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Cytotoxicity of abietane diterpenoids from *Perovskia abrotanoides* and of their semisynthetic analogues

Yutaka Aoyagi,[a] Yoshinao Takahashi,[a] Yudai Satake,[a] Koichi Takeya,[a,*] Ritsuo Aiyama,[b] Takeshi Matsuzaki,[b] Shusuke Hashimoto[b] and Teruo Kurihara[c]

[a]*School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan*
[b]*Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi-Shi, Tokyo 186-8650, Japan*
[c]*Faculty of Science, Josai University, 1-1 Keyakidai, Sakado, Saitama, 350-0295, Japan*

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Abstract—Seven known abietane diterpenoids and 11-*O*- and 12-*O*-acetylcarnosic acids were isolated from a methanol extract of *Perovskia abrotanoides* (Labiatae). Structure and cytotoxic activity relationships (SARs) of the natural and semisynthetic analogues of the presently isolated abietane diterpenoids were studied by using P388 murine leukemia cells.

1. Introduction

Perovskia abrotanoides (Labiatae) is an Iranian folk-medicinal herb, used for treatment of leishmaniasis.[1] The plants of this genus are known to contain irregular triterpenes, perdione[2] and perovskone,[3] and a large amount of tanshinones, and their reported biological activities include effects on cardiac function,[4–6] antioxidant,[7,8] aldose reductase inhibitory,[9] cytotoxic,[10] binding activities with the benzodiazepine receptor,[11] and apoptosis induction.[12] In the present study, we isolated seven known abietane diterpenoids and 11-*O*- and 12-*O*-acetylcarnosic acids from *P. abrotanoides*, the latter two being separated after conversion to methyl 11-*O*-acetyl-12-*O*-methyl- and 12-*O*-acetyl-11-*O*-methyl carnosates, respectively. Then are performed the structure and cytotoxic activity relationship (SAR) study of the presently isolated natural diterpenoids and their semisynthetic derivatives by using P388 murine leukemia cells (Fig. 1).

2. Results and discussion

By SiO_2 column chromatography, a hot EtOAc extract (238 g) of air-dried aerial parts of *P. abrotanoides* (2.9 kg) gave carnosic acid (**1**),[13] 12-*O*-methylcarnosic acid (**2**),[14,15] carnosol (**5**),[16] isorosmanol (**6**),[17] rosmanol (**7**),[16] 7-*O*-methylrosmanol (**8**),[18] and 7-*epi*-7-*O*-methylrosmanol (**9**),[19] and a mixture of two carnosic acids (**A**). Acetylation of the mixture **A** gave a single compound **10**, which on methylation gave methyl 11,12-*O*-diacetylcanosate (**11**) as the sole product in 79% yield. The spectral data of **11** were identical with those of carnosic acid diacetate methyl ester reported.[20,21] Methylation of the mixture **A** with methyl iodide in the presence of potassium carbonate gave a mixture of 11-*O*- and 12-*O*-methylated compounds, which was separated by silica gel chromatography into 12-*O*-acetyl-11-*O*-methyl- (**12**) and 11-*O*-acetyl-12-*O*-methylcarnosic acids (**13**) in 57 and 12% yields, respectively (Scheme 1). Selected NOE correlations are shown in Figure 2. In **12**, the NOEs were observed between the methyl protons of 20-ester group and 11-*O*-methyl protons; the methyl protons of 12-*O*-acetyl group and 15-H, and 16- and 17-methyl protons. In the minor component **13**, the NOEs were observed between the methyl protons of 20-acetyl group and 12-*O*-methyl protons and between the methyl protons of 12-*O*-acetyl group and 15-H, and 16- and 17-methyl protons. Thus, on the basis of the structures of **12** and **13** established, the mixture A was shown to consist of 12-*O*-acetyl- (**3**) and 11-*O*-acetylcarnosic acids (**4**)[22] in a ratio of 83:17. The structure of **3** and **4** may suggest a possibility that they are artifacts derived from **1** during the extraction and purification process. However, as seen in Figure 2, describing the steric interaction between the acetyl group at C-12 and the *i*-propyl group

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* Corresponding author; E-mail: takeyak@ps.toyaku.ac.jp

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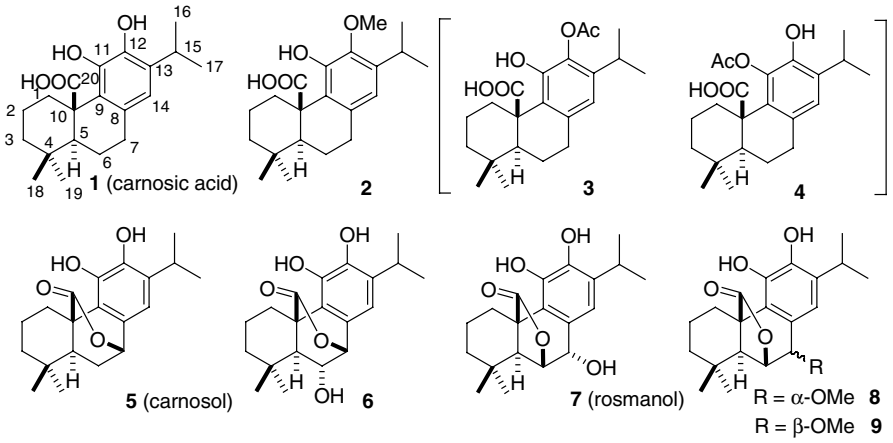
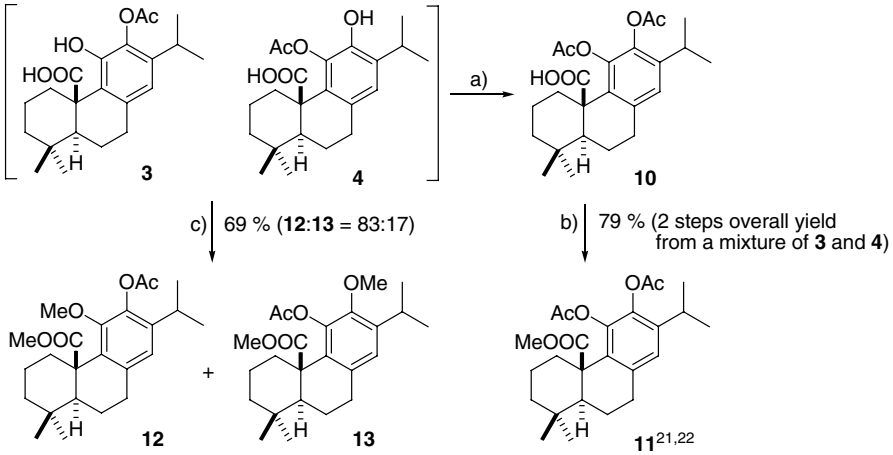


Figure 1. Abietane-type diterpenoids from *Perovskia abrotanoides*.



Scheme 1. Separation and structure elucidation of **3** and **4**. Reagents: (a) Ac_2O/Py; (b) $TMSCHN_2$/benzene–MeOH (1:1); (c) K_2CO_3/MeI/acetone.

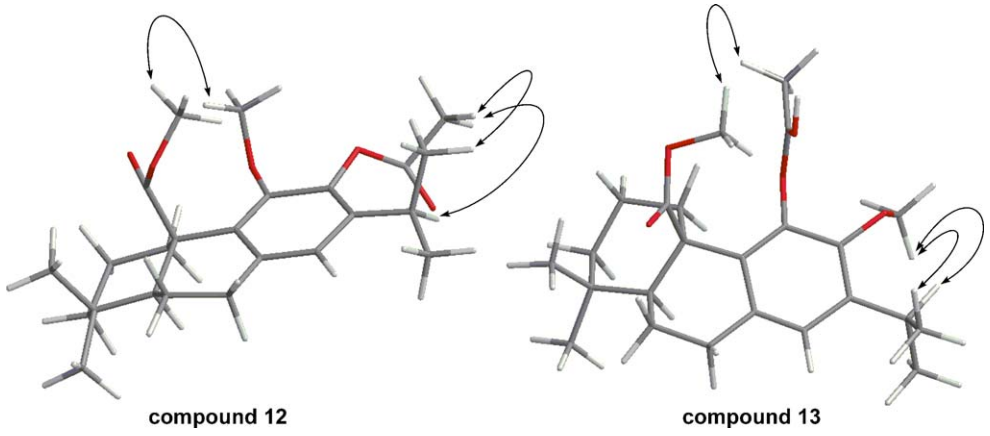
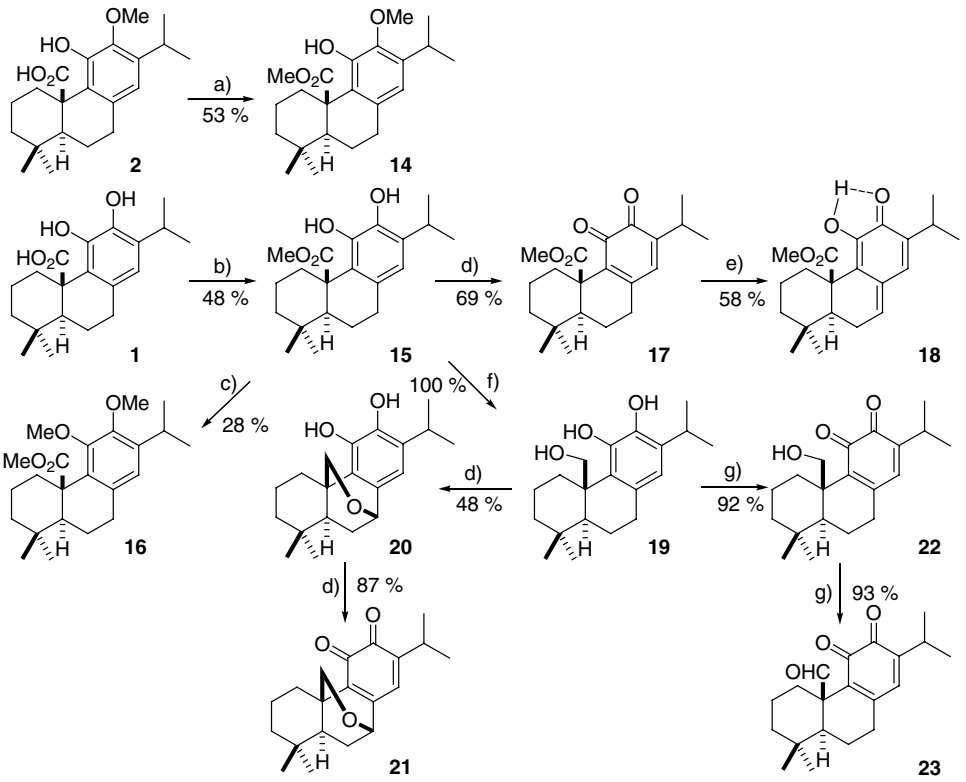


Figure 2. Selected NOESY correlations of compounds **12** and **13**.

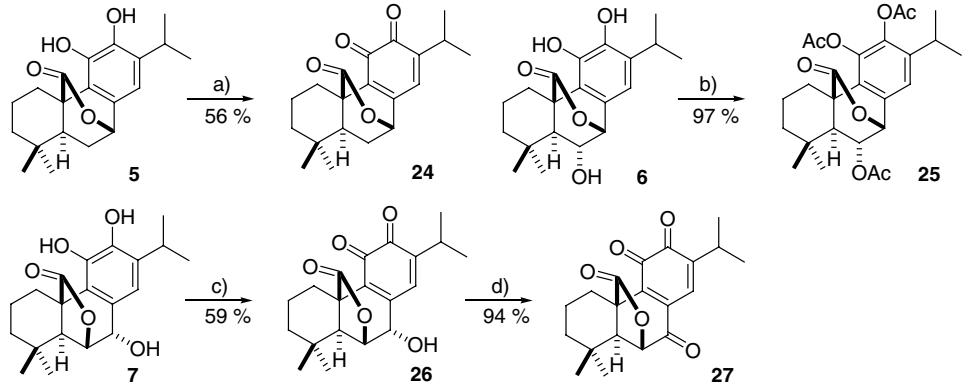
at C-13, the acetyl group at C-12 is under much bigger steric hindrance than that at C-11, and yet **3** is present in a lager amount than **4**. This fact may imply that, of the two, at least **3** is not an artifact.

In the present paper, a number of analogues of this series of compounds were prepared for the studies on the structure–activity relationship (SAR) by modification of their B- and/or C-rings and/or 20-substituents as shown in Schemes 2 and 3.

By methylation with trimethylsilyldiazomethane ($TMSCHN_2$), **2** and **1** gave the corresponding methyl esters **14**[14] and **15**,[23] respectively. The methylation of



Scheme 2. Chemical transformation of carnosic acid-type diterpenes. Reagents: (a) $TMSCHN_2$/benzene–MeOH (5:1); (b) $TMSCHN_2$/benzene–MeOH (2:1); (c) K_2CO_3/MeI/acetone; (d) DDQ/dioxane; (e) TBSOTf/2,6-lutidine/CH_2Cl_2; (f) $LiAlH_4$/THF; (g) Dess–Martin periodinane/CH_2Cl_2.



Scheme 3. Chemical transformation of carnosol and rosmanol-type diterpenes. Reagents: (a) Ag_2O/THF; (b) Ac_2O/pyridine; (c)DDQ/dioxane; (d) Dess–Martin periodinane/CH_2Cl_2.

the catechol unit in **15** by the usual method gave **16**.[24] Gonzalez et al.[25] reported that oxidation of **15** with two equivalents of DDQ did not produce *o*-quinone **17** but a mixture of quinone methide **18** and 5,6-dehydro compound in a ratio 1:9. We used one equivalent of DDQ in the reaction, which produced *o*-quinone **17** as the sole product in 69% yield. The efficient conversion of *o*-quinone **17** to quinone methide **18**[25] was accomplished by the treatment of **17** with a combination of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) and 2,6-lutidine. Reduction of **15** with lithium aluminum hydride in THF by the procedure described by Gonzalez et al.[25] gave an alcohol **19** in good yield. When **19** was reacted with one equivalent of DDQ, an unexpected intramolecular etherification took place to give **20**[25] in 48% yield. Further treatment of **20** with one equivalent of DDQ gave *o*-quinone **21**[26] in 87% yield. When **19** was treated with one equivalent of Dess–Martin periodinane in CH_2Cl_2 at room temperature, it gave the desired *o*-quinone **22** in good yield. Further reaction of **22** with one equivalent of Dess–Martin periodinane gave an aldehyde **23** in 93% (Scheme 2).

Analogues of carnosols (**5** and **6**) and rosmanol (**7**) were prepared as shown in Scheme 3. Catechol **5** was converted to *o*-quinone **24**[27,28] under the conventional oxidation conditions (silver(I) oxide in THF), **6** was acetylated to its triacetyl derivative **25**,[17] and rosmanol **7** was oxidized to **26**[18] with DDQ in 59%, which was further oxidized

with Dess–Martin periodinane to an *o*-quinone **27** in 94% (Scheme 3).

3. Cytotoxic activities and structure–activity relationships

These natural diterpenes from this plant source and their analogues (**1**, **2**, and **5**–**27**) prepared in the present study were assayed for their cytotoxic activities on P388 murine leukemia cells. The results are shown in Table 1.

The cytotoxic activity of the mother catechols (**1**, **15**, and **6**) tends to decrease when the phenolic hydroxy groups are acetylated or methylated, and the activity of those analogues having an *o*-quinone system on the C-ring is about the same as or higher than that of the corresponding catechols (**15** vs **11**, **13**, **14**, and **16**; **6** vs **25**). This fact suggests that the *o*-quinone-bearing C-ring or its catechol with two hydroxyls that can be easily oxidized to *o*-quinone is the essential structural requirement or pharmacophore required for this series of compounds to show cytotoxicity. As regards the effect of C-20 substituents on the activity, an ester group seems to enhance or augment the activity of the analogues having the above-specified pharmacophore. Hydroxymethyl, lactone, cyclic ether, and formyl groups give various less significant effects implying that stereo and electrostatic effects of C-20 substituents may play some role in the activity. Masuda et al. suggested that the low cytotoxic activity of carnosic acid *o*-quinone and its analogues having carboxylic acids at C-20 on K562 human leukemia cells is considered to be due to its negative charge which dose not allow the compounds to permeate into the cells.[27] Our present results demonstrate that if the cytotoxic activity is affected by dissociation of carboxylic acid functionality at C-20, the effect should be of a secondary order. Of the analogues assayed in the present study, abietane-type analogues **17** and **18**, having both 20-ester group and *o*-quinone or *o*-quinone equivalent structure in the C-ring, were shown to be the most active.

Table 1. IC_{50} values of natural and semisynthetic analogues **1**, **2**, and **5**–**27** against P388 murine leukemia cells

Compound	IC_{50} (μg/mL)
1	1.2
2	6.7
5	2.0
6	4.0
7	0.75
8	2.0
9	2.1
10	7.5
11	2.8
12	6.2
13	5.7
14	5.9
15	0.6
16	7.1
17	0.27
18	0.22
19	1.9
20	5.2
21	2.1
22	0.94
23	0.92
24	1.9
25	8.2
26	0.61
27	5.4
Cryptotanshinone	8.3
Mitomycin	0.035

4. Experimental

4.1. General method

Melting points were determined on a Yanaco MP-3 apparatus and are recorded uncorrected. IR spectra were recorded on a JASCO FT/IR 620 spectrophotometer, optical rotation on a JASCO DIP-360 automatic digital polarimeter, and Mass spectra on a Micromass LCT (Manchester, UK) spectrometer. NMR spectra were recorded in $CDCl_3$ or pyridine-d_5 on a Brucker AM-400 and DRX-500 spectrometer at 300 K and the *J* values were given in Hz. Preparative HPLC was carried out on a JASCO PU-986 equipped with a UV-970 UV detector (λ 220 nm) and an Inertsil PREP-ODS column (10 μm, 20 × 250 mm), by using a MeOH/H_2O or MeCN/H_2O solvent system at a flow rate of 10mL/min.

4.1.1. Plant material. The aerial parts of *P. abrotanoides* (Labiatae) were purchased from Kordes Jungpflanzen (Bilsen, Germany, http://www.kordes-jungpflanzen.de). The botanical identification was made by Prof. K. Takeya, School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo, Japan. A voucher specimen has been deposited in the herbarium of Tokyo University of Pharmacy and Life Science (03JCP12).

4.1.2. Extraction and isolation. Air-dried aerial parts of *P. abrotanoides* (2.9 kg) were extracted with hot AcOEt (3× 45 mL). The combined AcOEt extract was evaporated in vacuo to give a residue (238 g), which was applied to a silica gel column (Silica Gel 60N gel®, Kanto Chemical Co. Ltd., 63–210 μm) and eluted with toluene/AcOEt (1:0, 9:1, 9:1, 8:1, and 4:1) and AcOEt/MeOH (5:1 and 0:1) to yield fractions I–VII. Fraction **III** (64 g) was further subjected to a series of silica gel column chromatography eluting with toluene containing various amounts of AcOEt or Me_2CO to afford carnosic acid **1**[13] (7.3 g), carnosol **5**[16] (3.1 g), isorosmanol **6**[17] (54 mg), rosmanol **7**[16] (72 mg), 7-*O*-methylrosmanol **8**[18] (210 mg), and 7-*O*-methyl-7-epirosmanol **9**[19] (19 mg). Silica gel column chromatography of fraction **IV** (12 g) eluting with toluene with an increasing amount of Me_2CO afforded a mixture **A** (1.3 g) (mixture of 12-*O*- (**3**) and 11-*O*-acetylcarnosic acids (**4**)[22]) and 12-*O*-methyl carnosic acid **2**[14,15] (415 mg). Analogous chromatography of fraction **V** (32 g) eluting with $CHCl_3$ with an increasing amount of Me_2CO afforded a mixture **A** (3.1 g)(mixture of 12- (**3**) and 11-acetoxycarnosic acids (**4**)[22]).

4.1.3. Acetylation and methylation of mixture A (mixture of 11- (3) and 12-acetoxycarnosic acid (4)). A solution of the mixture **A** (0.021 g, 0.055 mmol), Ac_2O (0.25 mL, 2.7 mmol), and dry pyridine (0.5 mL, 6.2 mmol) was

stirred at rt for 17 h under an Ar atmosphere. After the reaction mixture was poured into ice-cooled H_2O (10 mL), the mixture was extracted with AcOEt (3× 10 mL). The combined organic layer was washed with 5% HCl aq (3× 10 mL), satd aq $NaHCO_3$ (3× 10 mL), and brine (3× 10 mL), successively. The organic phase was then dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue (0.023 g), which was used for the subsequent reaction without further purification. 2.0 M $TMSCHN_2$ in hexanes (1 mL, 2 mmol) was added to a solution of the crude diacetate (**10**) (0.023 g) in a mixture of benzene (0.5 mL) and MeOH (0.25 mL) at rt. After the reaction mixture was stirred at rt for 5 min, the solvent was evaporated in vacuo to give an oily residue, which was subjected to silica gel column chromatography eluting with hexanes/AcOEt (9:1) to yield **11** as a colorless amorphous solid (0.0187 g, 79% yield), whose spectral data were identical with those of methyl carnosate diacetate, reported.[20,21]

4.1.4. Methylation of mixture A (11- (3) and 12-acetoxycarnosic acid (4)). The mixture **A** (0.06 g, 0.16 mmol) was dissolved in dry Me_2CO (3 mL), and the solution was treated with powdered potassium carbonate (0.6 g, 4.3 mmol) and methyl iodide (0.6 mL, 9.6 mmol). After the reaction mixture was stirred at rt for 17 h under an Ar atmosphere, the solvent was evaporated under reduced pressure. Ice-cooled H_2O (10 mL) was added to the residue and the aqueous phase was extracted with AcOEt (3× 10 mL). The combined organic phase was washed with satd aq NaCl (3× 15 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which by ODS-HPLC (MeOH/H_2O = 85:15) gave **12** (37 mg, 57%) and **13** (8 mg, 12%).

Compound **12**: Colorless amorphous solid, mp 114–118 °C; $[\alpha]_D$ +149° (c = 0.10, $CHCl_3$); ^1H NMR (400 MHz, 300 K, acetone-d_6) δ: 6.82 (1H, s), 3.60 (3H, s), 3.54 (3H, s), 3.41 (1H, dt), 2.91 (1H, sept, 6.9), 2.76 (2H, dd, 4.3, 3.9), 2.30 (3H, s), 2.23 (1H, m), 2.22 (1H, m), 1.82 (1H, m), 1.52 (1H, m), 1.50 (1H, m), 1.48 (1H, m), 1.30 (1H, ddd, 4.9), 1.18 (3H, d, 6.9), 1.11 (3H, d, 6.9), 1.09 (1H, m), 0.97 (3H, s), 0.76 (3H, s); ^{13}C NMR (100 MHz, 300 K, acetone-d_6) δ: 176.5 (s), 169.0 (s), 152.1 (s), 140.9 (s), 140.6 (s), 136.8 (s), 134.8 (s), 60.5 (q), 54.5 (d), 51.7 (q), 48.7 (s), 41.6 (t), 35.7 (t), 34.3 (s), 32.9 (q), 32.6 (t), 27.9 (d), 23.3 (q), 23.2 (q), 20.7 (t), 20.6 (q), 20.0 (q), 19.0 (t); IR (film): ν_{max} 1768, 1727 (C=O) cm^{-1}; HRMS (ESI): Calculated for $C_{24}H_{34}O_5Na$ (M^++Na): 425.2304. Found: 425.2267.

Compound **13**: Colorless amorphous solid, mp 45–48 °C; $[\alpha]_D$ +139° (c = 0.11, $CHCl_3$); ^1H NMR (400 MHz, 300 K, acetone-d_6) δ: 6.91 (1H, s), 3.67 (3H, s), 3.45 (3H, s, overlapped), 3.40 (1H, m, overlapped), 3.19 (1H, sept, 6.9), 2.85 (1H, m), 2.31 (1H, m), 2.21 (3H, s), 2.15 (1H, m), 1.83 (1H, m), 1.55 (1H, m), 1.53 (1H, m, overlapped), 1.47 (1H, m), 1.31 (1H, m), 1.181 (3H, d, 6.9), 1.178 (3H, d, 6.9), 1.16 (1H, m), 0.97 (3H, s), 0.74 (3H, s); ^{13}C NMR (100 MHz, 300 K, acetone-d_6) δ: 148.9 (s), 144.2 (s), 141.3 (s), 134.8 (s), 132.8 (s), 125.4 (d), 54.7 (d), 48.5 (s), 41.9 (t), 35.8 (t), 34.5 (s), 32.8 (q), 32.4 (t), 27.3 (d), 23.8 (q), 23.6 (q), 20.7 (t), 20.1 (q), 19.1 (t); IR (film): ν_{max} 1778, 1720 (C=O) cm^{-1}; HRMS (ESI): Calculated for $C_{24}H_{34}O_5Na$ (M^++Na): 425.2304. Found: 425.2326.

4.1.5. Methylation of 12-*O*-methylcarnosic acid (2). 2.0 M $TMSCHN_2$ in hexane (0.7 mL, 1.4 mmol) was added to a solution of 12-*O*-methylcarnosic acid (**2**) (0.031 g, 0.09 mmol) in a mixture of benzene (2.0 mL) and MeOH (1.0 mL) at rt. After the reaction mixture was stirred at rt for 15 min, the solvent was evaporated in vacuo to give an oily residue, which was purified by PTLC (toluene/MeCN = 19:1) to yield **14** as a colorless amorphous solid (0.017 g, 53% yield). Its spectral data were identical with the data of methyl carnosate 12-methyl ether, reported.[14]

4.1.6. Methylation of carnosic acid (1). 2.0 M $TMSCHN_2$ in hexane (0.15 mL, 3.0 mmol) was added to a solution of carnosic acid (**1**) (0.10 g, 0.3 mmol) in a mixture of benzene (2.5 mL) and MeOH (0.5 mL) at 0 °C. After stirring at 0 °C for 15 min, the solvent was evaporated in vacuo to give an oily residue, which was purified by MPLC (hexane/AcOEt = 9:1) to yield **15** as a colorless amorphous solid (0.050 g, 48% yield). Its spectral data were identical with those of methyl carnosate, reported.[23]

4.1.7. Methylation of methyl carnosate (15). Powdered potassium carbonate (0.059 g, 0.43 mmol) and methyl iodide (0.3 mL, 4.8 mmol) were added to a solution of **15** (0.029 g, 0.09 mmol) in dry Me_2CO (1.4 mL). After the reaction mixture was stirred at 50 °C for 28 h under an Ar atmosphere and light shielding, the solvent was evaporated under reduced pressure. Ice-cooled H_2O (10 mL) was added to the residue and the aqueous phase was extracted with AcOEt (3× 10 mL). The combined organic phase was washed with satd aq NaCl (3× 15 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give an oily residue, which was purified by MPLC (hexane/AcOEt = 9:1) to give **16** (0.0089 g, 28%). Its spectral data were identical with those of methyl carnosate 11, 12-dimethyl ether, reported.[24]

4.1.8. Oxidation of catechol (15) with DDQ. DDQ (0.014 g, 0.062 mmol) was added to a solution of catechol (**15**) (0.020 g, 0.062 mmol) in dry dioxane (1 mL) under an Ar atmosphere. After the reaction mixture was stirred at rt for 40 min, the mixture was filtered through Celite 545® and the Celite 545® was washed with AcOEt (30 mL). The combined organic washing was evaporated in vacuo to give an oily residue, which by MPLC (toluene/MeCN = 49:1) gave a quinone (**17**) (0.014 g, 69%). Quinone **17**: Yellow solid, mp: 115–118 °C. ^1H NMR (400 MHz, $CDCl_3$) δ: 6.46 (1H, s), 3.66 (3H, s), 3.05 (1H, br d, J = 13.4 Hz), 2.90 (1H, sept, J = 6.8 Hz), 2.61 (1H, dd, J = 5.7 Hz), 2.48 (1H, m), 2.19 (2H, m), 1.86 (1H, m), 1.45–1.57 (2H, m), 1.35 (1H, m), 1.29–1.17 (1H, m), 1.10 (3H, d, J = 6.8 Hz), 1.09 (3H, d, J = 6.8 Hz), 0.98 (1H, m), 0.94 (3H, s), 0.73 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 180.8, 180.0, 174.8, 150.4, 147.3, 139.6, 137.4, 52.4, 52.0, 46.3, 41.3, 33.70, 33.47, 33.15, 32.3, 27.0, 21.39, 21.36, 19.8, 19.2, 17.4; IR (film):

ν_{max} 1732, 1659 (C=O) cm^{-1}; HRMS (ESI): Calculated for $C_{21}H_{29}O_4$ (M^++H): 345.2066. Found: 345.2075.

4.1.9. Reaction of quinone (17) with TBSOTf. TBSOTf (0.013 mL, 0.056 mmol) and 2,6-lutidine (0.016 mL, 0.14 mmol) were added to a solution of quinone (**17**) (0.013 g, 0.036 mmol) in dry CH_2Cl_2 (0.56 mL) at −78 °C under an Ar atmosphere. After stirring at rt for 15 h, satd aq NH_4Cl (5 mL) was added to the reaction mixture. The mixture was then extracted with AcOEt (3× 10 mL) and the combined organic phase was washed with 5% HCl (3× 10 mL), satd aq $NaHCO_3$ (3× 10 mL), and satd aq NaCl (3× 10 mL), successively, dried over $MgSO_4$, filtered, and evaporated in vacuo. The oily residue was subjected to MPLC (toluene/MeCN = 50:1) to give **18** (0.0075 g, 58%) as a colorless solid, whose spectral data were identical with those reported one.[25]

4.1.10. Reduction of methyl carnosate (15) with $LiAlH_4$. $LiAlH_4$ (0.053 g, 1.43 mmol) was added slowly to a solution of methyl carnosate (**15**) (0.165 g, 0.48 mmol) in dry THF (15 mL). The suspension was refluxed for 4.5 h under an Ar atmosphere. The reaction mixture was diluted with AcOEt (15 mL) and acidified with 5% HCl aq to ca. pH 3. The aqueous phase was extracted with AcOEt (3× 10 mL). The combined organic phase was washed with satd aq NaCl (3× 30 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give **19** (0.152 g, 100%) as a colorless amorphous solid. Its spectral data were identical with those of 11,12,20-trihydroxy-abieta-8,11,13-triene, reported.[25]

4.1.11. Reaction of 19 with DDQ. DDQ (0.0074 g, 0.033 mmol) was added to a solution of **19** (0.010 g, 0.033 mmol) in dry dioxane (0.3 mL) under an Ar atmosphere. After the reaction mixture was stirred at rt for 30 min, the organic solvent was evaporated in vacuo to give an oily residue, which was passed through a short silica gel column (hexane/AcOEt = 4:1) to give **20** (0.005 g, 48%). Its spectral data were identical with those of 20-deoxocarnosol, reported.[25]

4.1.12. Oxidation of catechol (20) with DDQ. DDQ (0.012 g, 0.0512 mmol) was added to a solution of catechol (**20**) (0.010 g, 0.032 mmol) in dry dioxane (0.3 mL) under an Ar atmosphere. After the reaction mixture was stirred at rt for 40 min, the mixture was filtered through Celite 545® and the Celite 545® was washed with AcOEt (30 mL). The organic solvent was evaporated in vacuo to give an oily residue, which was passed through a short silica gel column (hexane/AcOEt = 9:1) to give the quinone (**21**) (0.0087 g, 87%), whose spectral data were identical with those of 20-deoxocarnosol quinone, reported.[26]

4.1.13. Oxidation of catechol (19) with Dess–Martin periodinane (DMP). A mixture of catechol **19** (0.050 g, 0.16 mmol), DMP (0.068 g, 0.16 mmol), and CH_2Cl_2 (5 mL) was stirred at rt for 45 min. Then AcOEt (30 mL), satd aq $NaHCO_3$ (10 mL), and satd aq Na_2SO_3 (10 mL) were added to the reaction mixture. The organic phase was separated and the aqueous phase was extracted with AcOEt (2× 10 mL). The combined organic phase was washed with satd aq NaCl (3× 25 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give a residue, which was triturated with hexane to give *o*-quinone **22** (0.046 g, 92%). Compound **22**: Reddish prisms (hexane), mp 100–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.44 (1H, s), 4.14 (1H, d, 11.0), 3.72 (1H, d, 11.0), 2.90 (1H, m), 2.77 (1H, m), 2.62–241 (3H, m), 1.85 (1H, m), 1.77–1.47 (5H, m), 1.25–1.14 (2H, m), 1.11 (3H, d, 6.5), 1.09 (3H, d, 6.5), 0.95 (3H, s), 0.92 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 183.1, 181.5, 151.2, 147.0, 142.0, 137.6, 65.6, 51.2, 43.4, 41.3, 33.8, 33.6, 33.4, 32.9, 27.0, 21.9, 21.39, 21.36, 18.8, 17.8; IR (film): ν_{max} 3399 (OH), 1677, 1647 (C=O) cm^{-1}; HRMS (ESI): Calculated for $C_{20}H_{29}O_3$ (M^++H): 317.2117. Found: 317.2126.

4.1.14. Oxidation of *o*-quinone (22) with DMP. A mixture of *o*-quinone **22** (0.010 g, 0.032 mmol), DMP (0.013 g, 0.032 mmol), and CH_2Cl_2 (1 mL) was stirred at rt for 25 min. Then AcOEt (6 mL), satd aq $NaHCO_3$ (2 mL), and satd aq Na_2SO_3 (2 mL) were added to the reaction mixture. The organic phase was separated and the aqueous phase was extracted with AcOEt (2× 5 mL). The combined organic phase was washed with satd aq NaCl (3× 10 mL), dried over $MgSO_4$, filtered, and evaporated in vacuo to give a residue, which was triturated with hexane to give aldehyde **23** (0.009 g, 93%). Compound **23**: Reddish prisms (hexane), mp 127–128 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 6 10.1 (1H, s), 6.47 (1H, s), 3.00 (1H, m), 2.90 (1H, m), 2.59 (1H, m), 2.47 (1H, m), 1.97–1.80 (3H, m), 1.73–1.66 (1H, m), 1.53 (1H, m), 1.40 (1H, d, 11.0), 1.28 (1H, ddd, 13.5, 13.5, 4.2), 1.15 (1H, dd, 13.5, 4.2), 1.10 (3H, d, 6.5), 1.08 (3H, d, 6.5), 0.96 (3H, s), 0.72 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 203.9, 181.0, 179.7, 151.3, 147.6, 139.2, 136.9, 53.6, 50.4, 40.9, 33.6, 33.0, 32.0, 31.5, 26.9, 22.6, 21.4, 21.3, 19.0, 17.1. IR (film): ν_{max} 1712, 1672, 1658 (C=O) cm^{-1}; HRMS (ESI): Calculated for $C_{20}H_{27}O_3$ (M^++H): 315.1936. Found: 315.1943.

4.1.15. Oxidation of catechol (5) with silver(I) oxide. Silver(I) oxide (0.035 g, 0.15 mmol) was added to a solution of catechol **5** (0.010 g, 0.03 mmol) in dry THF (1 mL). After the mixture was stirred at rt for 19 h, the mixture was filtered through Celite 545. The filtrate was evaporated in vacuo to give a residue, which was with PTLC (toluene/MeCN = 19:1) to give *o*-quinone **24** (0.0056 g, 56%).[27,28]

4.1.16. Preparation of triacetate (25). Compound **25** was prepared according to the reported procedure by Nakatani et al.[17]

4.1.17. Oxidation of catechol (15) with DDQ. DDQ (0.021 g, 0.09 mmol) was added to a solution of catechol (**7**) (0.032 g, 0.09 mmol) in dry dioxane (0.8 mL) under an Ar atmosphere. After the reaction mixture was stirred at rt for 15 min, the mixture was filtered through Celite 545 and the residue was washed with AcOEt (30 mL). The organic solvent was evaporated in vacuo to give an oily residue, which was purified with silica gel short column (hexane/Me_2CO = 4:1) to give the quinone (**26**) (0.019 g, 59%), whose spectral data were identical with the reported one.[18]

4.1.18. Oxidation of catechol (26) with DMP. A mixture of *o*-quinone **26** (0.013 g, 0.037 mmol), DMP (0.024 g, 0.056 mmol), and CH_2Cl_2 (1 mL) was stirred at rt for 50 min. Another DMP (0.016 g, 0.037 mmol) was added to the mixture. After the reaction mixture was stirred at the same temperature, AcOEt (6 mL), satd aq $NaHCO_3$ (2 mL), and satd aq Na_2SO_3 (2 mL) were added to the reaction mixture. The organic phase was separated and the aqueous phase was extracted with AcOEt (2× 10 mL). The combined organic phase was washed with satd aq NaCl (3× 25 mL) and dried over $MgSO_4$, filtered, and evaporated in vacuo to give a residue, which was purified with silica gel short column chromatography (hexane/Me_2CO = 4:1) to give ketone **27** (0.012 g, 94%). Compound **27**: Reddish prisms ($CHCl_3$), mp 147–150 °C. ^1H NMR (500 MHz, $CDCl_3$) δ: 7.28 (1H, s), 4.78 (1H, s), 3.27 (1H, br d, J = 9.8 Hz), 2.97 (1H, sept, J = 5.9 Hz), 2.48 (1H, s), 1.2-1.7 (5H, m), 1.15 (3H, d, J = 7.0 Hz), 1.13 (3H, d, J = 7.0 Hz), 1.04 (3H, s), 0.97 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 190.1, 182.0, 179.0, 173.2, 151.3, 145.6, 136.5, 128.0, 80.0, 60.8, 49.4, 37.7, 32.5, 31.5, 27.9, 25.2, 21.8, 21.29, 21.28, 18.11. IR (film): ν_{max} 1790, 1713, 1669 (C=O) cm^{-1}; HRMS (ESI): Calculated for $C_{20}H_{22}O_5Li$ (M^++Li): 349.1627. Found: 349.1627.

4.2. Assay for cytotoxic activity

The cytotoxic assays were performed by using the MTT assay method. The murine P388 leukemia cells were precultured in RPMI 1640 medium (Nissui Co. Ltd., Japan) supplemented with 5% heat-inactivated fetal bovine serum (FBS) and kanamycin (5.3 mL/L) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C and adjusted to 3×10^4 cells/mL. The cell suspension (100 μL) was added to each well (3×10^3 cells/well) of a 96-microwell plate (flat-bottomed, polystyrene treated) and incubated for 24 h. Test compounds were dissolved in DMSO in various concentrations (100, 10, 1, and 0.1 μg/mL) and 10 μL of the test solution or DMSO (control) was added to each well. The plate was kept in an incubator for 48 h. After termination of cell culture by adding 5% MTT in PBS (20 μL) to each well, the plate was kept in the incubator for 4 h. To each well was added 100 μL of 10% SDS-0.01N HCl and the plate was read on a microplate reader (MPR A4i, Toso) at 550 nm. A dose-response curve was plotted for each compound, and the concentrations giving 50% inhibition of the cell growth (IC_{50}) were recorded.

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