Diterpenes from the Fruits of *Vitex rotundifolia*

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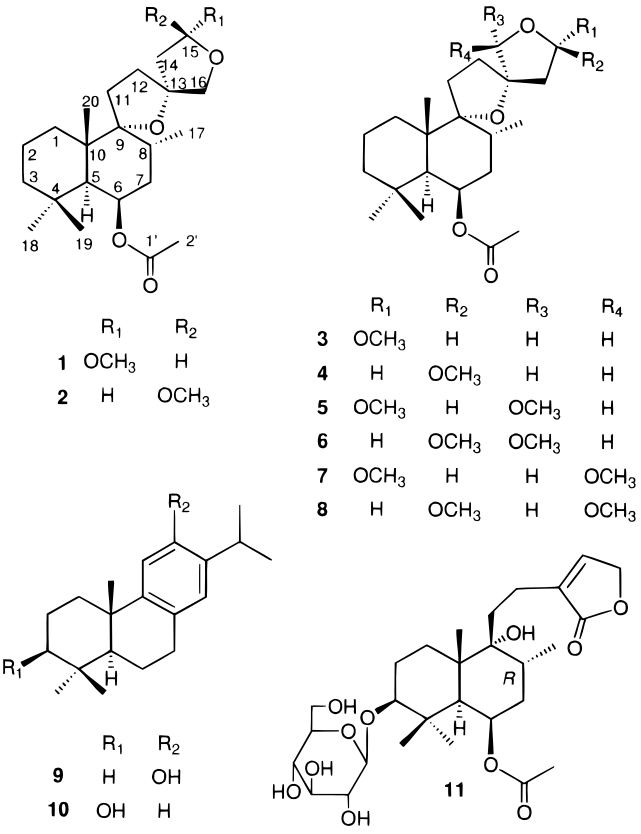
Eight new labdane-type diterpenes (**1**−**8**) were isolated from the fruit of *Vitex rotundifolia* along with two known abietane-type diterpenoids (**9**, **10**), and their structures were characterized on the basis of spectroscopic data and X-ray crystallographic analysis. Among them, the abietane-type diterpenoid ferruginol (**9**) exhibited a stronger antioxidative activity than the standard antioxidant, 3-*tert*-butyl-4-hydroxyanisole (BHA), using a ferric thiocyanate method.

Vitex rotundifolia L. f. (Verbenaceae) is widely distributed in Asia, and its fruit, "Viticis Trifoliae Fructus" is used as a folk medicine for headaches.[1] During the course of our studies on natural antioxidants from crude drugs,[2−5] the methanolic extract of this fruit showed a stronger antioxidative activity than 3-*tert*-butyl-4-hydroxyanisole (BHA), which is a synthetic antioxidant, using a ferric thiocyanate method. In preceding papers, we have reported the isolation and structure elucidation of iridoids, phenylpropanoids, a flavanone, lignans, and a diterpene glycoside from the methanolic extract of *V. rotundifolia* fruits. Several of these compounds were found to have a stronger antioxidative activity than BHA.[5−7] In a further investigation of this extract, we now report the isolation and structure determination of eight new labdane-type diterpenes (**1**−**8**) and two known abietane-type diterpenes (**9**, **10**). Additionally, we describe the antioxidative activity of three compounds (**6**, **9**, and **10**) among these *V. rotundifolia* constituents.



Results and Discussion

The methanolic extract of the fruits of *V. rotundifolia* was purified by Diaion HP-20, Si gel, and Chromatorex ODS column chromatography as well as HPLC on ODS and Si gel, to afford eight new labdane-type diterpenes (**1**−**8**), along with two known abietane-type diterpenoids (**9** and **10**). Compounds **9** and **10** were identified as ferruginol[8] and abietatrien-3β-ol,[9,10] respectively, using spectral and physical data comparison with literature values.

Compound **1** was obtained as colorless needles and analyzed for the molecular formula $C_{23}H_{38}O_5$ from its NMR and HRFABMS data. It did not show a parent ion peak in the EIMS but showed an intense fragment ion peak at *m*/*z* 334 $[M - CH_3COOH]^+$. The ^{1}H NMR spectrum indicated signals due to three tertiary methyl groups (δ 1.22, 0.97, 0.93), one secondary methyl group (δ 0.78), one acetyl group (δ 2.03), one methoxyl group (δ 3.39), two oxygenated methylene protons (δ 3.91, 3.56), and two oxygenated methine protons (δ 5.36, 4.96). The ^{13}C NMR spectrum exhibited 23 carbon signals, including a carbonyl carbon (δ 170.5), an acetal carbon (δ 105.2), a methoxyl carbon (δ 55.2), an oxygenated methine carbon (δ 70.7), an oxygenated methylene carbon (δ 75.3), and two oxygenated quaternary carbons (δ 89.1, 92.0). These ^{1}H and ^{13}C NMR signals (Tables 1 and 2, respectively) were assigned with the aid of ^{1}H−^{1}H COSY and HMQC spectra, and the planar structure of **1**, which was a labdane-type diterpene possessing two spiro-tetrahydrofuran rings, could be deduced from these observations. The relative stereochemistry in **1** was examined from its difference NOE NMR spectra (Figure 1). From these data, the configurations at C-5, C-6, C-8, C-9, C-10, and C-13 were determined to be *S**, *R**, *R**, *R**, *S**, and *R**, respectively. However, the configuration of the methoxyl group at C-15 could not be confirmed. Finally, the gross relative stereochemistry of **1** was elucidated by X-ray crystallography (Figure 2, Table 3). Con-

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Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**8** in $CDCl_3$[a]

position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1a	ca. 1.45	ca. 1.46	ca. 1.33	ca. 1.32	ca. 1.71	ca. 1.70	ca. 1.37	ca. 1.37
1b	ca. 1.32	ca. 1.39	ca. 1.27	ca. 1.32	ca. 1.28	ca. 1.30	ca. 1.37	ca. 1.37
2a	ca. 1.62	ca. 1.65	ca. 1.63	ca. 1.63	ca. 1.68	ca. 1.66	ca. 1.65	ca. 1.63
2b	ca. 1.45	ca. 1.47	ca. 1.45	ca. 1.48	ca. 1.46	ca. 1.46	ca. 1.47	ca. 1.45
3a	ca. 1.31	1.31 ddd (2,2,13)	ca. 1.30	ca. 1.31	ca. 1.28	ca. 1.30	1.31, br d (13)	1.30, br d (13)
3b	1.14 ddd (4,13,13)	1.14 ddd (4,13,13)	1.14 ddd (4,13,13)	1.16 ddd (4,13,13)	ca. 1.25	1.26 ddd (3,12,12)	1.17 ddd (4,13,13)	ca. 1.20
5	1.55 d (2)	1.51 d (2)	1.52 d (2)	1.50 d (2)	1.64 d (2)	1.58 d (2)	1.53 d (2)	1.49 d (2)
6	5.36 ddd (2,3,3)	5.37 ddd (2,3,3)	5.37 ddd (2,3,3)	5.37 ddd (2,3,3)	5.38 ddd (2,3,3)	5.38 ddd (2,3,3)	5.37 ddd (2,3,3)	5.37 ddd (2,3,3)
7a	ca. 1.62	ca. 1.65	ca. 1.67	ca. 1.70	ca. 1.73	ca. 1.76	ca. 1.70	ca. 1.67
7b	ca. 1.48	ca. 1.47	ca. 1.45	ca. 1.48	ca. 1.46	ca. 1.46	ca. 1.47	ca. 1.45
8	ca. 2.03	ca. 2.06	ca. 2.04	ca. 2.04	ca. 2.05	ca. 2.07	ca. 2.04	ca. 2.07
11a	ca. 2.09	ca. 2.15	ca. 2.10	ca. 2.08	ca. 2.11	ca. 2.09	ca. 2.08	ca. 2.02
11b	1.76 m	ca. 1.70	ca. 1.69	ca. 1.71	ca. 1.68	ca. 1.69	ca. 1.65	ca. 1.64
12a	ca. 2.07	ca. 2.17	ca. 2.05	ca. 2.14	1.94 ddd (1,10,13)	ca. 2.04	2.42 ddd (2,10,13)	2.40 ddd (3,10,13)
12b	1.89 m	ca. 2.13	1.89 ddd (9,10,10)	ca. 2.05	1.84 ddd (11,12,13)	ca. 2.04	ca. 1.68	1.89 ddd (9,9,13)
14a	2.20 dd (6,13)	2.37 dd (6,13)	2.25 dd (4,14)	2.44 dd (6,13)	2.39 dd (6,13)	2.57 dd (6,13)	2.27 dd (4,13)	2.43 dd (6,13)
14b	2.15 dd (4,13)	1.90 dd (1,13)	2.21 dd (6,14)	1.94 dd (2,13)	2.11 dd (6,13)	1.83 d (13)	2.23 dd (6,13)	1.99 dd (4,13)
15	4.96 dd (4,6)	5.07 dd (1,6)	4.95 dd (4,6)	5.05 dd (2,6)	4.94 dd (6,6)	5.03 d (6)	5.00 dd (4,6)	5.12 dd (4,6)
16a	3.91 d (9)	4.02 d (9)	3.80 d (9)	3.95 d (9)	4.24 s	4.32 s	4.95 s	4.89 s
16b	3.56 d (9)	3.83 d (9)	3.46 d (9)	3.69 d (9)				
17	0.78 d (7)	0.78 d (7)	0.82 d (7)	0.84 d (7)	0.82 d (7)	0.83 d (7)	0.84 d (7)	0.86 d (7)
18	0.93 s	0.94 s	0.94 s	0.94 s	0.95 s	0.96 s	0.94 s	0.94 s
19	0.97 s	0.97 s	0.96 s	0.97 s	0.97 s	0.98 s	0.97 s	0.97 s
20	1.22 s	1.21 s	1.19 s	1.20 s	1.20 s	1.20 s	1.19 s	1.20 s
2′	2.03 s	2.03 s	2.03 s	2.03 s	2.03 s	2.03 s	2.03 s	2.03 s
OCH_3-15	3.39 s	3.32 s	3.38 s	3.34 s	3.42 s	3.36 s	3.41 s	3.43 s
OCH_3-16					3.42 s	3.40 s	3.44 s	3.40 s

[a] Chemical shifts (δ) are in ppm relative to TMS. Coupling constants (J) in Hz are given in parentheses. Assignments are based on ^{1}H−^{1}H COSY, HMQC, HMBC, and difference NOE experiments. Values are recorded at 500 MHz.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**8** in $CDCl_3$[a]

position	**1**[b]	**2**[c]	**3**[b]	**4**[b]	**5**[b]	**6**[c]	**7**[c]	**8**[c]
1	34.0 t	33.9 t	33.1 t	33.7 t	32.1 t	32.0 t	34.1 t	34.2 t
2	18.8 t	18.8 t	18.8 t	18.8 t	19.1 t	19.0 t	19.0 t	18.9 t
3	44.0 t	44.1 t	43.9 t	44.0 t	43.8 t	43.8 t	44.1 t	44.0 t
4	34.2 s	34.1 s	34.1 s	33.7 s	34.1 s	34.1 s	34.1 s	34.1 s
5	48.4 d	48.7 d	48.7 d	48.7 d	48.6 d	48.7 d	48.9 d	48.9 d
6	70.7 d	70.8 d	70.8 d	70.8 d	70.9 d	70.8 d	70.8 d	70.8 d
7	36.8 t	36.6 t	36.4 t	36.6 t	36.3 t	36.2 t	36.5 t	36.6 t
8	31.0 d	31.5 d	31.6 d	31.4 d	31.7 d	31.6 d	31.5 d	31.4 d
9	92.0 s	92.4 s	91.7 s	92.3 s	93.2 s	93.0 s	92.7 s	93.1 s
10	43.1 s	42.9 s	42.9 s	42.9 s	42.8 s	42.8 s	43.1 s	43.1 s
11	28.9 t	29.7 s	29.6 t	29.7 t	29.2 t	29.4 t	30.2 t	29.7 t
12	39.8 t	38.4 t	39.7 t	37.9 t	37.5 t	38.0 t	32.8 t	31.2 t
13	89.1 s	89.9 s	89.3 s	89.4 s	89.3 s	88.1 s	90.3 s	92.3 s
14	46.9 t	47.6 t	46.6 t	46.9 t	41.7 t	41.8 t	45.3 t	44.6 t
15	105.2 d	105.8 d	104.4 d	104.9 d	103.5 d	103.1 d	102.1 d	105.1 d
16	75.3 t	78.0 t	74.7 t	77.4 t	105.3 d	106.6 d	108.3 d	108.3 d
17	16.8 q	17.3 q	17.2 q	17.3 q	16.9 q	16.8 q	17.4 q	17.7 q
18	33.1 q	33.1 q	33.1 q	33.1 q	32.8 q	32.7 q	33.1 q	33.1 q
19	23.7 q	23.8 q	23.8 q	23.8 q	23.8 q	23.7 q	23.8 q	23.9 q
20	19.9 q	19.8 q	19.6 q	19.9 q	19.6 q	19.6 q	19.8 q	19.9 q
1′	170.5 s	170.5 s	170.5 s	170.5 s	170.5 s	170.4 s	170.5 s	170.5 s
2′	22.0 q	22.0 q	22.0 q	22.0 q	22.0 q	21.8 q	21.9 q	22.0 q
OCH_3	55.2 q	54.8 q	55.0 q	54.9 q	55.3 q	55.3 q	56.6 q	55.6 q
OCH_3					54.5 q	54.6 q	54.9 q	55.1 q

[a] Chemical shifts (δ) are in ppm relative to TMS. Assignments are based on DEPT, HMQC, and HMBC experiments. [b]Values are recorded at 100 MHz. [c]Values are recorded at 125 MHz.

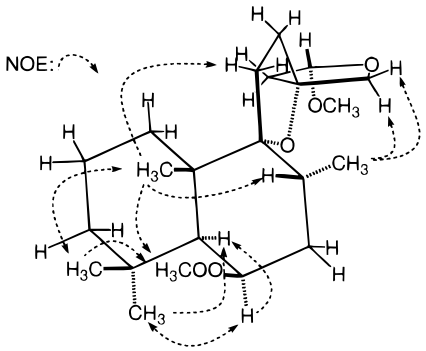

Figure 1. Selected NOE correlations observed in the NOE difference spectra of compound **1**.

sequently, the structure of **1** was concluded to be (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,15*R*)-6-acetoxy-9,13;15,16-diepoxy-15-methoxylabdane.

Compound **2** was obtained as a colorless syrup. The EIMS of **2** was superimposable on that of **1**, indicating a dominant ion peak at *m*/*z* 334. The ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively) of **2** were very similar to those of **1**, although the signals due to the two spiro-tetrahydrofuran rings moiety were slightly shifted. Therefore, **2** was recognized as an isomer at C-13 and/or C-15 of **1**. The difference NOE NMR spectra of **2** showed the same correlations as those of **1**, including correlations between Ha-16 and H_3-17 as well as between Hb-16 and H_3-17. Therefore, **2** was defined as the 15-epimer of **1**.

Compound **3** was obtained as colorless needles. The EIMS and ^1H and ^{13}C NMR data (Tables 1 and 2, respectively) for **3** were quite similar to those of **1**, expect for the H-1 signal, which appeared upfield. In the difference NOE spectrum of **3**, NOEs were observed between Ha-14 and H_3-17 and between Hb-14 and H_3-17, instead of the NOE correlations between Ha-16 and H_3-17 and between Hb-16 and H_3-17, as in **1**. Other NOE correlations were same as those of **1**. From these data, **3** was assumed to be the 13-epimer of **1** or **2**. To define the configuration of the methoxyl group at C-15 and to confirm the structure, the X-ray analysis of **3** was undertaken (Figure 2, Table 3). Accordingly, **3** was concluded to be the 13-epimer of **2**.

Compound **4** was obtained as a white powder. The EIMS and ^1H and ^{13}C NMR data (Tables 1 and 2, respectively) were superimposable on those of **2**, apart from the signal due to H-1, which was observed at a similar position to that of **3**. The correlations in the difference NOE spectra were same as those of **3**. In particular, NOEs were observed between Ha-14 and H_3-17 as well as between Hb-14 and H_3-17. Therefore, **4** was concluded to be the 15-epimer of **3**.

Compound **5** was obtained as a colorless syrup. The ^1H NMR spectrum (Table 1) was closely comparable to those of **1**−**4**, except for the appearance of the signals due to one more methoxyl group and one oxygenated methine proton, and the loss of signals assignable to Ha-16 and Hb-16. The EIMS of **5** showed an intense fragment ion peak at *m*/*z* 364, which was 30 mass units [OCH_3 − H] larger than those of **1**−**4**. From these data, **5** was considered to be a derivative of **1**−**4**, in which an additional methoxyl group was attached to C-16. This assumption was confirmed by the ^{13}C NMR and difference NOE spectra. In comparing the ^{13}C NMR signals (Table 2) of **4** and **5**, the signal due to C-16 in **5** was shifted downfield by 27.9 ppm, while, in contrast, the signals due to C-1, C-14, and C-15 were shifted upfield by 1.6, 5.2, and 1.4 ppm, respectively, and the chemical shifts of the other carbon signals were almost the same as those of **4**. In the NOE spectrum of **5**, the same correlations as those of **4** were observed, and, in addition, irradiation of the signal due to H-16 showed NOEs of the signals due to Ha-1, Hb-1, Ha-12, and H-15. From these NOE correlations, both of the configurations of the methoxyl group at C-15 and C-16 were determined to be α. Consequently, **5** was elucidated as (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,-13*S*,15*S*,16*R*)-6-acetoxy-9,13;15,16-diepoxy-15,16-dimethoxylabdane.

Compound **6** was obtained as a colorless syrup. The EIMS gave a dominant fragment ion peak similar to that of **5** at *m*/*z* 364, and the ^1H and ^{13}C NMR spectra (Tables 1 and 2, respectively) were quite similar to those of **5**, except for the slight shift of the signals due to the spiro-

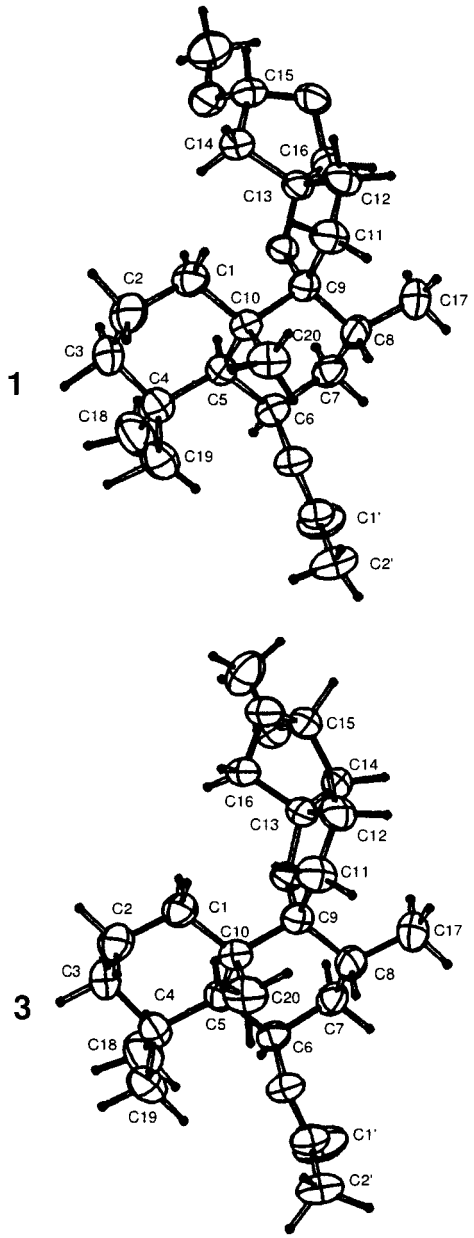

Figure 2. ORTEP drawings of **1** and **3**. The ellipsoid probability level of **1** and **3** are 50%.

tetrahydrofuran ring moiety. The difference NOE spectra indicated correlations similar to those of **5**, whereas a correlation between H-16 and H-15 was not detected, suggesting **6** to be the 15-epimer of **5**. The β-orientation of the methoxyl group at C-15 was also supported on the basis of the comparison of the ^{1}H NMR chemical shift difference ($\Delta\delta$: δHa-14 − δHb-14) between Ha-14 and Hb-14 with that of the 15-epimer. In the case of **6**, the chemical shift difference ($\Delta\delta$ 0.74) was larger than that of **5** ($\Delta\delta$ 0.28), which was in agreement with those observed for **1** ($\Delta\delta$ 0.05), **2** ($\Delta\delta$ 0.47), **3** ($\Delta\delta$ 0.04), and **4** ($\Delta\delta$ 0.50). Accordingly, **6** was concluded to be the 15-epimer of **5**.

Compound **7** was obtained as a white powder, and **8** was obtained as colorless needles. These compounds were inferred as being the 16-epimers of **5** or **6**, respectively, from the EIMS data and ^{1}H and ^{13}C NMR spectra (Tables 1 and 2, respectively), as well as from the NOE spectra, in which irradiation of the signal due to H-16 showed no NOE of the signal due to Ha-12, in each case. The β-configurations for the methoxyl group at C-16 of **7** and **8** were

Table 3. Experimental Data for X-Ray Diffraction Studies of **1** and **3**

data	**1**	**3**
crystal data		
formula	$C_{23}H_{38}O_5$	$C_{23}H_{38}O_5$
formula weight	394.55	394.55
system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
Z	4	4
a (Å)	14.885 (1)	14.769 (3)
b (Å)	18.460 (2)	18.744 (2)
c (Å)	8.037 (1)	8.0519 (4)
V (Å^3)	2208.5 (4)	2229.0 (4)
μ(Cu Kα) (mm^{-1})	0.620	0.614
Dx(Mg cm^{-3})	1.186	1.175
F(000)	864	864
refinement		
total no. of reflcns	1907	1923
no. of obsd reflcns	1756 [$F > 3.0\sigma(F)$]	1772 [$F > 3.0\sigma(F)$]
R	0.041	0.040
R_w	0.045	0.054

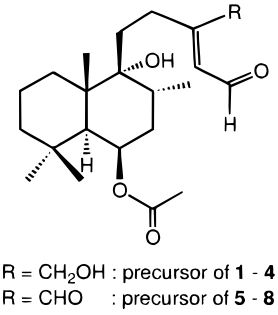

Figure 3. Structures of deduced precursors of compounds **1**−**8**.

confirmed from the lowfield resonance of Ha-12, which was deshielded by the methoxyl group at C-16;[11] the Ha-12 signals of **7** and **8** were observed at a lower field (δ 2.42 in **7**; δ 2.40 in **8**) when compared to **5** and **6** (δ 1.94 in **5**; δ 2.04 in **6**). Comparison of the ^{1}H NMR chemical shift difference ($\Delta\delta$ 0.04) of the geminal protons at C-14 of **7** with that of **8** ($\Delta\delta$ 0.44) led to the configurational determination for the methoxyl group at C-15 of **7** to be α and that of **8** to be β. The β-orientation of the methoxyl groups at C-15 and C-16 of **8** was also supported by the observed NOE correlation between H-15 and H-16. The structures of **7** and **8** were therefore defined as the 16-epimers of **5** and **6**, respectively.

Although the absolute configurations of **1**−**8** have not been confirmed, from a biogenetic point of view they are probably the same as that of viteoside A (**11**), which was previously isolated from "Viticis Trifoliae Fructus".[7] However, it is possible that **1**−**8** are artifacts formed from aldehydes (Figure 3) during the extraction and/or isolation procedures, because of the co-occurrence of epimers at C-13. In addition, it is noted that the sign and the value of the specific rotations of **1**−**8** are correlated with their stereochemistry in the tetrahydrofuran ring. In the case of an R* configuration at C-13, the sign of the optical rotations of **1** ([α]$_D$ −64.7°, OCH$_3$-15α) and **2** ([α]$_D$ +55.9°, OCH$_3$-15β) were negative and positive, respectively. In contrast, in the case of an S* configuration at C-13, those of **3** ([α]$_D$ +60.6°, OCH$_3$-15α) and **4** ([α]$_D$ −70.9°, OCH$_3$-15β) were positive and negative, respectively, and the absolute values of the specific rotation of these four compounds were similar to each other. On the other hand, the α-methoxyl group at C-16 in **5** and **6** shifted the value of the optical rotation toward the negative side (**5**, [α]$_D$ +17.4°; **6**, [α]$_D$

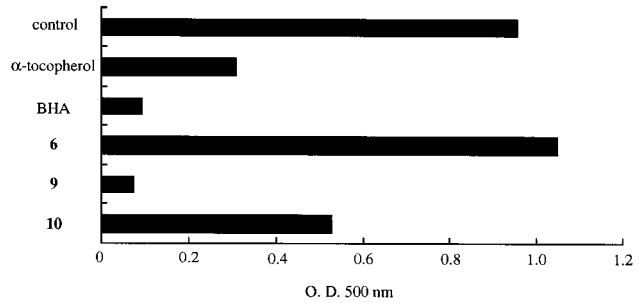

Figure 4. Antioxidative activities of compounds **6**, **9**, **10**, α-tocopherol, and BHA after 5 days of lipid peroxidation. The final concentration of the sample tested was 0.5 mM.

Table 4. Reduction of 1,1-Diphenyl-2-picrylhydrazyl[a]

compound	concentration ($\times 10^{-5}$ M)	ΔO.D.
9	2	0.081
	4	0.153
	8	0.255
α-tocopherol	1	0.252
	2	0.471
	4	0.934
L-cysteine	2	0.161
	4	0.355
	8	0.754

[a] For protocol used, see Experimental Section. ΔO.D. = O.D. of control at 517 nm (1.059) − O.D. of sample. 1,1-Diphenyl-2-picrylhydradyl; 1×10^{-4} M.

−79.7°), and the *β*-methoxyl group at C-16 in **7** and **8** shifted toward the positive side (**7**, $[\alpha]_D$ +67.5°; **8**, $[\alpha]_D$ −19.5°). The absolute values of the specific rotation of **5** and **6** were similar to those of **8** and **7**, respectively.

The antioxidative activity of **6**, **9**, and **10** was evaluated using linoleic acid as the substrate by the ferric thiocyanate method,[12] and the activity was compared with α-tocopherol and BHA, each at a 0.5-mM concentration. Among them, **9** showed a stronger antioxidative activity than BHA (Figure 4). Furthermore, the scavenging effect of **9** on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was examined.[13] The effect of **9** was almost half that of L-cysteine (Table 4). Thus, the presence of a phenolic hydroxyl group at C-12 in the potent antioxidative compound **9** may be responsible for its observed radical-scavenging effects.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were measured with a JASCO DTP-1000 KUY digital polarimeter. Visible absorptions were measured with a Shimadzu UV-140−02 spectrometer. ^{1}H NMR spectra were recorded in $CDCl_3$ solution using a JEOL alpha 500 spectrometer at 500 MHz, and chemical shifts are given on a *δ* (ppm) scale with tetramethylsilane (TMS) as an internal standard. ^{13}C NMR spectra were recorded in $CDCl_3$ solution using JEOL JNM-GX-400 and JEOL alpha 500 spectrometers at 100 MHz (compounds **1**, **3**−**5**, **9**, **10**) or 125 MHz (compounds **2**, **6**−**8**), respectively. EIMS were collected on a JEOL JMS-DX-303HF instrument, and HRFABMS was obtained with a JEOL JMS−HX110 mass spectrometer. Column chromatography was carried out by passage over Si gel 60 (Merck, Art. 7734 and Art. 9385), Diaion HP-20 (Mitsubishi Chemical Industries Co., Ltd.), and Chromatorex ODS (Fuji Silysia Chemical, Ltd.). HPLC separations were run on a Shimadzu Micro pump LC-10AS with a Shimadzu RI-Detector RID-6A. For HPLC column chromatography, TSK-GEL ODS-120T (Tosoh Co., Ltd., 21.5 mm i.d. × 300 mm), YMC-pack D-ODS-5 (YMC, 20 mm i.d. × 250 mm), YMC-pack SIL-06 (YMC, 20 mm i.d. × 250 mm), and Kusano C. I. G. prepacked Si-5 (Kusano Kagakukikai Co., 22 mm i.d. × 100 mm) were used.

Plant Material. Fruit of *V. rotundifolia* was purchased in January 1995, from Uchida Wakanyaku Co., Ltd., a commercial outlet of traditional medicines in Tokyo, Japan, and identified by Mr. Kouki Kitaoka, Medical Plant Garden, Kumamoto University. A voucher specimen (lot 232418) is deposited at the Laboratory of Chemistry, Research Institute of General Education, Kyushu Tokai University.

Extraction and Isolation. The dried, powdered fruit of *V. rotundifolia* (2914 g) was extracted with MeOH. The MeOH extract (185.2 g) was partitioned between hexane and MeOH. The MeOH layer was filtered through absorbent cotton. The filtrate was evaporated under reduced pressure, and crude material (158 g) was subjected to passage over Diaion HP 20 (40% MeOH, 70% MeOH, 90% MeOH, MeOH, acetone) to give fractions 1−4. Fraction 3 (36.1 g) was chromatographed over Si gel [Art. 7734, hexane, hexane−EtOAc (5:1, 1:1, 1:2,1:4, 1:8, 1:16), EtOAc, MeOH] to afford fractions 5−10. Chromatography of fraction 5 (5.68 g) over Chromatorex ODS (70% MeOH, 75% MeOH, 80% MeOH, 85% MeOH, 90% MeOH, 95% MeOH) furnished fractions 11−21. Fraction 17 (1.30 g) was subjected to Si gel column chromatography [Art. 9385, hexane−acetone (20:1, 5:1, 3:1), acetone] to give fractions 22−27. Fraction 23 (769 mg) was subjected to HPLC (TSK-GEL ODS-120T, 88% MeOH and YMC pack SIL-06, hexane−acetone, 30:1, in turn) to give **1** (33 mg), **2** (32 mg), **3** (23 mg), **4** (27 mg), **6** (67 mg), **7** (55 mg), and **8** (63 mg). HPLC (TSK-GEL ODS-120T, 88% MeOH) of fraction 24 (118 mg) afforded **5** (7 mg) and fractions 28−33. Fraction 25 (193 mg) was subjected to HPLC (TSK-GEL ODS-120T, 88% MeOH) to give **10**[9,10] (17 mg) and fractions 34−40. HPLC (Kusano CIG prepacked Si-5, hexane−EtOAc, 20:1) of fraction 18 (687 mg) afforded fractions 41−48. Fraction 44 (25 mg) was subjected to HPLC (YMC-pack D-ODS-5, 90% MeOH) to give **9**[8] (6 mg).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,15*R*)-6-Acetoxy-9,13;15,16-diepoxy-15-methoxylabdane (1): colorless needles (hexane−EtOAc); mp 116−117 °C; $[\alpha]_D^{17}$ −64.7° (*c* 0.8, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 334 (100) $[M - CH_3COOH]^+$, 197 (55), 184 (35), 165 (97), 81 (79); HRFABMS *m*/*z* 417.2614 $[M + Na]^+$ (calcd for $C_{23}H_{38}O_5Na$ 417.2617).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*R*,15*S*)-6-Acetoxy-9,13;15,16-diepoxy-15-methoxylabdane (2): colorless syrup; $[\alpha]_D^{17}$ +55.9° (*c* 1.6, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 334 (100) $[M - CH_3COOH]^+$, 197 (58), 184 (40), 165 (92), 81 (63).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*S*)-6-Acetoxy-9,13;15,16-diepoxy-15-methoxylabdane (3): colorless needles (hexane−EtOAc); mp 166−168 °C; $[\alpha]_D^{17}$ +60.6° (*c* 0.8, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 334 (100) $[M - CH_3COOH]^+$, 197 (46), 184 (32), 165 (75), 81 (43).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*R*)-6-Acetoxy-9,13;15,16-diepoxy-15-methoxylabdane (4): colorless syrup; $[\alpha]_D^{17}$ −70.9° (*c* 1.3, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 334 (100) $[M - CH_3COOH]^+$, 197 (38), 184 (34), 165 (57), 81 (32).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*S*,16*R*)-6-Acetoxy-9,13;15,-16-diepoxy-15,16-dimethoxylabdane (5): colorless syrup; $[\alpha]_D^{17}$ +17.4° (*c* 0.7, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 364 (100) $[M - CH_3COOH]^+$, 304 (37), 227 (46).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*R*,16*R*)-6-Acetoxy-9,13;15,-16-diepoxy-15,16-dimethoxylabdane (6): colorless syrup; $[\alpha]_D^{17}$ −79.7° (*c* 1.5, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 364 (100) $[M - CH_3COOH]^+$, 304 (20), 227 (44), 154 (51).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*S*,16*S*)-6-Acetoxy-9,13;15,-16-diepoxy-15,16-dimethoxylabdane (7): a white powder; $[\alpha]_D^{17}$ +67.5° (*c* 1.3, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 364 (100) $[M - CH_3COOH]^+$, 304 (37), 227 (46).

(*rel* 5*S*,6*R*,8*R*,9*R*,10*S*,13*S*,15*R*,16*S*)-6-Acetoxy-9,13;15,-16-diepoxy-15,16-dimethoxylabdane (8): colorless needles (hexane−EtOAc); mp 96−97 °C; $[\alpha]_D^{17}$ −19.5° (*c* 2.1, acetone); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; EIMS *m*/*z* $[M]^+$ absent, 364 (100) $[M - CH_3COOH]^+$, 304 (14), 227 (20), 195 (23), 154 (51).

X-ray Structure Analysis of 1 and 3. The reflection data were collected on an Enraf−Nonius CAD4 diffractometer using graphite-monochromated Cu Kα radiation ($\lambda = 1.5418$ Å) with the ω-2θ scan technique to a maximum θ of 60° at room temperature (23 °C). The structures were solved by the direct method using SIR88.[14] All atomic parameters, with anisotropic temperature factors for non-hydrogen atoms and isotropic ones for hydrogen atoms, were refined by a block-diagonal least-squares method. Experimental data for **1** and **3** are shown in Table 3.

Assay for Antioxidative Activity. A mixture of 2.51% linoleic acid EtOH solution (0.80 mL), 0.05 M phosphate buffer (pH 7.0, 1.60 mL), EtOH (0.60 mL), and H_2O (0.80 mL) were added to 10 mM of EtOH solution (0.20 mL) of each sample in a vial with a cap and placed in darkness at 40 °C to accelerate oxidation. After the fifth day of incubation, this assay solution (0.05 mL) was diluted with 75% EtOH (4.85 mL), which was followed by adding 30% ammonium thiocyanate (0.05 mL). Precisely 3 min after the addition of 0.02 M ferrous chloride in 3.5% hydrochloric acid (0.05 mL) to the reaction mixture, the absorbance of the red color developed was measured at 500 nm. The control sample was prepared in the same manner by mixing all the same chemicals and ingredients and by excluding the test compounds. α-Tocopherol and BHA were used as standard samples.

Assay of Scavenging Effect on DPPH. The method of Uchiyama et al. [13] was applied in a slightly modified manner. The EtOH solution (1.00 mL) of each test sample was added to a mixture of 0.1 M acetic acid buffer (pH 5.5, 1.00 mL) and 0.5 mM DPPH EtOH solution (0.50 mL) in a test tube and left to stand at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm. α-Tocopherol and L-cysteine were used as standard samples.

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