Antitumor-Promoting Effects and Cytotoxic Activities of Dammar Resin Triterpenoids and Their Derivatives

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Nineteen known triterpenoids, **1**–**19**, and one known sesquiterpenoid, **20**, were isolated from dammar resin obtained from *Shorea javanica* K. & V. (Dipterocarpaceae). One of the acidic triterpenoids, dammarenolic acid (**1**), was converted to fourteen derivatives, namely, an alcohol, **21**, an aldehyde, **22**, and twelve L-amino acid conjugates, **23**–**34**. Compounds **1**–**34** were examined for their inhibitory effects on the induction of *Epstein–Barr* virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in *Raji* cells, a known primary screening test for antitumor promoters. All of the compounds tested, except for compounds **4**, **5**, **12**–**14**, **16**, and **17**, showed inhibitory effects against EBV-EA activation with potencies either comparable with or stronger than that of β-carotene, a known natural antitumor promoter. In addition, (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al (**22**) exhibited inhibitory effects on skin tumor promotion in an *in vivo* two-stage mouse skin carcinogenesis test based on 7,12-dimethylbenz[*a*]anthracene (DMBA) as initiator, and with TPA as promoter. Furthermore, evaluation of the cytotoxic activities of compounds **1**–**34** against human cancer cell lines showed that reduction (*i.e.*, **21** and **22**) or conjugation with L-amino acids (*i.e.*, **23**–**34**) of compound **1** enhanced the cytotoxicity against human melanoma cell line CRL1579.

Introduction. – Dammar resin, the exudate of various species of Dipterocarpaceae family, has long been used as varnish for paintings, and remain widely used today [1–3]. Dammar resin is called triterpenoid resin because it contains a large amount of functionalized triterpenoids with dammarane, oleanane, and ursane skeletons [1–5]. Dammar resin triterpenoids have been reported to possess antiviral activities against *Herpes simplex* virus types I and II *in vitro* [6], and protective effects against *in vitro* LDL oxidation [7]. In the course of our search for potential bioactive compounds from natural sources [8][9], we were especially interested to investigate the terpenoid constituents of dammar resin. This article describes the isolation of twenty terpenoids, **1**–**20**, from dammar resin obtained from *Shorea javanica* K. & V. (Dipterocarpaceae), and preparation of 14 derivatives of dammarenolic acid (**1**), *i.e.*, **21**–**34**. In addition, we report their inhibitory effects on the activation of *Epstein–Barr* virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in *Raji* cells, and their cytotoxic activity against human leukemia (HL60) and human melanoma (RL1579)

cells. Furthermore, we report the inhibitory effect of (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al (**22**) on *in vivo* two-stage mouse-skin carcinogenesis.

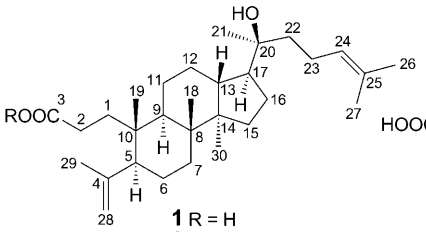
Results and Discussion. – *Isolation.* Toluene-soluble portion of the resin was subjected to column chromatography on silica gel, followed by preparative reversed-phase (RP) HPLC, to yield 19 triterpenoids, *i.e.*, 13 dammarane-type, **1**–**13**, four ursane-type, **14**–**17**, one oleanane-type, **18**, and one hopane-type, **19**, triterpenoid, and one eudesmane-type sesquiterpenoid, **20**. All of them are known compounds except for compound **15** which is a methyl ester of 19-dehydroxycecropiacic acid (**14**). Compound **14** is a known compound (*CAS* Reg. No. 25278-88-0), but its structure remained not fully characterized, and, hence, we have accomplished characterization of compound **14** as well as **15** as described below.

The molecular formula of compound **14** was determined as $C_{30}H_{46}O_6$ from its HR-EI-MS (*m*/*z* 502.3286, M^+) and ^{13}C-DEPT-NMR data. Electron ionization of **14** gave a fragmentation typical for a Δ^{12}-unsaturated ursane-type triterpene where cleavage of the C(8)–C(14) and the C(9)–C(11) bonds, as a result of a *retro-Diels–Alder* reaction [10], yielded a major fragment ion (*m*/*z* 248) corresponding to the *D*- and *E*-ring portion of the triterpene molecule bearing one COOH group. The compound has a trisubstituted C=C bond (δ(H) 5.53 (br. *s*); δ(C) 126.1), three COOH groups ($\tilde{\nu}_{max}$ 1698 cm^{-1}; δ(C) 174.4, 180.0, and 182.3), two secondary Me groups (δ(H) 0.89 (*d*, *J*= 6.2) and 0.90 (*d*, *J*=6.5)), and five tertiary Me groups (δ(H) 1.09, 1.17, 1.36, 1.55, and 1.57 (each *s*)). These data suggested that compound **14** is a tetracyclic 2,3-secours-12-ene-type triterpenoid with three COOH groups at C(2), C(3), and C(28). The above evidence, coupled with the ^{13}C- and ^{1}H-NMR data (*Table 1*), and analyses of ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY spectra, indicated that **14** was 2,3-secours-12-ene-2,3,28-trioic acid (=19-dehydroxycecropiacic acid).

Compound **15** was assigned a molecular formula $C_{31}H_{48}O_6$ as determined from the HR-EI-MS (*m*/*z* 516.3449, M^+) and ^{13}C-DEPT NMR data. ^{1}H- and ^{13}C-NMR signals of compound **15** were very close to those of compound **14** except for an additional MeO signal (δ(C) 51.9; δ(H) 3.70 (*s*, 3 H); *Table 1*). The MeO group was shown to be present as a methyl ester group at C(3) by the presence of a significant cross-correlation (^3J(C,H)) between signals of δ(H) 3.70 and δ(C) 179.8 (C(3) or C(28)) in the HMBC spectrum, and a fragment ion at *m*/*z* 248 in the EI-MS corresponding to the *D*- and *E*-ring portion of the ursane-type triterpene bearing one COOH group. Based on the above evidence, coupled with analysis of the IR, EI-MS, ^{13}C-DEPT, ^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY spectra, compound **15** was characterized as 2,3-secours-12-ene-2,3,28-trioic acid 3-*O*-methyl ester (=19-dehydroxycecropiacic acid 3-*O*-methyl ester).

Preparation of Dammarenolic Acid (**1**) *Derivatives.* Dammarenolic acid (**1**) was reduced with $LiAlH_4$ in Et_2O, after methyl esterification, to give (20*S*)-3,4-secodammara-4(28),24-diene-3,20-diol (**21**) in 46% yield. The alcohol **21** was oxidized with pyridinium chlorochromate (PCC) in CH_2Cl_2 to afford (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al (**22**) in 83% yield.

Amino acid conjugates were prepared by the treatment of **1** with H_2O-soluble carbodiimide (WSCD) and 1-hydroxybenzotriazole (HOBt) in the presence of each L-amino acid methyl ester hydrochloride (Ala, Asp, Gly, Ile, Leu, Met, Phe, Pro, Ser, Trp,



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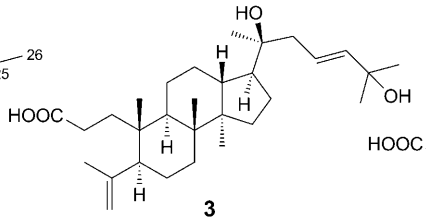

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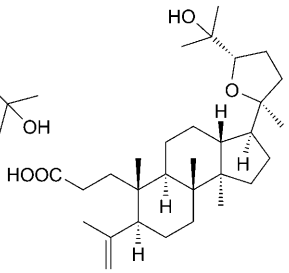

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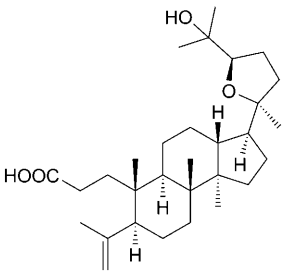

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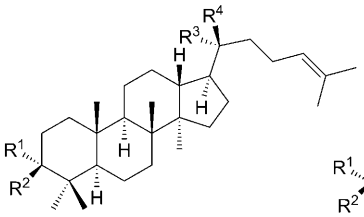
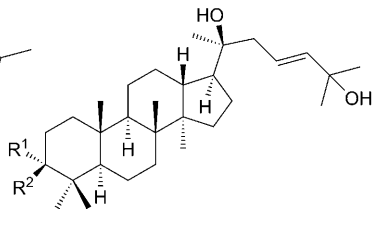
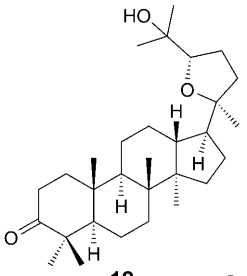


	R^1	R^2	R^3	R^4
6	H	OH	OH	Me
7	H	OH	Me	OH
8	=O		OH	Me
9	=O		Me	OH



	R^1	R^2
10	H	OH
11	=O	



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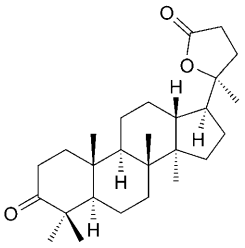

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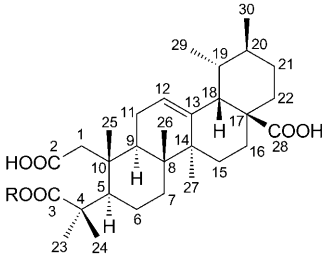

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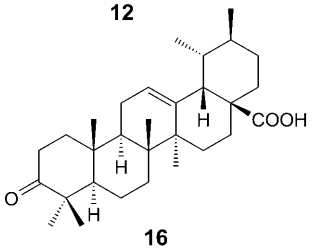

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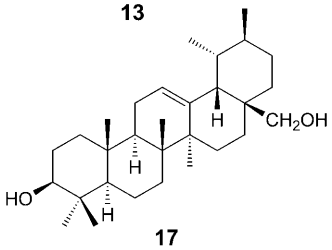

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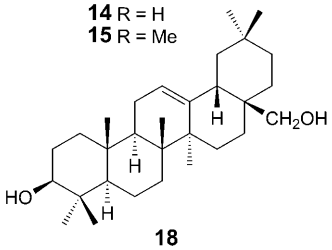

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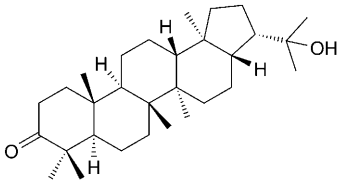

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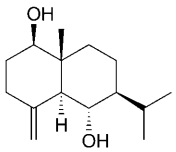

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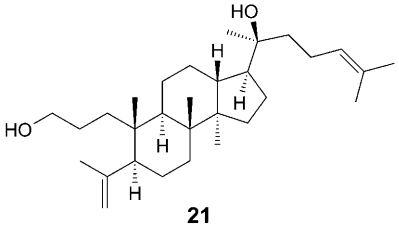

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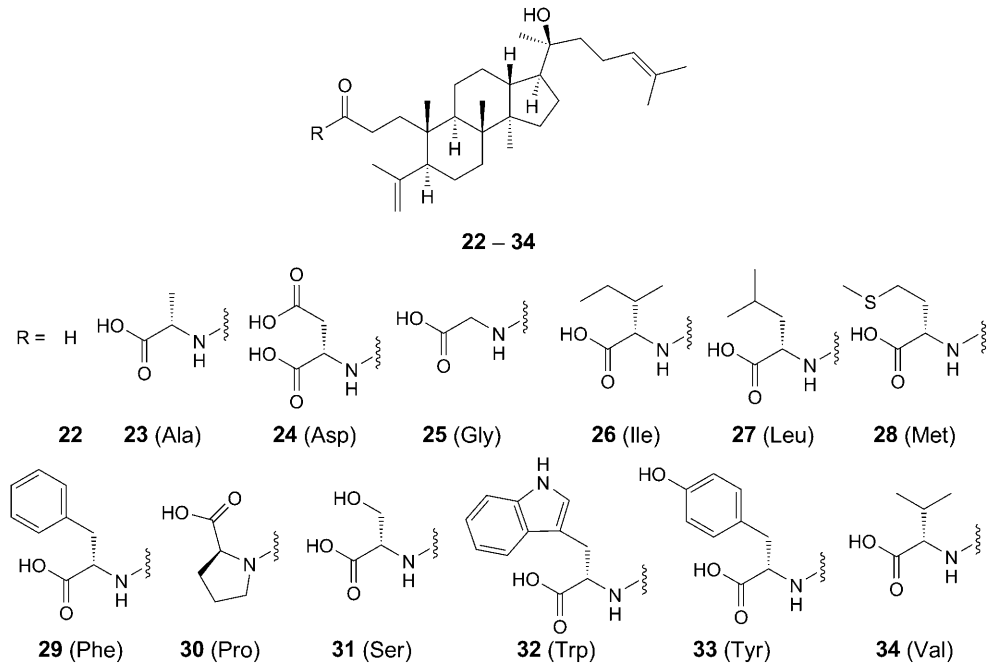
Table 1. *^{13}C-, ^{1}H-, and HMBC-NMR Spectral Data for Two Ursane-type Triterpenoids* **14** *and* **15** *from Dammar Resin* (C_5D_5N)

Position	**14**			**15**		
	δ(C)	δ(H)[a])	HMBC (H→C)	δ(C)	δ(H)[a])	HMBC (H→C)
CH_2(1)	42.8	2.70 (*d*, *J*=17.9), 3.06 (*d*, *J*=17.9)	2, 5, 9, 10, 25	42.4	2.61 (*d*, *J*=18.2), 2.66 (*d*, *J*=18.2)	2, 5, 9, 10, 25
C(2)	174.4			174.1		
C(3)	182.3			179.8[b])		
C(4)	46.9			46.6		
CH(5)	48.8	3.14 (*d*, *J*=11.7)	3, 4, 6, 7, 10, 23, 24, 25	49.0	2.97 (*t*, *J*=7.2)	3, 4, 6, 7, 9, 10, 23, 24, 25
CH_2(6)	21.9	1.71 (*m*)		21.4	1.58 (*m*)	
CH_2(7)	33.0	1.42 (*m*), 1.85 (*m*)		32.8	1.40 (*m*), 1.79 (*m*)	
C(8)	40.1			40.0		
CH(9)	39.3	3.25 (*dd*, *J*=5.8, 11.7)	8, 10, 11, 25, 26	39.3	3.18 (*dd*, *J*=5.8, 11.7)	8, 10, 11, 25, 26
C(10)	42.2			41.8		
CH_2(11)	24.1	2.22 (*m*, H_α), 2.06 (*m*, H_β)		24.1	2.19 (*dt*, *J*=5.1, 17.9, H_α), 2.05 (*m*, H_β)	
CH(12)	126.1	5.53 (br. *s*)	9, 11, 14, 18	125.9	5.24 (br. *s*)	9, 11, 14, 18
C(13)	139.0			139.0		
C(14)	43.4			43.3		
CH_2(15)	28.8	1.25 (*m*, H_α), 2.28 (*m*, H_β)		28.7	1.26 (*m*, H_α), 2.29 (*dt*, *J*=5.4, 13.4, H_β)	
CH_2(16)	25.0[b])	2.09 (*m*, H_α), 1.96 (*m*, H_β)		25.0	2.09 (*dt*, *J*=4.1, 13.4, H_α), 2.00 (*m*, H_β)	
C(17)	48.2			48.2		
CH(18)	53.6	2.61 (*d*, *J*=11.3)	12, 13, 14, 16, 17, 19, 20, 28	53.6	2.62 (*d*, *J*=8.0)	12, 13, 14, 16, 17, 19, 20, 28, 29
CH(19)	39.5[c])	1.42 (*m*)		39.5[c])	1.43 (*m*)	
CH(20)	39.6[c])	0.98 (*m*)		39.6[c])	0.99 (*m*)	
CH_2(21)	31.1	1.32 (*m*, H_α), 1.45 (*m*, H_β)		31.1	1.32 (*m*, H_α), 1.43 (*m*, H_β)	
CH_2(22)	37.4	1.92 (*m*)		37.4	1.93 (*m*)	
Me(23)	25.1[b])	1.57 (*s*)	3, 4, 5, 24	24.4	1.40 (*s*)	3, 4, 5, 24
Me(24)	27.6	1.55 (*s*)	3, 4, 5, 23	27.7	1.40 (*s*)	3, 4, 5, 23
Me(25)	19.6	1.17 (*s*)	1, 5, 9, 10	19.4	1.05 (*s*)	1, 5, 9, 10
Me(26)	17.7	1.09 (*s*)	7, 8, 9, 14	17.6	1.09 (*s*)	7, 8, 9, 14
Me(27)	23.8	1.36 (*s*)	8, 13, 14, 15	23.8	1.35 (*s*)	8, 13, 14, 15
Me(28)	180.0			179.9[b])		
Me(29)	17.5	0.89 (*d*, *J*=6.2)	18, 19, 20	17.5	0.88 (*d*, *J*=6.5)	18, 19, 20
Me(30)	21.3	0.90 (*d*, *J*=6.5)	19, 20, 21	21.3	0.89 (*d*, *J*=6.5)	19, 20, 21
COO*Me*				51.9	3.70 (*s*)	3

[a]) *J* Values in Hz. [b]), [c]) Values bearing the same superscript in each column are interchangeable.



Tyr, and Val) in DMF. The products were hydrolyzed and purified by preparative HPLC to afford the corresponding L-amino acid conjugates **23**–**34** in 26–57% yield.

The structures of resulting products were confirmed by IR, HR-ESI-MS, and ^{1}H-NMR data.

The molecular formula of compound **21** was determined to be $C_{30}H_{52}O_2$ on the basis of its HR-ESI-MS (positive-ion mode; $[M+Na]^+$, m/z 467.3846). Compound **21** had the same ^{1}H-NMR signals as those of **1** with an additional signal at δ(H) 3.58 (*m*, 2 H), which are consistent with the reduction of the C(3)OOH group of **1** to a HO–CH_2(3) group. These results, coupled with IR data, confirmed that compound **21** was (20*S*)-3,4-secodammara-4(28),24-diene-3,20-diol.

Compound **22** was assigned a molecular formula $C_{30}H_{50}O_2$, as determined from its $[M+Na]^+$ peak at m/z 465.3712 in the HR-ESI-MS (positive-ion mode). Compound **22** showed the same ^{1}H-NMR signals as those of **1** with an additional signal at δ(H) 9.74 (*s*, 1 H) and the absorption band at 1722 cm^{-1} in the IR, which are consistent with the reduction of the C(3)OOH group of **1** to a C(3)HO group. Thus, compound **22** was determined as (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al.

Compounds **23**–**34** exhibited absorption bands of NH groups (3300–3500 cm^{-1}) and CONH groups (1640–1660 cm^{-1}) characteristic for the amide groups in the IR spectra, and ^{1}H signals of CONH group at δ(H) 5.79–6.74 along with the signals of amino acid side chain in the ^{1}H-NMR spectra. These IR and ^{1}H-NMR data, as well as the HR-ESI-MS data are consistent with the L-amino acid conjugates **23**–**34** of **1**. An example for the structure elucidation of the amino acid conjugates is described for dammarenoyl-L-alanine (**23**). The molecular formula of compound **23** was determined

to be $C_{33}H_{55}NO_4$ on the basis of its HR-ESI-MS (positive-ion mode; $[M+Na]^+$, m/z 552.4014). The absorption bands of NH group (3421 cm^{-1}) and CONH group (1648 cm^{-1}) in the IR spectrum, and the ^1H signal at δ(H) 6.14 (*d*, $J=7.1$) in the ^1H-NMR spectrum indicated that compound **23** had an amide group. In addition, ^1H-NMR spectrum of compound **23** displayed signals with the same chemical shifts as those of **1** along with the signals ascribed to an alanine moiety, *i.e.*, one CH signal at δ(H) 4.56 (*sext.*, $J=7.1$) and one Me signal at 1.45 (*d*, $J=7.1$). From these results, compound **23** was determined to be dammarenoyl-L-alanine.

Inhibitory Effects on EBV-EA Induction. The inhibitory effects of the compounds isolated from dammar resin and dammarenolic acid derivatives on EBV-EA activation induced by TPA (32 pmol) were examined as a preliminary evaluation of their potential to inhibit tumor promotion, and the results are summarized in *Table 2*. The inhibitory effects were compared with those of the reference compound, β-carotene, a vitamin A precursor studied widely in cancer-chemoprevention animal models. Among the compounds tested, 27 compounds, *i.e.*, **1**–**3**, **6**–**11**, **15**, and **18**–**34**, exhibited potent inhibitory effects with IC_{50} values (concentration of 50% inhibition with respect to positive control) in the range 208–392 mol ratio/32 pmol TPA with preservation of high viability (60–70%) of *Raji* cells. As such, these compounds were comparable or more potent than the reference compound, β-carotene (IC_{50} 397 mol ratio/32 pmol TPA). In addition, among the potent inhibitory compounds, dammarenolic acid (**1**; IC_{50} 226 mol ratio/32 pmol TPA), a major component of dammar resin, and its two reduction products, **21** (IC_{50} 208 mol ratio/32 pmol TPA) and **22** (IC_{50} 212 mol ratio/32 pmol TPA), exhibited the highest inhibitory effects. On the basis of the results collected in *Table 2*, we can draw some conclusions about the structure–activity relationship of the compounds: dammarane-type triterpenoids, those possessing a linear side chain at C(17), *i.e.*, **1**–**3** and **6**–**11**, exhibited more potent inhibitory effects than those with a cyclic side chain, *i.e.*, **4**, **5**, **12**, and **13**, and 3-oxo group exerts almost no influence on the activity when compared with 3β-OH group (*i.e.*, **6** *vs.* **8**, **7** *vs.* **9**, and **10** *vs.* **11**). In addition, as far as dammarenolic acid (**1**) derivatives are concerned, conjugation with L-amino acids at C(3), (*i.e.*, **23**–**34**) reduces activity, whereas reduction to alcohol (*i.e.*, **21**) and aldehyde (*i.e.*, **22**) at C(3) enhances activity. Since the inhibitory effects against EBV-EA activation have been demonstrated to closely parallel those against tumor promotion *in vivo* [11], the highly inhibitory compounds against EBV-EA activation could be valuable antitumor promoters.

Two-Stage Carcinogenesis. Subsequently, we determined the inhibitory effects of one dammarenolic acid derivative, (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al (**22**), in a two-stage carcinogenesis test on mouse skin using 7,12-dimethylbenz-[*a*]anthracene (DMBA) as an initiator and TPA as a promoter. The incidence [%] of papilloma-bearing mice and the average numbers of papillomas per mouse are presented in the *Figure*, *a* and *b*, respectively. The incidence of papillomas in group I (untreated) was highly significant, at 100% of mice at 10 weeks of promotion. Further, 3.7 and 8.6 papillomas were formed per mouse at 10 and 20 weeks of promotion, respectively. The formation of papillomas in mouse skin was delayed, and the mean numbers of papillomas per mouse were reduced by treatment with **22**. Thus, in group II (treated with **22**), the ratio of papilloma-bearing mice was 20% at 10 weeks and 90% at 20 weeks, and the mean papillomas per mouse were 1.1 at 10 weeks and 3.3 at 20 weeks.

Table 2. *Inhibitory Effects on the Induction of* Epstein–Barr *Virus Early Antigen* (EBV-EA) *and Cytotoxicity Against Two Human Cancer Cell Lines of Compounds from Dammar Resin*

Compound	Percentage EBV-EA induction[a])						Cytotoxicity	
	Drug concentration[b])					IC_{50}[c])	EC_{50} [μM]	
	1000		500	100	10		HL60	CRL1579
1	0	(70)	20.4	68.7	90.4	226	13.5	>100
2	0	(70)	22.5	69.8	93.5	279	10.8	38.2
3	0	(70)	20.7	67.4	91.2	272	13.4	13.1
4	2.7	(70)	43.8	74.7	100	467	17.5	21.7
5	3.3	(70)	46.9	76.1	100	472	8.9	13.7
6	0	(70)	22.7	70.1	94.1	341	16.9	97.3
7[d])	0	(70)	27.5	71.3	90.2	300	20.3	47.1
8	0	(70)	25.8	73.2	97.3	341	19.2	>100
9	0	(70)	24.1	72.1	97.9	339	9.3	57.4
10	0	(70)	22.4	70.1	90.3	270	31.9	68.2
11	0	(70)	23.5	71.4	91.3	272	12.8	12.6
12	3.7	(70)	46.9	75.9	100	476	94.2	71.4
13	11.3	(70)	51.4	81.7	100	483	12.4	16.4
14	3.1	(70)	52.5	78.4	96.7	511	>100	>100
15	0	(70)	39.0	75.1	98.3	385	41.2	>100
16	10.3	(70)	41.6	73.2	100	460	13.8	17.0
17[d])	13.4	(70)	57.2	82.3	100	488	>100	>100
18[d])	0	(70)	42.6	74.8	96.6	392	82.9	96.6
19	2.3	(70)	31.4	73.5	95.7	347	17.7	>100
20	0	(70)	23.4	71.1	95.3	276	87.5	>100
21	0	(70)	16.0	57.0	84.6	208	21.7	80.8
22	0	(70)	18.5	58.6	86.0	212	17.7	13.4
23	0	(70)	31.5	74.2	96.4	300	9.0	11.3
24	0	(70)	36.8	79.2	98.8	330	99.3	57.4
25	0	(70)	32.7	75.8	97.0	318	8.0	10.1
26	0	(70)	34.9	77.1	97.6	319	15.0	13.7
27	0	(70)	35.1	78.6	98.2	328	14.3	11.6
28	2.8	(60)	38.2	80.6	100	381	10.5	10.0
29	2.1	(70)	37.8	79.1	100	372	4.7	7.5
30	0	(70)	37.2	79.6	98.7	361	10.2	10.4
31	0	(70)	35.0	78.1	97.2	326	6.8	14.5
32	4.6	(60)	40.2	81.2	100	388	12.1	11.2
33	0	(70)	36.2	78.3	98.3	329	12.2	11.0
34	0	(70)	30.2	71.8	93.6	290	11.5	10.7
β-Carotene[e])	8.6	(70)	34.2	82.1	100	397		
Cisplatin[e])							1.9	21.1

[a]) Values represent the relative percentage to the positive control, with TPA (32 pmol, 20 ng) representing 100% induction. [b]) Concentrations in terms of molar ratio/32 pmol TPA. [c]) IC_{50} represents the molar ratio of compound, relative to TPA, required to inhibit 50% of the positive control activated with 32 pmol TPA. [d]) Values for EBV-EA induction taken from [15][16]. [e]) Reference compounds.

The inhibitory effects of (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al (**22**) on papilloma formation on mouse skin were almost equivalent to or more potent than those of curcumin [12] and glycyrrhetic acid [13].

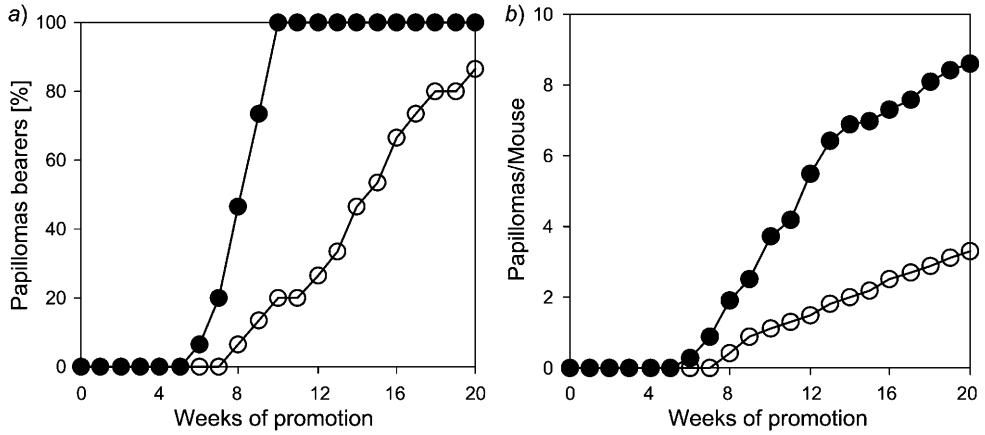


Figure. *Inhibitory effect of (20*S*)-20-hydroxy-3,4-secodammara-4(28),24-dien-3-al* (**22**) *on DMBA-TPA mouse-skin carcinogenesis. a*) Percentage of mice with papillomas; *b*) average number of papillomas per mouse. Tumor formation in all mice was initiated with DMBA (390 nmol) and promoted with TPA (1.7 nmol) twice weekly, starting one week after initiation. Filled circles (●) represent the untreated control group (TPA alone); open circles (○) refer to TPA + **22** (85 nmol). After 20 weeks of promotion, a significant difference in the number of papillomas per mouse between the groups treated with compound **22** and the control group was evident ($p < 0.05$).

Cytotoxic Activity. The cytotoxic activities of compounds **1**–**34** and cisplatin, which is one of the most effective and widely used chemotherapeutic drugs employed in the treatment of human cancers, against two human cell lines, HL60 (leukemia) and CRL1579 (melanoma), were determined by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay, and the results are summarized in *Table 2*. Six compounds, **5**, **9**, **23**, **25**, **29**, and **31**, showed potent activity (EC_{50} 4.7–9.3 μM), which is of the same order of potency as cisplatin (1.9 μM) against HL60. On the other hand, 18 compounds, **3**–**5**, **11**, **13**, **16**, **22**, **23**, and **25**–**34**, exhibited potent cytotoxic activity against CRL1579 (EC_{50} 7.5–21.7 μM), which is comparable with or higher activity than cisplatin (21.1 μM). It appears that reduction (*i.e.*, **21** and **22**) and conjugation with amino acids (*i.e.*, **23**–**34**; except for **24**) of **1** (EC_{50} 13.5 μM) are not responsible for the improvement of cytotoxicity (EC_{50} 4.7–21.7 μM) against HL60. On the other hand, reduction and conjugation with amino acids (except for **21** and **24**) of **1** ($EC_{50} > 100$ μM) give rise to higher activity (EC_{50} 7.5–14.5 μM) against CRL1579. These results suggest that reduction and amino acid conjugation at C(3)OOH group of dammarenolic acid (**1**) enhance cytotoxic activity against CRL1579.

Conjugation of a triterpenoid, betulinic acid, with a series of amino acids has been reported previously to improve H_2O solubility and selective cytotoxicity against human melanoma (MEL-2) and mouse fibrosarcoma (KB) cell lines [14].

Conclusions. – It can be concluded that triterpenoids isolated from dammar resin, especially dammarenolic acid (**1**) and its derivatives, are valuable as potential cancer chemopreventive agents in chemical and environmental carcinogenesis. In addition, it may be suggested that structural modification of the C(3)OOH group of 3,4-seco-triterpene acid may be useful to develop effective antitumor drugs. The results of this

study will be of value for further utilization of dammar resin in product application in pharmaceutical field in the future.

This work was supported, in part, by a grant '*Academic Frontier*' *Project for Private Universities* and matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology of Japan), 2007–2010.

Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2, 230–400 mesh; *Merck*) and *Chromatorex-ODS* (100–200 mesh; *Fuji Silysia Chemical, Ltd.*, Aichi, Japan). LC: Reversed-phase (RP) prep. high-performance liquid chromatography (HPLC) was carried out on an octadecyl silica column (*Pegasil ODS II column*, 25 cm × 10 cm i.d.; *Senshu Scientific Co., Ltd.*, Tokyo, Japan) at 25° with MeOH (4 ml/min; HPLC system *I*), MeOH/H_2O 95:5 (4 ml/min; HPLC system *II*), MeOH/H_2O 9:1 (4 ml/min; HPLC system *III*), or MeOH/H_2O 8:2 (3 ml/min; HPLC system *IV*) as mobile phase. Normal-phase HPLC was carried out on a silica column (*Pegasil Silica 60-5 column,* 25 cm × 4.6 mm i.d.; *Senshu Scientific Co., Ltd.*) at 25° with hexane/AcOEt 6:1 (1 ml/min) as mobile phase. M.p.: *Yanagimoto Micro Mp* apparatus; uncorrected. IR Spectra: *Perkin-Elmer Spectrum One* FT-IR spectrophotometer in KBr pellets; in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *JEOL ECA-600* or *JEOL ECX-400* spectrometer, in $CDCl_3$ unless otherwise indicated; δ in ppm, *J* in Hz. HR-ESI-MS: *Agilent 1100 LC/MSD TOF* (time-of-flight) system (ionization mode: positive; cap. voltage: 3000 V; fragmentor voltage: 225 V). EI-MS and HR-EI-MS: *JEOL JMS-GC mate* spectrometer (70 eV) using direct inlet system. Microplate reader: *Sunrise-Basic* (*Tecan Japan Co., Ltd.*, Kawasaki, Japan).

Chemicals and Materials. Dammar resin was obtained from the tree of *Shorea javanica* K. & V. (Dipterocarpaceae) at a forest near Palembang (Sumatra, Indonesia) in 2005. A voucher specimen has been deposited with the laboratory of *Arakawa Chemical Industries Ltd.* (Ibaraki, Japan). Compounds were purchased as follows: TPA, DMBA, MTT, and DMSO from *Sigma-Aldrich Japan Co.* (Tokyo, Japan), WSCD from *Peptide Institute Inc.* (Osaka, Japan), HOBt from *Dojindo Laboratories* (Kumamoto, Japan), PCC and $LiAlH_4$ from *Tokyo Chemical Industry Co., Ltd.* (Tokyo, Japan), DMF from *Wako Pure Chemical Industries, Ltd.* (Osaka, Japan), the EBV cell culture reagents and butanoic acid from *Nacalai Tesque, Inc.* (Kyoto, Japan), and *RPMI-1640* medium, fetal bovine serum (FBS) (for *RPMI-1640* medium), and penicillin-streptomycin from *Invitrogen Co.* (Auckland, NZ). All other chemicals and reagents were of anal. grade. Three compounds, dammarenediol II (**7**) [15], uvaol (**17**) [16], and erythrodiol (**18**) [16] were used as the reference specimens.

Extraction, Isolation, and Identification. Dammar resin (40 g) was powdered and dissolved in toluene. The toluene-soluble portion was chromatographed on a SiO_2 (500 g) column with a stepwise gradient of hexane/AcOEt (1:0 (2.6 l), 9:1 (7.0 l), 4:1 (6.0 l), 1:1 (2.7 l), 0:1 (1.8 l)) as eluent, which yielded three fractions containing triterpenoids: *Fr. A* (6.2 g; hexane/AcOEt 9:1), *B* (12.1 g; hexane/AcOEt 4:1), and *C* (4.6 g; hexane/AcOEt 1:1). A portion of *Fr. A* (164 mg) was subjected to HPLC (system *I*) to yield a mixture of hydroxydammarenone I [17] and II (**8** and **9**, resp.) [17] (61.2 mg; t_R 7.7 min), and *hydroxyhopanone* (**19**; 8.5 mg; t_R 11.6 min) [18]. The mixture **8**/**9** was subjected to normal-phase HPLC to give **8** (8.6 mg; t_R 14.3 min) and **9** (4.3 mg; t_R 13.8 min). A portion of *Fr. B* (7.8 g) was chromatographed on an *ODS* (*Chromatorex-ODS*) column with a 90% MeOH as eluent, which yielded three fractions, *Fr. B1* (588 mg), *B2* (3.8 g), and *B3* (1.1 g). *Fr. B1* was subjected to HPLC (system *IV*) to yield *(23*E*)-23-dehydro-25-hydroxydammarenolic acid* (**3**; 45.0 mg; t_R 29.7 min) [19], *isofouquierol* (**10**; 9.0 mg; t_R 33.6) [20], *cabralealactone* (**13**; 29.2 mg; t_R 27.9 min) [15][21], *19-dehydroxycecropiacic acid* (**14**; 53.2 mg; t_R 12.9 min), *19-dehydroxycecropiacic acid 3*-O-*methyl ester* (**15**; 24.2 mg, t_R 19.2 min), and *1β,16α-dihydroxyeudesm-4(14)-ene* (**20**; 12.5 mg; t_R 3.3 min) [22]. A portion of *Fr. B2* (885 mg) was subjected to HPLC (system *II*) to afford *dammarenolic acid* (**1**, 716 mg; t_R 16.5 min) [6], *dammarenolic acid methyl ester* (**2**, 20.0 mg; t_R 36.0 min) [21], *eichlerianic acid* (**4**; 27.9 mg; t_R 15.3 min) [6][15], and *ursonic acid* (**16**; 66.3 mg; t_R 25.2 min) [6]. A portion of *Fr. B3* (558 mg) was subjected to HPLC (system *II*) to give *dammarenediol I* (**6**; 48.1 mg; t_R 18.2 min) [17], *dammarenediol II* (**7**; 153.7 mg; t_R 19.2 min) [15], *ocotillone* (**12**; 22.9 mg; t_R 11.7 min) [6], *uvaol* (**17**; 9.9 mg; t_R 30.6 min) [16], and *erythrodiol* (**18**;

11.1 mg; t_R 32.0 min) [16]. *Fr. C* was chromatographed on an *ODS* (200 g) column with a 95% MeOH as eluent, which yielded purified *Fr. C* (3.1 g). A portion of *Fr. C* (201 mg) was subjected to HPLC (system *IV*) to yield **1**, **4**, *shoreic acid* (**5**; 5.8 mg; t_R 75.9 min) [6][15], **10**, and *isofouquierone* (**11**; 7.4 mg; t_R 31.8 min) [20]. Identification of **7**, **17**, and **18** was performed by chromatographic (HPLC) and spectroscopic (MS and ^{1}H-NMR) comparison with reference compounds, and of all of the other compounds described above, except for **14** and **15**, by spectral comparison with literature. Characterization of **14** and **15** was performed by spectroscopic methods, and their physical characteristics and spectral data are given below.

19-Dehydroxycecropiacic Acid (=*(1*R,*2*R,*4a*R,*4b*S,*6a*S,*9*S,*10*R,*10a*S,*12a*R*)-1-(Carboxymethyl)-2-(2-carboxypropan-2-yl)-1,3,4,4a,4b,5,6,7,8,9,10,10a,12,12a-tetradecahydro-1,4a,4b,9,10-pentamethylchrysene-6a(2*H*)-carboxylic Acid*; **14**): Amorphous powder. $[\alpha]_D^{25} = 55.8$ ($c = 0.24$, MeOH). IR (KBr): 3424, 2972, 2927, 2633, 1698, 1650, 1554, 1455, 1389, 1310, 1186, 970, 660, 534. ^{1}H-(600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): see *Table 2*. EI-MS: 502 (2), 484 (5), 248 (100), 203 (45), 189 (10), 133 (36). HR-EI-MS: 502.3286 (M^+, $C_{30}H_{46}O_6^+$; calc. 502.3294).

*19-Dehydroxycecropiacic Acid 3-*O*-Methyl Ester* (=*(1*R,*2*R,*4a*R,*4b*S,*6a*S,*9*S,*10*R,*10a*S,*12a*R*)-1-(Carboxymethyl)-1,3,4,4a,4b,5,6,7,8,9,10,10a,12,12a-tetradecahydro-2-(1-methoxy-2-methyl-1-oxopropan-2-yl)-1,4a,4b,9,10-pentamethylchrysene-6a(2*H*)-carboxylic Acid*; **15**): Amorphous powder. $[\alpha]_D^{25} = 40.9$ ($c = 0.92$, MeOH). IR (KBr): 3406, 2975, 2950, 1725, 1697, 1640, 1455, 1390, 1238, 1188, 1143, 964, 939, 659. ^{1}H-(600 MHz, C_5D_5N) and ^{13}C-NMR (150 MHz, C_5D_5N): see *Table 2*. EI-MS: 516 (6), 498 (10), 248 (58), 203 (43), 189 (3), 133 (40). HR-EI-MS: 516.3449 (M^+, $C_{31}H_{48}O_6^+$; calc. 516.3451).

*Preparation of Dammarenolic Acid Derivatives. (20*S*)-3,4-Secodammara-4(28),24-diene-3,20-diol* (=*(2*S*)-2-[(3*S,*3a*R,*5a*R,*6*S,*7*S,*9a*R,*9b*R*)-Dodecahydro-6-(3-hydroxypropyl)-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-3-yl]-6-methylhept-5-en-2-ol*; **21**). To a soln. of **1** (200 mg, 0.44 mmol) in dried benzene (6 ml), (trimethylsilyl)diazomethane (10% hexane soln.; 0.5 ml) was added and stirred at r.t. for 1 h. The mixture was evaporated to give **2** (198 mg, 95%). To a soln. of **2** (180 mg, 0.38 mmol) in dried Et_2O (15 ml), $LiAlH_4$ (84 mg, 2.2 mmol) was added and refluxed for 6 h under N_2. After addition of 1M HCl (2–3 ml), the mixture was stirred for few min, then extracted twice with Et_2O (20 ml). The Et_2O extract was washed with aq. $NaHCO_3$ (20 ml) and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude mixture was subjected to CC (SiO_2 (5 g); hexane/AcOEt 8:2) to afford purified **21** (81 mg, 48%). Amorphous solid. IR (KBr): 3367, 2940, 1637, 1452, 1376, 1113, 1056, 1022, 890. ^{1}H-NMR (400 MHz): 0.82 (*s*, 3 H); 0.90 (*s*, 3 H); 1.00 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.72 (*s*, 3 H); 3.58 (*m*, 2 H); 4.65 (*d*, $J = 1.9$, 1 H); 4.82 (br. *s*, 1 H); 5.12 (*t*, $J = 7.1$, 1 H). HR-ESI-MS: 467.3846 ($[M+Na]^+$, $C_{30}H_{52}NaO_2^+$; calc. 467.3865).

*(20*S*)-20-Hydroxy-3,4-secodammara-4(28),24-dien-3-al* (=*3-[(3*S,*3a*R,*5a*R,*6*S,*7*S,*9a*R,*9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]propanal*; **22**). To a soln. of **21** (36 mg, 0.08 mmol) in dried CH_2Cl_2 (2.7 ml), pyridinium chlorochromate (87 mg, 0.40 mmol) was added, and the mixture was stirred for 2 h at r.t. After addition of Et_2O (3 ml), the mixture was passed through CC (SiO_2 (5 g); Et_2O) to afford **22** (30 mg, 83%) without further purification: Amorphous solid. IR (KBr): 3436, 2962, 2726, 1722, 1634, 1453, 1376, 1115, 892. ^{1}H-NMR (400 MHz): 0.88 (*s*, 3 H); 0.90 (*s*, 3 H); 1.01 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.72 (*s*, 3 H); 4.63 (br. *s*, 1 H); 4.84 (br. *s*, 1 H); 5.12 (*t*, $J = 6.8$, 1 H); 9.74 (*s*, 1 H). HR-ESI-MS: 465.3712 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3708).

*Dammarenoyl-*L*-alanine* (=N-*{3-[(3*S,*3a*R,*5a*R,*6*S,*7*S,*9a*R,*9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]propanoyl}-*L*-alanine*; **23**). To a soln. of **1** (51 mg, 0.11 mmol), HOBt (20 mg, 0.15 mmol), and L-alanine methyl ester hydrochloride (16 mg, 0.11 mmol) in dried DMF (150 µl) was added WSCD (21 µl) at −20°, and the mixture was left to stand overnight at r.t. The mixture was diluted with H_2O and extracted with AcOEt (3 ml) twice. The extract was washed with H_2O, 1M HCl, and aq. $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure to afford a crude product (56 mg). The crude product was purified by HPLC (system III) to give dammarenoyl-L-alanine methyl ester (**23M**; 19 mg, 32%, t_R 8.0 min). Compound **23M** (14 mg, 0.02 mmol) was dissolved in 4M NaOH (0.6 ml)/THF (1.5 ml)/MeOH (1 ml) and left to stand overnight. The mixture was diluted with H_2O and extracted with Et_2O (3 ml) twice. The Et_2O extract was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure to give **23**

(13 mg, 95%). Amorphous solid. IR (KBr): 3421, 2963, 1731, 1648, 1534, 1458, 1375, 1212, 1154, 892. ^{1}H-NMR (400 MHz): 0.86 (*s*, 3 H); 0.89 (*s*, 3 H); 1.00 (*s*, 3 H); 1.15 (*s*, 3 H); 1.45 (*d*, *J* = 7.1, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.74 (*s*, 3 H); 4.56 (*sext.*, *J* = 7.1, 1 H); 4.68 (br. *s*, 1 H); 4.86 (br. *s*, 1 H); 5.12 (*t*, *J* = 7.1, 1 H); 6.14 (*d*, *J* = 7.1, 1 H). HR-ESI-MS: 552.4014 ($[M+Na]^+$, $C_{33}H_{55}NNaO_4^+$; calc. 552.4028).

*Dammarenoyl-*L*-aspartic Acid* (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]-propanoyl}-*L*-aspartic Acid*; **24**). Compound **24** and the other nine amino acid conjugates, **25**–**34**, of **1** were prepared in the same way as described above for the preparation of **23**. Thus, treatment of **1** (50 mg, 0.11 mmol) with L-aspartic acid dimethyl ester hydrochloride (22 mg, 0.11 mmol) afforded dammarenoyl-L-aspartic acid dimethyl ester (**24M**; 32 mg, 49%; t_R 11.2 min on HPLC system *III*) and hydrolysis of **24M** (22 mg, 0.04 mmol) gave **24** (20 mg, 95%). White solid. M.p. 120–122°. IR (KBr): 3419, 2962, 1730, 1646, 1519, 1453, 1385, 1217, 892. ^{1}H-NMR (400 MHz): 0.85 (*s*, 3 H); 0.90 (*s*, 3 H); 1.00 (*s*, 3 H); 1.18 (*s*, 3 H); 1.63 (*s*, 3 H); 1.70 (*s*, 3 H); 1.75 (*s*, 3 H); 2.90 (*dd*, *J* = 4.9, 17.8, 1 H); 3.10 (*dd*, *J* = 4.4, 18.3, 1 H); 4.68 (br. *s*, 1 H); 4.85 (*m*, 1 H); 4.86 (br. *s*, 1 H); 5.13 (*t*, *J* = 6.3, 1 H); 6.74 (*d*, *J* = 7.3, 1 H). HR-ESI-MS: 596.3909 ($[M+Na]^+$, $C_{34}H_{55}NNaO_6^+$; calc. 596.3927).

Dammarenoyl-glycine (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]propanoyl}glycine*; **25**). Treatment of **1** (50 mg, 0.11 mmol) with glycine methyl ester hydrochloride (15 mg, 0.12 mmol) gave dammarenoyl-glycine methyl ester (**25M**; 26 mg, 45%; t_R 9.6 min on HPLC system *III*) and hydrolysis of **25M** (25 mg, 0.05 mmol) yielded **25** (14 mg, 57%). Amorphous solid. IR (KBr): 3308, 2945, 1729, 1658, 1635, 1539, 1456, 1385, 1209, 891. ^{1}H-NMR (400 MHz): 0.86 (*s*, 3 H); 0.89 (*s*, 3 H); 1.00 (*s*, 3 H); 1.16 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.73 (*s*, 3 H); 4.05 (*d*, *J* = 4.1, 2 H); 4.68 (br. *s*, 1 H); 4.85 (br. *s*, 1 H); 5.12 (*t*, *J* = 7.1, 1 H); 6.42 (br. *s*, 1 H). HR-ESI-MS: 538.3839 ($[M+Na]^+$, $C_{32}H_{53}NNaO_4^+$; calc. 538.3872).

*Dammarenoyl-*L*-isoleucine* (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]-propanoyl}-*L*-isoleucine*; **26**). Treatment of **1** (51 mg, 0.11 mmol) with L-isoleucine methyl ester hydrochloride (20 mg, 0.11 mmol) afforded dammarenoyl-L-isoleucine methyl ester (**26M**; 28 mg, 43%; t_R 14.2 min on HPLC system *III*) which (21 mg, 0.04 mmol) upon hydrolysis yielded **26** (20 mg, 98%). White solid. M.p. 93–95°. IR (KBr): 3421, 2964, 1717, 1651, 1525, 1375, 1212, 892. ^{1}H-NMR (400 MHz): 0.86 (*s*, 3 H); 0.89 (*s*, 3 H); 0.95 (*t*, *J* = 7.6, 3 H); 0.96 (*d*, *J* = 7.1, 3 H); 1.01 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.74 (*s*, 3 H); 4.57 (*dd*, *J* = 4.9, 8.0, 1 H); 4.68 (br. *s*, 1 H); 4.86 (br. *s*, 1 H); 5.13 (*t*, *J* = 7.3, 1 H); 5.92 (*d*, *J* = 8.5, 1 H). HR-ESI-MS: 594.4469 ($[M+Na]^+$, $C_{36}H_{61}NNaO_4^+$; calc. 594.4498).

*Dammarenoyl-*L*-leucine* (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]propanoyl}-*L*-leucine*; **27**). Treatment of **1** (51 mg, 0.11 mmol) with L-leucine methyl ester hydrochloride (20 mg, 0.11 mmol) gave dammarenoyl-L-leucine methyl ester (**27M**; 32 mg, 49%; t_R 14.8 min on HPLC system *III*), and hydrolysis of **27M** (21 mg, 0.04 mmol) afforded **27** (20 mg, 97%). White solid. M.p. 98–100°. IR (KBr): 3423, 2959, 1718, 1656, 1522, 1458, 1385, 1235, 1207, 892. ^{1}H-NMR (400 MHz): 0.86 (*s*, 3 H); 0.89 (*s*, 3 H); 0.95 (*d*, *J* = 6.1, 3 H); 0.97 (*d*, *J* = 6.1, 3 H); 1.01 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.70 (*s*, 3 H); 1.74 (*s*, 3 H); 4.56 (*dd*, *J* = 3.9, 7.6, 1 H); 4.67 (br. *s*, 1 H); 4.85 (br. *s*, 1 H); 5.12 (br *t*, *J* = 7.1, 1 H); 5.79 (*d*, *J* = 7.1, 1 H). HR-ESI-MS: 594.4474 ($[M+Na]^+$, $C_{36}H_{61}NNaO_4^+$; calc. 594.4498).

*Dammarenoyl-*L*-methionine* (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[*a*]naphthalen-6-yl]-propanoyl}-*L*-methionine*; **28**). Treatment of **1** (50 mg, 0.11 mmol) with L-methionine methyl ester hydrochloride (22 mg, 0.11 mmol) afforded dammarenoyl-L-methionine methyl ester (**28M**; 34.2 mg, 52%; t_R 13.0 min on HPLC system *III*), which (24 mg, 0.04 mmol) upon hydrolysis gave **28** (23 mg, 98%). Amorphous solid. IR (KBr): 3421, 2962, 1718, 1648, 1524, 1458, 1375, 1226, 1115, 954, 892. ^{1}H-NMR (400 MHz): 0.87 (*s*, 3 H); 0.89 (*s*, 3 H); 1.01 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.74 (*s*, 3 H); 2.12 (*s*, 3 H); 4.65 (*m*, 1 H); 4.66 (br. *s*, 1 H); 4.86 (br. *s*, 1 H); 5.13 (*t*, *J* = 7.1, 1 H); 6.34 (*d*, *J* = 7.3, 1 H). HR-ESI-MS: 612.4030 ($[M+Na]^+$, $C_{35}H_{59}NNaO_4S^+$; calc. 612.4062).

Dammarenoyl-L-phenylalanine (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-6-yl]propanoyl}-L-phenylalanine*; **29**). Treatment of **1** (46 mg, 0.10 mmol) with L-phenylalanine methyl ester hydrochloride (22 mg, 0.10 mmol) gave dammarenoyl-L-phenylalanine methyl ester (**29M**; 26 mg, 42%; t_R 14.4 min on HPLC system *III*), and hydrolysis of **29M** (14 mg, 0.02 mmol) afforded **29** (11 mg, 80%). White solid. M.p. 84–86°. IR (KBr): 3422, 2962, 1731, 1650, 1522, 1458, 1375, 1212, 892, 736, 700. ^{1}H-NMR (400 MHz): 0.85 (*s*, 3 H); 0.88 (*s*, 3 H); 1.01 (*s*, 3 H); 1.18 (*s*, 3 H); 1.65 (*s*, 3 H); 1.71 (*s*, 3 H); 1.72 (*s*, 3 H); 3.15 (*dd*, *J* = 6.3, 14.1, 1 H); 3.26 (*dd*, *J* = 5.6, 13.9, 1 H); 4.62 (br. *s*, 1 H); 4.81 (br. *s*, 1 H); 4.86 (*dd*, *J* = 6.1, 13.4, 1 H); 5.15 (*t*, *J* = 7.1, 1 H); 5.91 (*d*, *J* = 7.3, 1 H); 7.18 (*m*, 2 H); 7.30 (*m*, 3 H). HR-ESI-MS: 628.4308 ($[M+Na]^+$, $C_{39}H_{59}NNaO_4^+$; calc. 628.4341).

Dammarenoyl-L-proline (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-6-yl]propanoyl}-L-proline*; **30**). Treatment of **1** (51 mg, 0.11 mmol) with L-proline methyl ester hydrochloride (19 mg, 0.11 mmol) yielded dammarenoyl-L-proline methyl ester (**30M**; 30 mg, 47%; t_R 14.0 min on HPLC system *III*), and hydrolysis of **30M** (21 mg, 0.04 mmol) afforded **30** (20 mg, 98%). White solid. M.p. 95–97°. IR (KBr): 3450, 2962, 1731, 1637, 1449, 1375, 1194, 888. ^{1}H-NMR (400 MHz): 0.89 (*s*, 3 H); 0.90 (*s*, 3 H); 1.02 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.74 (*s*, 3 H); 3.47 (*m*, 1 H); 3.61 (*m*, 1 H); 4.61 (*dd*, *J* = 1.7, 8.0, 1 H); 4.68 (br. *s*, 1 H); 4.86 (br. *s*, 1 H); 5.12 (*t*, *J* = 7.3, 1 H). HR-ESI-MS: 578.4175 ($[M+Na]^+$, $C_{35}H_{57}NNaO_4^+$; calc. 578.4185).

Dammarenoyl-L-serine (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-6-yl]propanoyl}-L-serine*; **31**). Treatment of **1** (50 mg, 0.11 mmol) with L-serine methyl ester hydrochloride (17 mg, 0.11 mmol) afforded dammarenoyl-L-serine methyl ester (**31M**; 29 mg, 47%; t_R 9.6 min on HPLC system *III*), and hydrolysis of **31M** (20 mg, 0.04 mmol) yielded **31** (19 mg, 97%). White solid. M.p. 96–99°. IR (KBr): 3394, 2963, 1731, 1650, 1528, 1458, 1375, 1228, 1077, 892. ^{1}H-NMR (400 MHz): 0.87 (*s*, 3 H); 0.90 (*s*, 3 H); 1.01 (*s*, 3 H); 1.16 (*s*, 3 H); 1.63 (*s*, 3 H); 1.70 (*s*, 3 H); 1.74 (*s*, 3 H); 3.82 (*dd*, *J* = 3.7, 11.7, 1 H); 4.16 (*dd*, *J* = 3.2, 11.5, 1 H); 4.53 (*dd*, *J* = 2.7, 5.4, 1 H); 4.68 (br. *s*, 1 H); 4.87 (br. *s*, 1 H); 5.13 (*t*, *J* = 7.3, 1 H); 6.59 (*d*, *J* = 5.1, 1 H). HR-ESI-MS: 568.3949 ($[M+Na]^+$, $C_{33}H_{55}NNaO_5^+$; calc. 568.3977).

Dammarenoyl-L-tryptophan (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-6-yl]propanoyl}-L-tryptophan*; **32**). Treatment of **1** (51 mg, 0.11 mmol) with L-tryptophan methyl ester hydrochloride (28 mg, 0.11 mmol) afforded dammarenoyl-L-tryptophan methyl ester (**32M**; 29 mg, 40%; t_R 6.0 min on HPLC system *III*), and hydrolysis of **32M** (19 mg, 0.03 mmol) gave **32** (18 mg, 97%). White solid. M.p. 108–110°. IR (KBr): 3401, 2945, 1731, 1648, 1518, 1458, 1375, 1228, 1104, 892, 741. ^{1}H-NMR (400 MHz): 0.78 (*s*, 3 H); 0.83 (*s*, 3 H); 0.97 (*s*, 3 H); 1.15 (*s*, 3 H); 1.64 (*s*, 3 H); 1.66 (*s*, 3 H); 1.70 (*s*, 3 H); 3.38 (br. *d*, *J* = 5.8, 2 H); 4.52 (br. *s*, 1 H); 4.72 (br. *s*, 1 H); 4.89 (*dd*, *J* = 5.8, 12.7, 1 H); 5.14 (*t*, *J* = 7.3, 1 H); 5.94 (*d*, *J* = 6.8, 1 H); 7.07 (*d*, *J* = 1.9, 1 H); 7.13 (br. *t*, *J* = 7.1, 1 H); 7.21 (br. *t*, *J* = 7.1, 1 H); 7.37 (*d*, *J* = 8.0, 1 H); 7.58 (*d*, *J* = 7.3, 1 H); 8.21 (br. *s*, 1 H). HR-ESI-MS: *m/z* 667.4407 ($[M+Na]^+$, $C_{41}H_{60}N_2NaO_4^+$; calc. 667.4450).

Dammarenoyl-L-tyrosine (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-6-yl]propanoyl}-L-tyrosine*; **33**). Treatment of **1** (46 mg, 0.10 mmol) with L-tyrosine methyl ester hydrochloride (22 mg, 0.10 mmol) yielded dammarenoyl-L-tyrosine methyl ester (**33M**; 38 mg, 59%; t_R 9.1 min on HPLC system *III*), and hydrolysis of **33M** (23 mg, 0.04 mmol) afforded **33** (22 mg, 97%). White solid. M.p. 102–105°. IR (KBr): 3421, 2963, 1719, 1644, 1517, 1450, 1375, 1230, 1112, 893, 829. ^{1}H-NMR (400 MHz): 0.82 (*s*, 3 H); 0.87 (*s*, 3 H); 0.99 (*s*, 3 H); 1.18 (*s*, 3 H); 1.64 (*s*, 3 H); 1.69 (*s*, 3 H); 1.70 (*s*, 3 H); 2.96 (*dd*, *J* = 6.3, 14.1, 1 H); 3.15 (*dd*, *J* = 4.9, 13.9, 1 H); 4.63 (br. *s*, 1 H); 4.80 (br. *s*, 1 H); 4.86 (*dd*, *J* = 5.8, 13.7, 1 H); 5.13 (*t*, *J* = 6.8, 1 H); 6.12 (br. *d*, *J* = 7.1, 1 H); 6.60 (*d*, *J* = 8.3, 2 H); 6.94 (*d*, *J* = 8.3, 2 H). HR-ESI-MS: 644.4259 ($[M+Na]^+$, $C_{39}H_{59}NNaO_5^+$; calc. 644.4290).

Dammarenoyl-L-valine (= N-*{3-[(3*S*,3a*R*,5a*R*,6*S*,7*S*,9a*R*,9b*R*)-Dodecahydro-3-[(2*S*)-2-hydroxy-6-methylhept-5-en-2-yl]-6,9a,9b-trimethyl-7-(prop-1-en-2-yl)-1*H*-cyclopenta[a]naphthalen-6-yl]propanoyl}-L-valine*; **34**). Treatment of **1** (51 mg, 0.11 mmol) with L-valine methyl ester hydrochloride (19 mg, 0.11 mmol) afforded dammarenoyl-L-valine methyl ester (**34M**; 19 mg, 30%; t_R 14.0 min on HPLC

system *III*), and hydrolysis of **34M** (16 mg, 0.03 mmol) gave **34** (15 mg, 96%). White solid. M.p. 98–102°. IR (KBr): 3421, 2964, 1723, 1657, 1523, 1458, 1375, 1209, 1184, 1148, 892. ^{1}H-NMR (400 MHz): 0.87 (*s*, 3 H); 0.90 (*s*, 3 H); 0.95 (*d*, *J* = 6.8, 3 H); 0.98 (*d*, *J* = 7.1, 3 H); 1.01 (*s*, 3 H); 1.15 (*s*, 3 H); 1.63 (*s*, 3 H); 1.69 (*s*, 3 H); 1.74 (*s*, 3 H); 4.69 (br. *s*, 1 H); 4.86 (br. *s*, 1 H); 4.54 (*dd*, *J* = 5.1, 8.5, 1 H); 5.12 (*t*, *J* = 7.8, 1 H); 5.92 (*d*, *J* = 8.5, 1 H). HR-ESI-MS: 580.4311 ($[M+Na]^+$, $C_{35}H_{59}NNaO_4^+$; calc. 580.4341).

In vitro *EBV-EA Activation.* The EBV genome-carrying lymphoblastoid cells, *Raji* cells, derived from *Burkitt*'s lymphoma, were cultured in *RPMI-1640* medium. The *Raji* cells were incubated for 48 h at 37° in a medium containing 4 mM butanoic acid, 32 pM TPA, and various amounts of each test compound. Smears were made from the cell suspension, and the EBV-EA-inducing cells were stained by means of an indirect immunofluorescence technique. Details of this *in vitro* assay on EBV-EA induction have been reported in [23].

In vivo *Two-Stage Carcinogenesis Assay on Mouse Skin Papillomas.* Each group of specific pathogen-free ICE mice obtained from *Japan SLC* (Shizuoka, Japan) was composed of 15 mice housed five per cage and given H_2O *ad libitum*. The back of each mouse was shaved with surgical clippers, and the mouse was treated topically with DMBA (100 μg, 390 nmol) in acetone (0.1 ml) for the initiation treatment. One week after the initiation, papilloma formation was promoted by the application of TPA (1 μg, 1.7 nmol) in acetone (0.1 ml) on the skin twice a week for 20 weeks. Group I received the TPA treatment alone, and group II received a topical application of test sample (85 nmol) in acetone (0.1 ml) 1 h before each TPA treatment. The incidence and numbers of papillomas were observed and detected weekly for 20 weeks; only typical papillomas larger than *ca.* 1 mm in diameter were counted. Details of this *in vivo* two-stage carcinogenesis test have been reported in [24].

Cytotoxicity Assay. Cytotoxicity assay was performed according to the method described in [25][26]. Briefly, HL60 (human leukemia) and CRL1579 (human melanoma) cell lines (each 3×10^3 cells/well) were treated with compounds for 48 h, and then MTT soln. was added to the well. After incubation for 3 h, the generated blue formazan was solubilized with 0.04M HCl in i-PrOH. The absorbances at 570 (top) and 630 nm (bottom) were measured with a microplate reader.

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Received April 26, 2010