatment of **4** with 10% KOH in EtOH–H_2O yielded erythrodiol (**9**) (Xue, Lu, Konno, Soejarto, Cordell, Fong & Hodgson, 1988) and *p*-coumaric acid (**16**), which were identical in all respects with authentic samples. Connection of the *p*-coumaroyl unit was determined to be at the C-3 hydroxyl group of erythrodiol (**9**) via an ester linkage on the basis of the HMBC correlation between H-3 (δ_H 4.87) and C-1 (δ_C 166.6). Thus, the structure of **4** was established as 3-*O*-(*E*)-coumaroyl erythrodiol. Compound **5** has physicochemical data

comparable to those of **4** except for having a smaller J value (13.2 Hz) between H-2 and H-3 than in **4** (15.9 Hz). This single difference from **4** revealed that **5** as 3-*O*-(*Z*)-coumaroyl erythrodiol.

Compounds **6** and **7** had the same molecular formula ($C_{39}H_{54}O_5$), as established by HR-EIMS at m/z 602 $[M]^+$. Their IR spectra showed the presence of carboxyl (3343, 1703 cm^{-1}) and hydroxyl (3343 cm^{-1}) groups. Acetylation of **6** gave a monoacetate **6a** (m/z 644 $[M]^+$), which was then treated with diazomethane yielding the methyl ester **6b** (m/z 658 $[M]^+$). The above chemical transformations supported the presence of hydroxyl and carboxyl groups in **6**. In their EI-MS, fragment ion peaks at m/z 248 and 147, typical of an oleanolic acid skeleton and a coumaroyl unit were observed. The NMR spectral data (Table 2) of **6** and **7** indicated that they comprised of an oleanolic acid unit and a coumaroyl moiety. Alkaline treatment of **6** afforded oleanolic acid (**10**) (Maillard, Adewunmi & Hostettman, 1992) and *p*-coumaric acid (**16**), the spectral data of which were superimposable with those of authentic samples. The H-2 and H-3 doublet olefinic protons of the coumaroyl units in **6** and **7** appeared at δ_H 6.71 and 8.04 with $J=16.1$ Hz, and at δ_H 6.04 and 7.00 with $J=12.8$ Hz, respectively. Hence, compounds **6** and **7** were assigned as 3-*O*-(*E*)-coumaroyl oleanolic acid and 3-*O*-(*Z*)-coumaroyl oleanolic acid, respectively.

Compounds **1**–**16** were tested for their antioxidant properties using three in vitro assay systems, including anti-lipid peroxidation (ALP) (Stocks, Gutterige, Sharp & Dormandy, 1974), free radical scavenging of the α,α-diphenyl-β-picrylhydrazyl radical (DPPH) (Blois, 1958) and superoxidation anion (O_2^-) scavenging activities (McCord and Fridovich, 1969). The results are summarized in Table 1. Compound **11**, gallic acid, a known antioxidant (Larson, 1988), exhibited the most potent inhibitory activities in the three assays and significantly contributed to that observed in the methanol extracts and ethyl acetate extracts. Catechin was also used as a relation standard. Compounds **12** and **13** showed inhibitory activity only in the DPPH assay. The new coumaroyl triterpenoids **1**–**7** and *p*-coumaric acid (**16**), exhibited no antioxidant properties at 50 μg/ml. Thus, gallic acid (**11**) and its derivatives (**12**–**13**) could be regarded as the most important antioxidant principles present in *C. equisetifolia*.

To our knowledge, there are very few triterpenes having a coumaroyl ester group (Lee, Lin & Liu, 1996; Ito and Lai, 1978), and in particular, their co-occurrence with *Z*-coumaroyl and dihydrocoumaroyl groups is interesting from the perspective of their biosynthesis (Yagi, Okamura, Haraguchi, Noda & Nishioka, 1978a, b).

3. Experimental

Mps: uncorr. ^1H and ^{13}C NMR: TMS as int. standard; CC: silica gel (Merck, 230–400 mesh and Wakogel C-300) and Sephadex LH-20 (25–100 μm, Pharmacia); TLC: precoated silica gel 60 F_{254} (Merck, 0.25 mm) and RP-8 F_{254} (Merck, 0.25 mm). Spots were visualized by UV (254 nm) and 10% $CeSO_4$–H_2SO_4.

3.1. Plant material

C. equisetifolia was collected in Ishigaki Islands, Japan and identified by Dr Hiroyuki Murata (Kagoshima, Japan). A voucher specimen has been deposited in our institute.

3.2. Extraction and isolation

The dried and powdered leaves (4.6 kg) were immersed in MeOH at room temp. for 1 month. The MeOH extract was evaporated in vacuo to give a gummy extract (200 g). The MeOH extract was mixed with celite (200 g) and evapd in vacuo. The resultant powder was packed in a glass column and eluted with *n*-hexane (11), CH_2Cl_2 (11), CH_2Cl_2–EtOAc (4:1) (11), EtOAc (11), EtOAc–MeOH (9:1) (11) and MeOH (11) to give 6 frs (1–6). Fr. 4 (3.4 g) was chromatographed by CC on Si gel (Merck) eluting with CH_2Cl_2–EtOAc (4:1) to give 6 frs (11–16). Fr. 16 (1.3 g) was re-chromatographed by CC on Si gel ($CHCl_3$–MeOH, 29:1) and then on Sephadex LH-20 (MeOH) to give **11** (3.3 mg), **12** (2.5 mg) and **13** (3.0 mg). Fr. 15 (63.1 mg) was purified by CC on Sephadex LH-20 (MeOH) and prep. TLC (ODS) with MeOH–H_2O (1:1) to afford **14** (1.7 mg), **15** (1.0 mg) and **16** (2.7 mg). Fr. 1 (6.71 g) was purified by repeated CC on Si gel (*n*-hexane-EtOAc, 5:1) to give **1** (100.3 mg), **2** (10.1 mg) and **3** (11.0 mg). Fr. 3 (4.07 g) was purified by CC on Si gel (CH_2Cl_2–EtOAc, 9:1) and then on Sephadex LH-20 (MeOH) to give **4** (17.5 mg), **5** (3.6 mg), **6** (3.5 mg), **7** (17.1 mg), **9** (3.8 mg) and **10** (5.2 mg).

3.3. 3-O-(E)-Coumaroyl β-amyrin (1)

Colorless prisms, mp 188.5–190.0°C, $[\alpha]_D^{25.9}$ +60.8° (c 1.42, $CHCl_3$). EIMS m/z (rel. int.): 572.4229 $[M]^+$ (53) (calc. 572.4229 for $C_{39}H_{56}O_3$), 408 (11), 218 (100), 203 (34), 189 (22), 164 (11), 147 (26); IR ν_{film}^{FT} cm^{-1}: 3351 (OH), 1682 (C=O), 1605, 1514, 1454, 1277, 1171, 982, 831, 760; UV λ_{max}^{EtOH} nm (ϵ): 226 (8200), 311 (15,600); ^1H-NMR (400 MHz, $CDCl_3$): δ 0.83 (3H, *s*), 0.86 (3H, *s*), 0.88 (3H, *s*), 0.90 (3H, *s*), 0.94 (3H, *s*), 0.98 (3H, *s*), 0.99 (3H, *s*), 1.14 (3H, *s*), 4.64 (1H, *dd*, $J=8.3$, 7.8 Hz, H-3), 5.19 (1H, *dd*, $J=3.6$, 3.6 Hz, H-12), 5.68 (1H, *s*, OH), 6.30 (1H, *d*, $J=16.0$ Hz, H-2′),

6.85 (2H, *d*, $J=8.8$ Hz, H-6′ and H-8′), 7.43 (2H, *d*, $J=8.8$ Hz, H-5′ and H-9′), 7.61 (1H, *d*, $J=16.0$ Hz, H-3′); ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2.

3.4. 3-O-(*Z*)-Coumaroyl β-amyrin (*2*)

Colorless prisms, mp 106.0–109.0°C, $[\alpha]_D^{25.9}$ +38.6° (*c* 0.89, $CHCl_3$). EIMS *m/z* (rel. int.): 572.4229 $[M]^+$ (10) (calc. 572.4229 for $C_{39}H_{56}O_3$), 408 (5), 218 (100), 203 (27), 191 (20), 147 (32); IR ν_{film}^{FT} cm^{-1}: 3373 (OH), 1694 (C═O), 1605, 1514, 1454, 1171, 986, 851, 760; UV λ_{max}^{EtOH} nm (ϵ): 225 (6700), 309 (9300); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 0.83 (3H, *s*, H_3-23), 0.83 (3H, *s*, H_3-28), 0.85 (1H, *m*, H-5), 0.87 (3H, *s*, H_3-30), 0.88 (3H, *s*, H_3-29), 0.89 (3H, *s*, H_3-24), 0.96 (3H, *s*, H_3-25), 0.97 (3H, *s*, H_3-26), 1.09 (1H, *ddd*, $J=13.9$, 12.9, 3.2 Hz, H-21), 1.14 (3H, *s*, H_3-27), 1.22 (1H, *ddd*, $J=13.4$, 3.2, 3.2 Hz, H-22), 1.43 (1H, *ddd*, $J=13.9$, 13.4, 4.2 Hz, H-22), 1.57 (1H, *dd*, $J=9.3$, 5.9 Hz, H-9), 1.76 (1H, *ddd*, $J=13.9$, 13.7, 4.4 Hz, H-15), 1.95 (1H, *dd*, $J=13.6$, 4.2 Hz, H-18), 1.99 (1H, *ddd*, $J=13.7$, 12.9, 3.7 Hz, H-16), 4.56 (1H, *dd*, $J=11.2$, 4.9 Hz, H-3), 5.19 (1H, *dd*, $J=3.6$, 3.4 Hz, H-12), 5.39 (1H, *s*, OH), 5.84 (1H, *d*, $J=12.7$ Hz, H-2′), 6.78 (2H, *d*, $J=8.5$ Hz, H-6′ and H-8′), 6.83 (1H, *d*, $J=12.7$ Hz, H-3′), 7.62 (2H, *d*, $J=8.5$ Hz, H-5′ and H-9′); ^{13}C-NMR (150 MHz, $CDCl_3$): Table 2.

3.5. 3-O-Dihydrocoumaroyl β-amyrin (*3*)

Oil, $[\alpha]_D^{25.9}$ +49.5° (*c* 0.65, $CHCl_3$). EIMS *m/z* (rel. int.): 574.4365 $[M]^+$ (9) (calc. 574.4386 for $C_{39}H_{58}O_3$), 409 (14), 218 (100) 203 (26) 189 (17); IR ν_{film}^{FT} cm^{-1}: 3405 (OH), 1705 (C═O), 1614, 1516, 1453, 1217, 990, 828, 758; UV λ_{max}^{EtOH} nm (ϵ): 223 (6900), 274 (6000); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.80 (3H, *s*), 0.83 (6H, *s*), 0.87 (6H, *s*), 0.95 (3H, *s*), 0.97 (3H, *s*), 1.13 (3H, *s*), 2.59 (2H, *t*, $J=7.1$ Hz, H-2′), 2.88 (2H, *t*, $J=7.1$ Hz, H-3′), 4.50 (1H, *dd*, $J=7.1$, 2.2 Hz, H-3), 4.78 (1H, *s*, OH), 5.18 (1H, *dd*, $J=3.6$, 3.4 Hz, H-12), 6.74 (2H, *d*, $J=8.6$ Hz, H-6′ and H-8′), 7.07 (2H, *d*, $J=8.6$ Hz, H-5′ and H-9′); ^{13}C-NMR (100 MHz, $CDCl_3$): Table 2.

3.6. 3-O-(*E*)-Coumaroyl erythrodiol (*4*)

Colorless prisms, mp 241–244°C, $[\alpha]_D^{20.2}$ +64.7° (*c* 1.75, dioxane). EIMS *m/z* (rel. int.): 588.4202 $[M]^+$ (9) (calc. 588.4179 for $C_{39}H_{56}O_4$), 424 (12), 234 (17), 203 (100), 189 (30), 147 (41); IR ν_{film}^{FT} cm^{-1}: 3360 (OH), 1676 (C═O), 1609, 1516, 1464, 1385, 1186, 821; UV $\lambda_{max}^{dioxane}$ nm (ϵ): 225 (12,000), 308 (19,200); ^{1}H-NMR (600 MHz, py-d_5): δ 0.88 (3H, *s*, H_3-25), 0.92 (3H, *s*, H_3-26), 0.94 (3H, *s*, H_3-30), 0.96 (3H, *s*, H_3-24), 0.97 (3H, *s*, H_3-29), 0.98 (3H, *s*, H_3-23), 1.27 (3H, *s*, H_3-27), 3.56 (1H, *d*, $J=10.4$ Hz, H-28), 3.83 (1H, *d*, $J=10.4$ Hz, H-28), 4.87 (1H, *dd*, $J=11.5$, 4.7 Hz, H-3), 5.22 (1H, *dd*, $J=3.9$, 3.3 Hz, H-12), 6.69 (1H, *d*, $J=15.9$ Hz, H-2′), 7.17 (2H, *d*, $J=8.5$ Hz, H-6′ and H-8′), 7.65 (2H, *d*, $J=8.5$ Hz, H-5′ and H-9′), 8.02 (1H, *d*, $J=15.9$ Hz, H-3′); ^{13}C-NMR (150 MHz, py-d_5): Table 2.

3.7. 3-O-(*Z*)-Coumaroyl erythrodiol (*5*)

Colorless prisms, mp 137–138°C, $[\alpha]_D^{20.2}$ +41.9° (*c* 2.44, acetone). EIMS *m/z* (rel. int.): 588.4195 $[M]^+$ (5) (calc. 588.4179 for $C_{39}H_{56}O_4$), 570 (6), 234 (26), 203 (100), 191 (26), 147 (44), 32 (39); IR ν_{film}^{FT} cm^{-1}: 3341 (OH), 1711 (C═O), 1605, 1514, 1454, 1364, 1167, 831; UV $\lambda_{max}^{dioxane}$ nm (ϵ): 225 (14,300), 310 (21,400); ^{1}H-NMR (400 MHz, py-d_5): δ 0.87 (3H, *s*, H_3-25), 0.91 (3H, *s*, H_3-26), 0.93 (3H, *s*, H_3-30), 0.95 (3H, *s*, H_3-24), 0.96 (3H, *s*, H_3-29), 0.99 (3H, *s*, H_3-23), 1.27 (3H, *s*, H_3-27), 3.57 (1H, *d*, $J=10.2$ Hz, H-28), 3.84 (1H, *d*, $J=10.2$ Hz, H-28), 4.80 (1H, *dd*, $J=11.7$, 4.9 Hz, H-3), 5.23 (1H, *br s*, H-12), 6.04 (1H, *d*, $J=13.2$ Hz, H-2′), 6.99 (1H, *d*, $J=13.2$ Hz, H-3′), 7.18 (2H, *d*, $J=8.8$ Hz, H-6′ and H-8′), 8.10 (2H, *d*, $J=8.8$ Hz, H-5′ and H-9′); ^{13}C-NMR (100 MHz, py-d_5): Table 2.

3.8. 3-O-(*Z*)-Coumaroyl oleanolic acid (*6*)

Colorless prisms, mp 245–247°C, $[\alpha]_D^{20.3}$ +89.5° (acetone, *c* 0.21). EIMS *m/z* (rel. int.): 602.3978 $[M]^+$ (2) (calc. 602.3971 for $C_{39}H_{54}O_5$), 556 (6), 438 (12), 248 (54), 203 (36), 147 (27), 121 (100), 91 (48); IR ν_{film}^{FT} cm^{-1}: 3343 (OH), 1703 (C═O), 1605, 1514, 1447, 1364, 1275, 1169, 831; UV $\lambda_{max}^{dioxane}$ nm (ϵ): 225 (12,900), 308 (18,100); ^{1}H-NMR (400 MHz, py-d_5): δ 0.86 (3H, *s*), 0.96 (3H, *s*), 0.98 (6H, *s*), 1.01 (3H, *s*), 1.02 (3H, *s*), 1.30 (3H, *s*), 4.90 (1H, *dd*, $J=11.4$ Hz, 5.5 Hz, H-3), 5.49 (1H, *br s*, H-12), 6.71 (1H, *d*, $J=16.1$ Hz, H-2′), 7.18 (2H, *d*, $J=8.4$ Hz, H-6′ and H-8′), 7.67 (2H, *d*, $J=8.4$ Hz, H-5′ and H-9′), 8.04 (1H, *d*, $J=16.1$ Hz, H-3′); ^{13}C-NMR (100 MHz, py-d_5): Table 2.

3.9. 3-O-(*E*)-Coumaroyl oleanolic acid (*7*)

Colorless prisms, mp 141–142°C, $[\alpha]_D^{19.6}$ +23.6° (acetone, *c* 0.36). EIMS *m/z* (rel. int.): 602.3999 $[M]^+$ (4) (calc. 602.3971 for $C_{39}H_{54}O_5$), 556 (5), 438 (19), 248 (100), 203 (36), 191 (49), 147 (46), 121 (43), 91 (31); IR ν_{film}^{FT} cm^{-1}: 3387 (OH), 1701 (C═O), 1604, 1512, 1458, 1364, 1167, 850; UV $\lambda_{max}^{dioxane}$ nm (ϵ): 225 (23,700), 310 (22,400); ^{1}H-NMR (400 MHz, py-d_5): δ 0.83 (3H, *s*), 0.89 (3H, *s*), 0.95 (3H, *s*), 0.96 (3H, *s*), 0.99 (3H, *s*), 1.01 (3H, *s*), 1.27 (3H, *s*), 4.81 (1H, *m*, H-3), 5.49 (1H, *br s*, H-12), 6.04 (1H, *d*, $J=12.8$ Hz, H-2), 7.00 (1H, *d*, $J=12.8$ Hz, H-3), 7.18 (2H, *d*,

$J=8.4$ Hz, H-6′ and H-8′), 8.11 (2H, *d*, $J=8.4$ Hz, H-5′ and H-9′).

3.10. Acetylation of *4*, *5* and *6*

Compound **4** (14.0 mg), **5** (13.8 mg) and **6** (2.7 mg) were separately acetylated with Ac_2O and pyridine overnight, respectively. The usual work-up afforded an acetate **4a** (6.2 mg), **5a** (8.5 mg) and **6a** (1.8 mg).

3.11. 3-O-(*E*)-Coumaroyl β-amyrin diacetate (*4a*)

Colorless prisms, mp 74–79°C, $[\alpha]_D^{20.1}$ +39.5° ($CHCl_3$, *c* 0.80). EIMS *m/z* (rel. int.): 672.4406 $[M]^+$ (4) (calc. 672.4390 for $C_{43}H_{60}O_6$), 612 (15), 466 (34), 276 (16), 216 (16), 203 (100), 190 (42), 147 (51); IR ν_{film}^{FT} cm^{-1}: 1738 (C=O), 1601, 1506, 1464, 1167, 837; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.88 (3H, *s*), 0.90 (3H, *s*), 0.92 (3H, *s*), 0.94 (3H, *s*), 0.97 (3H, *s*), 0.98 (3H, *s*), 1.18 (3H, *s*), 2.06 (3H, *s*), 2.31 (3H, *s*), 3.71 (1H, *d*, $J=11.0$ Hz, H-28), 4.04 (1H, *d*, $J=11.0$ Hz, H-28), 4.64 (1H, *dd*, $J=7.0$, 8.8 Hz, H-3), 5.21 (1H, *br s*, H-12), 6.40 (1H, *d*, $J=16.1$ Hz, H-2′), 7.08 (2H, *d*, $J=8.4$ Hz, H-6′ and H-8′), 7.55 (2H, *d*, $J=8.4$ Hz, H-5′ and H-9′), 7.64 (1H, *d*, $J=16.1$ Hz, H-3′).

3.12. 3-O-(*Z*)-Coumaroyl β-amyrin diacetate (*5a*)

$[\alpha]_D^{21.0}$ +33.2° ($CHCl_3$, *c* 0.85). HR-FABMS *m/z*: Found 695.4274, calc. 695.4287 for $C_{43}H_{60}O_6Na$; IR ν_{film}^{FT} cm^{-1}: 1736 (C=O), 1601, 1506, 1464, 1366, 1017, 988, 912, 858, 824, 758; ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.70 (3H, *s*), 0.79 (3H, *s*), 0.81 (3H, *s*), 0.83 (3H, *s*), 0.88 (6H, *s*), 1.10 (3H, *s*), 1.99 (3H, *s*), 2.24 (3H, *s*), 3.64 (1H, *d*, $J=11.0$ Hz, H-28), 3.97 (1H, *d*, $J=11.0$ Hz, H-28), 4.55 (1H, *dd*, $J=9.9$, 5.9 Hz, H-3), 5.20 (1H, *br s*, H-12), 5.89 (1H, *d*, $J=12.8$ Hz, H-2′), 6.83 (1H, *d*, $J=12.8$ Hz, H-3′), 7.01 (2H, *d*, $J=8.8$ Hz, H-6′ and H-8′), 7.58 (2H, *d*, $J=8.8$ Hz, H-5′ and H-9′).

3.13. 3-O-(*Z*)-Coumaroyl oleanolic acid acetate (*6a*)

$[\alpha]_D^{21.0}$ +21.1° ($CHCl_3$, *c* 0.18). EIMS *m/z* (rel. int.): 644.4086 $[M]^+$ (6) (calc. 644.4077 for $C_{41}H_{56}O_6$), 598 (7), 438 (18), 248 (100), 203 (61), 147 (30), 32 (34); IR ν_{film}^{FT} cm^{-1}: 1766 (C=O), 1601, 1506, 1464, 1369, 1278, 837; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.79 (3H, *s*), 0.85 (3H, *s*), 0.91 (3H, *s*), 0.94 (6H, *s*), 0.97 (3H, *s*), 1.26 (3H, *s*), 2.31 (3H, *s*), 4.60 (1H, *m*, H-3), 5.30 (1H, *br s*, H-12), 6.40 (1H, *d*, $J=16.1$, H-2′), 7.12 (2H, *d*, $J=8.4$ Hz, H-6′ and H-8′), 7.55 (2H, *d*, $J=8.4$ Hz, H-5′ and H-9′), 7.64 (1H, *d*, $J=16.1$ Hz, H-3′).

3.14. Methylation of *6a*

Compound **6a** (1.5 mg) was treated with CH_2N_2 to give the methyl ester **6b** (1.0 mg). EIMS *m/z* (rel. int.): 658.4228 $[M]^+$ (3) (calc. 658.4233 for $C_{42}H_{58}O_6$), 452 (20), 262 (90), 203 (100), 189 (30), 147 (37); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.74 (3H, *s*), 0.85 (3H, *s*), 0.91 (3H, *s*), 0.93 (6H, *s*), 0.97 (3H, *s*), 1.26 (3H, *s*), 2.31 (3H, *s*), 3.63 (3H, *s*), 4.60 (1H, *m*, H-3), 5.27 (1H, *m*, H-12), 6.40 (1H, *d*, $J=16.1$ Hz, H-2′), 7.12 (2H, *d*, $J=8.4$ Hz, H-6′ and H-8′), 7.55 (2H, *d*, $J=8.4$ Hz, H-5′ and H-9′), 7.64 (1H, *d*, $JJ=16.1$ Hz, H-3′).

3.15. Hydrolysis of *1*, *4* and *6*

Compounds **1** (11.3 mg), **4** (12.5 mg) and **6** (5.7 mg) were separately hydrolyzed with 10% KOH/EtOH under reflux to afford β-amyrin (**8**) (4.6 mg), erythrodiol (**9**) (5.9 mg) and oleanolic acid (**10**) (1.2 mg), respectively, as well as *p*-coumaric acid. All products were identical in all respects to authentic samples.

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Appendix

Coumaroyl triterpenes from *Casuarina equisetifolia*

Hironobu Takahashi, Miho Iuchi, Yoshimi Fujita, Hiroyuki Minami and Yoshiyasu Fukuyama

Institute of Pharmacognosy, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro-cho, Tokushima 770-8514, Japan

Seven new oleanae-type triterpene esters **1**–**7** were isolated from *Casuarina equisetifolia*, together with two known triterpenoids. Gallic acid was identified as one of antioxidant substances in *C. equisetifolia*.

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