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Anti-inflammatory constituents from the fruits of *Vitex rotundifolia*

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ABSTRACT

Three new diterpenes (**7**, **15** and **17**) and two new neolignans (**19** and **20**) along with nineteen known compounds have been isolated from the fruits of *Vitex rotundifolia*. Their structures were elucidated by a combination of 1D and 2D NMR, HRESI-MS, and CD data. All isolates were tested for their inhibitory activities on LPS-induced nitric oxide production in RAW264.7 cells. Of these, compounds **3**, **4**, **7**, **13**, **15**, **19**, and **24** found to inhibit nitric oxide production with the IC_{50} values ranging from 11.3 to 24.5 μM.

Vitex rotundifolia L. f., belonging to the plant family Verbenaceae, is a medicinal plant that is widely used in Korea, China, and Japan for the treatment of inflammation, headache, migraine, chronic bronchitis, eye pain, and gastrointestinal infections.[1–3] *V. rotundifolia* has been reported to exhibit a broad range of pharmacological activities such as cytotoxic, anti-inflammatory, anti-microbial, anti-nociceptive, and anti-hyperprolactinemia activities.[4–7] Previous phytochemical research has resulted in the isolation of diverse compounds such as diterpenes, flavonoids, and lignans.[5–11]

As part of an ongoing research program searching for anti-inflammatory constituents from Korean medicinal plants, the MeOH extract of the fruits of *V. rotundifolia* showed the inhibitory effect on the nitric oxide (NO) production in RAW264.7 cells. The bioassay-guided fractionation of the CH_2Cl_2-soluble fraction led to the isolation of three new diterpenes (**7**, **15** and **17**) and two new neolignans (**19** and **20**) along with 19 known compounds. In this Letter, we describe the structural determination of the new compounds and the inhibitory activities of all 24 compounds against LPS-induced NO production in RAW264.7 cells.

The CH_2Cl_2-soluble fraction of the methanolic extract of the fruits of *V. rotundifolia* was subjected to repeated column chromatography over silica gel, RP-18, MCI gel, and semi-preparative HPLC, to afford a new halimane-type diterpene (**7**), two new labdane-type diterpenes (**15** and **17**), two new neolignans (**19** and **20**) together with nineteen known compounds (Fig. 1).[12] The known compounds were identified as vitexilactone (**1**),[10,11] (*rel* 5*S*,6*R*,8*R*,9*R*,10*S*)-6-acetoxy-9-hydroxy-13(14)-labden-16,15-olide (**2**),[10] viteagnusin I (**3**),[13] vitetrifolin D (**4**),[14] vitetrifolin E (**5**),[14] vitetrifolin F (**6**),[14] vitetrifolin G (**8**),[14] 9,13-epoxy-16-norlabda-13*E*-en-15-al (**9**),[15] 13-*epi*-2-oxokolavelool (**10**),[16] isolophanthin A (**11**),[17] vitedoin B (**12**),[15] viteagnusin F (**13**),[18] viteagnusin G (**14**),[18] (*rel* 3*S*,5*S*,8*R*,9*R*,10*S*)-3,9-dihydroxy-13(14)-labden-16,15-olide (**16**),[19] (7*S*,8*R*)-dihydrodehydrodiconiferyl alcohol (**18**),[20] ficusal (**21**),[21] (+)-lariciresinol (**22**),[22] fiscusesquilignan A (**23**),[21] and casticin (**24**),[23] by comparison of their UV, NMR, CD, and MS data with the reported values in the literature.

Compound **7**[24] was isolated as colorless syrup. The molecular formular was determined as $C_{21}H_{36}O_3$ by HRESIMS data at *m*/*z* 359.2554 $[M+Na]^+$ (calcd for $C_{21}H_{36}O_3Na$, 359.2556). The ^1H NMR spectrum revealed signals due to a vinylic group [δ_H 5.85 (1H, dd, J = 17.5, 11.0 Hz, H-14), 5.20 (1H, dd, J = 17.5, 1.5 Hz, H-15a), and 5.08 (1H, dd, J = 11.0, 1.5 Hz, H-15b)], four tertiary methyl groups [δ_H 1.26 (3H, s, H_3-16), 1.15 (3H, s, H_3-19), 1.07 (3H, s, H_3-18), and 1.04 (3H, s, H_3-20)], one secondary methyl group [δ_H 1.06 (3H, d, J = 6.5 Hz, H_3-17)], one methoxy group [δ_H 3.59 (3H, s, OCH_3-6)], and two oxygenated methine protons [δ_H 3.78 (1H, d, J = 4.0 Hz, H-6) and 3.62 (1H, dd, J = 13.5, 4.0 Hz, H-7)] (Table 1). The ^{13}C and DEPT NMR spectra showed 21 carbon signals including one vinylic group (δ_C 144.6 and 112.1), one tetra-substituted olefinic bond (δ_C 138.7 and 135.3), two oxygenated methine carbons (δ_C 77.2 and 74.1), one oxygenated quaternary carbon (δ_C 73.2),

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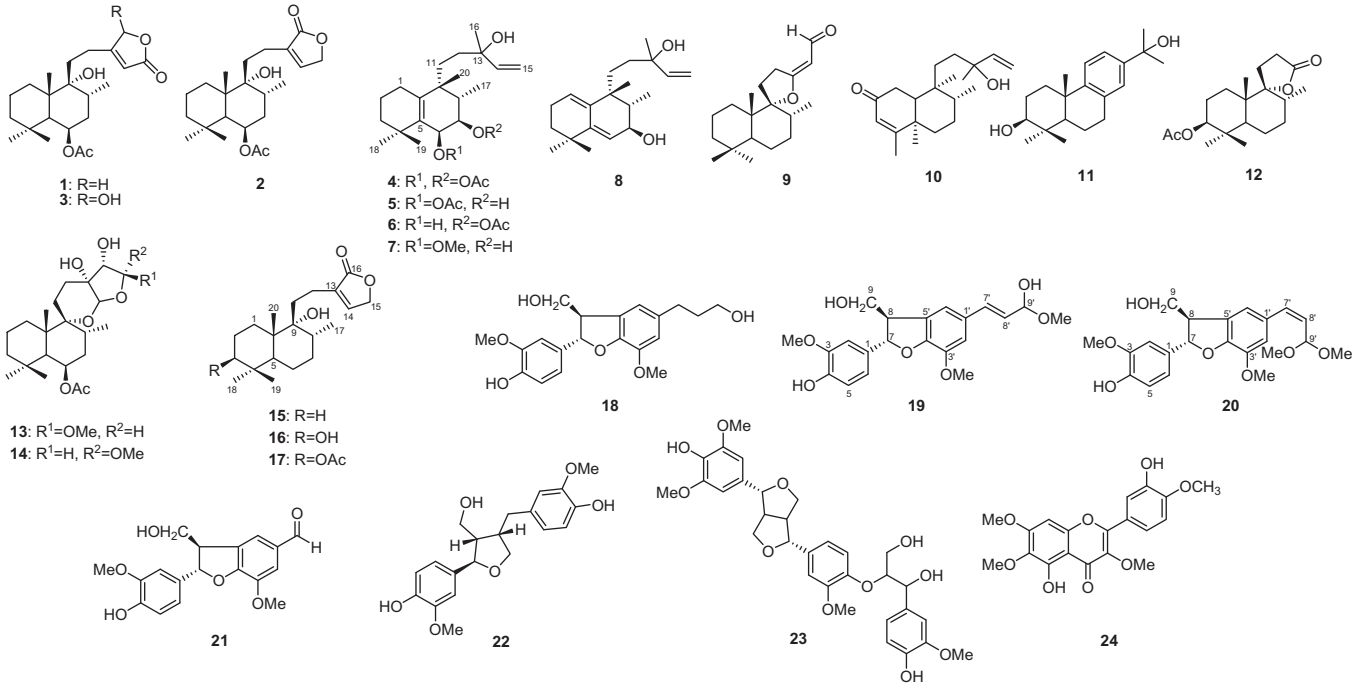

Figure 1. Chemical structure of the isolated compounds (**1**–**24**).

Table 1
^{1}H NMR data of compounds **7**, **15**, **17**, **19**, and **20**[a]

Carbon no.	**7**[b]	**15**[b]	**17**[b]	Carbon no.	**19**[c]	**20**[c]
1	1.95 m, 2.03 m	ca. 1.49	ca. 1.52, 1.43	1		
2	ca. 1.65	ca. 1.48	ca. 1.73, 1.62	2	6.96 d (2.0)	6.97 d (2.0)
3	ca. 1.47, 1.56	ca. 1.36, 1.17	4.51dd (12.0, 4.5)	3		
4				4		
5		1.43 m	1.58 m	5	6.80 d (8.0)	6.79 d (8.0)
6	3.78 d (4.0)	ca. 1.56, 1.31	ca. 1.56, 1.36	6	6.85 dd (8.0, 2.0)	6.85 dd (8.0, 2.0)
7	3.62 dd (13.5, 4.0)	ca. 1.49, 1.31	ca. 1.77, 1.51	7	5.56 d (6.5)	5.56 d (6.5)
8	1.92 dq-like (13.5, 6.5)	1.80 m	1.81 m	8	3.52 q (6.5)	3.52 q (6.5)
9				9	3.93 m, 3.83 m	3.92 m, 3.83 m
10				1′		
11	ca. 1.27, 1.43	ca. 1.87, 1.68	ca. 1.85, 1.70	2′	7.00 br s	7.01 br s
12	ca. 1.15, 1.42	2.41 m	2.41 m	3′		
13				4′		
14	5.85 dd (17.5, 11.0)	7.12 dd (1.5, 1.5)	7.12 dd (1.5, 1.5)	5′		
15	5.20 dd (17.5, 1.5)	4.79 d (1.5)	4.78 d (1.5)	6′	7.03 br s	6.98 br s
	5.08 dd (11.0, 1.5)	4.78 d (1.5)				
16	1.26 s			7′	6.68 d (16.5)	6.67 d (12.0)
17	1.06 d (6.5)	0.93d (7.0)	0.94 d (6.5)	8′	6.04 d (16.5, 5.5)	5.61 d (12.0, 7.0)
18	1.07 s	0.90 s	0.90 s	9′	4.93 d (5.5)	5.15 d (7.0)
19	1.15 s	0.85 s	0.90 s	3′-OCH_3	3.90 s	3.90 s
20	1.04 s	0.95 s	0.97 s	3-OCH_3	3.84 s	3.83 s
1′				9′-OCH_3	3.38 s	3.38 s
2′			2.07 s	9′-OCH_3		3.38 s
6-OCH_3	3.59 s					

[a] TMS was used as an internal standard; chemical shifts (δ) are expressed in ppm; *J* values are given in parentheses.
[b] Data was measured in $CDCl_3$ at 500 MHz.
[c] Data was measured in CD_3OD at 500 MHz.

and one methoxy group (δ_C 59.9) (Table 2). On the basis of the structures of diterpenoids previously isolated from the genus *Vitex*,[14,25] these data were similar to those of vitetrifolin F (**6**), which possessed a 3-hydroxy-3-methyl-1-propenyl group. The planar structure of **7** was confirmed by a combination of COSY, HSQC, and HMBC experiments. In the HMBC spectrum, the correlations between OCH_3 (δ_H 3.59) and C-6 (δ_C 77.2) indicated that the methoxy group were located at C-6 (Fig. 2). Furthermore, the relative configuration was determined by NOE experiment (Fig. 3). The key NOE correlations were observed as H-6/H-18, H-7/H-11, H-11/H-17, H-8/H-20, and OCH_3-6/H-19, corresponding the 6*S**,7*R**,8*S**, and 9*R**. Also, the coupling constant ($J_{6,7}$ = 4.0 Hz) further supported these orientation. However, the configuration at C-13 remains to be determined. Therefore, compound **7** was elucidated as (*rel* 6*S*,7*R*,8*S*,9*R*)-6-methoxy-5(10),14-halimadien-7,13-diol, named vitetrifolinH.

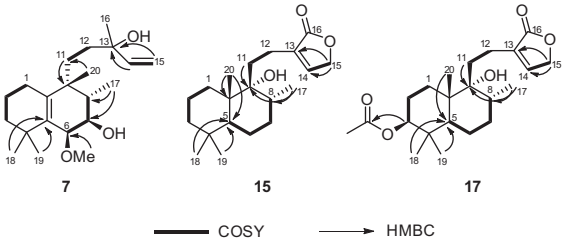

Figure 2. The COSY and HMBC correlations of compounds **7**, **15**, and **17**.

Compound **15**[26] was isolated as colorless syrup, with the molecular formular $C_{20}H_{32}O_3$, as determined by the HRESIMS at *m*/*z* 343.2245 $[M+Na]^+$ (calcd for $C_{20}H_{32}O_3Na$ 343.2243). The ^1H and ^{13}C NMR spectra were similar to those of compound **16**, except for the absence of a hydroxyl group. The ^1H NMR spectrum showed a typical α-substituted butenolide ring [δ_H 7.12 (1H, dd, J = 1.5, 1.5 Hz, H-14), 4.79 (1H, d, J = 1.5 Hz, H-15b), and 4.78 (1H, d, J = 1.5 Hz, H-15a)] along with three tertiary methyl groups [δ_H 0.95 (3H, s, H_3-20), 0.90 (3H, s, H_3-18), and 0.85 (3H, s, H_3-19)], and one secondary methyl group [δ_H 0.93 (3H, d, J = 7.0 Hz, H_3-17)] (Table 1). The ^{13}C and DEPT NMR spectra exhibited 20 carbon signals including three methine carbons, eight methylene carbons, and four methyl carbons (Table 2). According to the analysis of COSY, HSQC, and HMBC data, the planar structure of compound **15** was determined, as shown in Figure 2. The HMBC spectrum confirmed the correlations between H_2-11 and H_3-17 and C-9 and between H_3-18, H_3-19, and H_3-20 and C-5 (Fig. 2). In the NOESY spectrum of compound **15** (Fig. 3), the NOE correlations were observed between H-5/H_3-18, H_3-19/H_3-20, H_3-20/H_2-11, H-8/H_2-11, and H-8/H_3-20, indicating the configuration of 5*S**,8*R**,9*R**, and 10*S**. Therefore, compound **15** was determined as (*rel* 5*S*,8*R*,9*R*,10*S*)-9-hydroxy-13(14)-labden-16,15-olide, named viterotulin A.

Compound **17**[27] was isolated as colorless syrup, with the molecular formular $C_{22}H_{34}O_5$, as determined by the HRESIMS at *m*/*z* 401.2297 $[M+Na]^+$ (calcd for $C_{22}H_{34}O_5Na$ 401.2298). The ^1H, ^{13}C and DEPT NMR spectra were closely comparable to those of compound **16**, except for the additional acetoxy group [δ_H 2.07 (3H, s, H_3-2′); δ_C 170.9 (C-1′) and 21.3 (C-2′)], instead of a hydroxyl group (Tables 1 and 2). The acetoxy group was assigned at C-3 by the corresponding HMBC correlation between H-3 [δ_H 4.51 (1H, dd, J = 12.0, 4.5 Hz)] and C-1′ (δ_C 170.9) (Fig. 2). In the NOESY spectrum (Fig. 3), the NOE correlations were observed between H-3/H_3-18, H-3/H-5, H-5/H_3-18, H_3-19/H_3-20, H_3-20/H_2-11, H-8/H_2-11, H-8/H_3-19, and H-8/H_3-20, indicating the configuration of 3*S**,5*S**,8*R**,9-*R**, and 10*S**. Therefore, the compound **17** was elucidated as (*rel*

Table 2
^{13}C NMR data of compounds **7**, **15**, **17**, **19**, and **20**[a]

Position	**7**[b]	**15**[b]	**17**[b]	Position	**19**[c]	**20**
1	25.8	31.9	31.1	1	133.0	133.0
2	19.6	18.6	23.6	2	109.2	109.2
3	39.7	41.7	80.5	3	147.8	147.8
4	34.4	33.3	37.7	4	146.3	146.3
5	135.3	46.4	45.7	5	114.8	114.8
6	77.2	21.6	21.1	6	118.4	118.4
7	74.1	31.3	29.8	7	88.0	88.0
8	39.6	36.7	36.5	8	53.7	53.7
9	42.8	78.1	76.6	9	63.4	63.4
10	138.7	43.2	42.8	1′	130.2	129.9
11	29.6	32.3	32.4	2′	115.5	113.6
12	38.8	22.3	22.3	3′	144.2	143.8
13	73.2	135.3	135.0	4′	148.4	147.9
14	144.6	143.7	143.9	5′	129.1	128.8
15	112.1	70.1	70.2	6′	111.0	117.9
16	27.8	174.5	174.5	7′	133.6	133.4
17	11.5	16.4	16.2	8′	122.9	125.8
18	30.0	33.7	28.4	9′	103.8	100.1
19	28.7	22.0	16.7	3-OCH_3	55.0	55.0
20	28.3	16.2	16.1	3′-OCH_3	55.4	55.3
1′			170.9	9′-OCH_3	51.9	51.4
2′			21.3	9′-OCH_3		51.3
6-OCH_3	59.9					

[a] TMS was used as an internal standard; chemical shifts (δ) are expressed in ppm.
[b] Data was measured in $CDCl_3$ at 125 MHz.
[c] Data was measured in CD_3OD at 125 MHz.

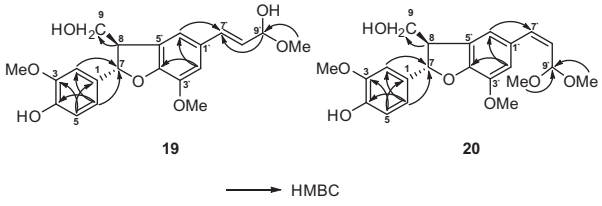

Figure 4. The HMBC correlations of compounds **19** and **20**.

3*S*,5*S*,8*R*,9*R*,10*S*)-3-acetoxy-9-hydroxy-13(14)-labden-16,15-olide, named viterotulin B.

Compound **19**[28] was obtained as yellow oil and its molecular formular $C_{21}H_{24}O_7$ was established by the HRESIMS data (*m*/*z* 411.1418, calcd for $[M+Na]^+$, 411.1414). The ^1H NMR spectrum exhibited an ABX-pattern aromatic proton signals [δ_H 6.96 (1H, d, J = 2.0 Hz, H-2), 6.85 (1H, dd, J = 8.0, 2.0 Hz, H-6), and 6.80 (1H, d, J = 8.0 Hz, H-5)], a 1,3,4,5-tetra-subsitituted aromatic proton signals [δ_H 7.03 (1H, br s, H-6′) and 7.00 (1H, br s, H-2′)], a –OCHCHCH$_2$O– moiety [δ_H 5.56 (1H, d, J = 6.5 Hz, H-7), 3.93

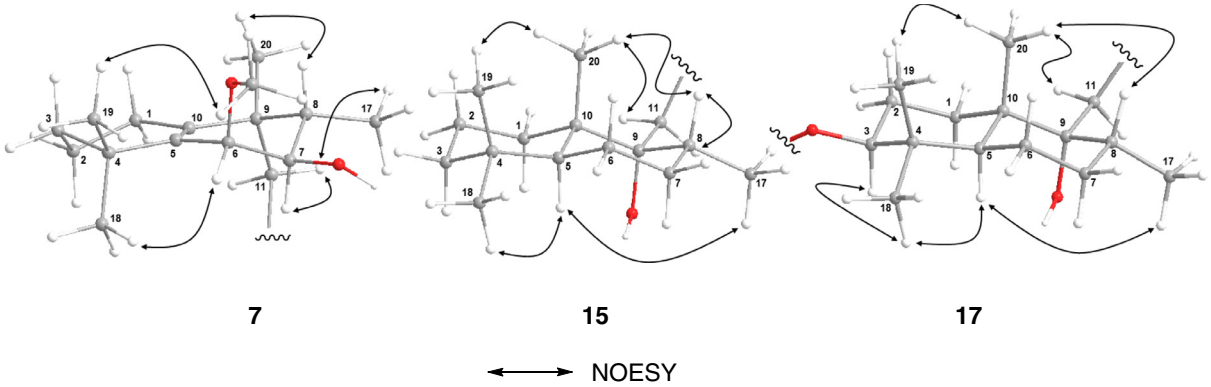

Figure 3. The key NOESY correlations of compounds **7**, **15**, and **17**.

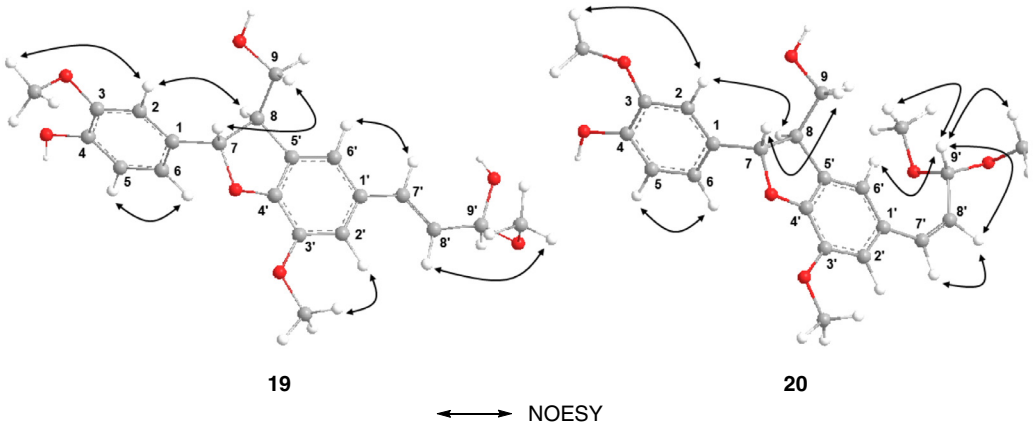

Figure 5. The key NOESY correlations of compounds **19** and **20**.

(1H, m, H-9a), 3.83 (1H, m, H-9b), and 3.52 (1H, q, J = 6.5 Hz, H-8)], an *E*-propenol group [δ_H 6.68 (1H, d, J = 16.5 Hz, H-7′), 6.04 (1H, dd, J = 16.5, 5.5 Hz, H-8′), and 4.93 (1H, d, J = 5.5 Hz, H-9′)], and three methoxy group (δ_H 3.90, 3.84, and 3.38, each 3H, s) (Table 1). The ^{13}C NMR data of **19** were similar to those of balanophnin,[29] except for the presence of a doubly-oxygenated carbon and an additional methoxy group instead of aldehyde carbon (Table 2). The location of three methoxy groups were assigned as C-3, C-3′, and C-9′ by the HMBC correlations between OCH_3-3 (δ_H 3.84)/C-3 (δ_C 147.8), OCH_3-3′ (δ_H 3.90)/C-3′ (δ_C 144.2), and OCH_3-9′ (δ_H 3.38)/C-9′ (δ_C 103.8) (Fig. 4). The coupling constant between H-7 and H-8 was 6.5 Hz, and NOE correlations H-2/H-8 and H-7/H_2-9 were observed in the NOESY spectrum compound of **19** (Fig. 5), which indicated that the geometry between H-7 and H-8 possessed a *trans*-configuration.[20,29] Furthermore, the absolute configuration of **19** was determined to be 7*S*,8*R* by the positive Cotton effects at 212 ($\Delta\varepsilon$ +0.43) and 260 ($\Delta\varepsilon$ +0.55) nm and negative Cotton effect at 234 ($\Delta\varepsilon$ −0.38) nm, in accordance with previously reported CD data.[30] Therefore, compound **19** was elucidated as (7*S*,8*R*)-9′-methoxydihydroconiferyl alcohol, named viterolignan A.

Compound **20**[31] was isolated as yellow oil and its molecular formular $C_{22}H_{26}O_7$ was determined by HRESIMS data (*m*/z 425.1576, calcd for $[M+Na]^+$, 425.1570). The ^{1}H and ^{13}C NMR spectra of **20** was closely resembled to those of **19**, except for the additional methoxy group and *Z*-propenol group [δ_H 6.67 (1H, d, J = 12.0 Hz, H-7′) and 5.61 (1H, dd, J = 12.0, 7.0 Hz, H-8′)] (Tables 1 and 2). The additional methoxy group was assigned as C-9′ position by the HMBC correlation from OCH_3-9′ (δ_H 3.38) to C-9′ (δ_C 100.1) (Fig. 4). The relative and absolute configuration of **19** was assigned by the NOESY and CD experiments, respectively. The NOESY spectrum showed the correlations H-2/H-8 and H-7/H_2-9 were observed (Fig. 5), furthermore, the CD spectrum showed the Cotton effects at 214 ($\Delta\varepsilon$ +0.37), 234 ($\Delta\varepsilon$ −0.59), and 260 ($\Delta\varepsilon$ +0.22) nm.[30] Therefore, compound **20** was elucidated as (7*S*,8*R*)-9′-dimethoxydihydroconifryl alcohol, named viterolignan B.

Table 3
Inhibition of NO production by compounds **1**–**24**

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	42.5	**10**	>50	**19**	21.1
2	45.4	**11**	>50	**20**	42.8
3	11.3	**12**	>50	**21**	>50
4	24.5	**13**	17.2	**22**	>50
5	>50	**14**	29.3	**23**	>50
6	>50	**15**	16.4	**24**	21.4
7	22.2	**16**	>50	AG[a]	16.6
8	34.1	**17**	49.1		
9	41.8	**18**	38.7		

[a] Aminoguanidine was used as the positive control.

The NO radical, synthesized by the oxidation of L-arginine catalyzed by nitric oxide synthase (NOS), is involved in a number of physiological and pathological processes in mammals. However, excess production of NO by iNOS in macrophages causes various inflammatory diseases.[32] Therefore, inhibitors of NO production might be considered as potential anti-inflammatory agents. All isolated compounds (**1**–**24**) were examined for their inhibitory effects on NO production in LPS-induced RAW264.7 cells by Griess method. Of these, compounds **3**, **4**, **7**, **13**, **15**, **19**, and **24** exhibited the significant inhibitory activity with the IC_{50} values ranging from 11.3 to 24.5 μM, as compared with the positive control aminoguanidine at 16.6 μM (Table 3).[33] It was found that none of the concentrations used in the experiment were cytotoxic (cell viability >90%). Although further investigations are needed to clarify the detailed structure–activity relationship, these results indicate that labdane-type diterpenoids possessing a butenolide ring (**1**–**3**, **15**, and **17**) and a five- and six-membered cyclic acetal group (**13**–**14**) seems to be more potent than the other diterpenoids. Moreover, neolignan (**18**–**20**) and flavonoid (**24**) also played a key role for the inhibitory activity. Recently, norlabdane-type diterpenes and phenyldihydronaphthalene-type lignans from the seeds of *V. negundo* were isolated and evaluated for their inhibitory effects on nitric oxide production in LPS-induced RAW264.7 cells.[34,35] Moreover, aqueous extract of *V. trifolia* leaves showed the inhibition of LPS-induced inflammatory mediators in RAW264.7 cells through the inhibition of NF-κB translocation and expression.[36]

Taken together, our findings and previously reported data[4] provide a potential explanation for the use of the fruits of *V. rotundifolia* in the treatment of inflammatory diseases.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.004.

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12. *Extraction and isolation*: The air-dried and powdered fruits of *V. rotundifolia* (6 kg) were extracted with MeOH (24 L × 3) at room temperature, and then the solution was evaporated under vacuo. The residue was suspended in H_2O and partitioned with *n*-hexane, CH_2Cl_2, and EtOAc, respectively. The CH_2Cl_2-soluble extract (54 g) was subjected to silica gel CC eluting with a stepwise gradient of CH_2Cl_2–MeOH (100:0 to 10:1) to give 5 sub-fractions (VRC1–VRC5). VRC2 (1.5 g) was further subjected to silica gel CC and eluted with *n*-hexane–EtOAc (10:1 to 2:1) to afford 6 sub-fractions (VRC21–VRC–26). VRC24 (0.4 g) was subjected to reversed-phase (ODS-A) CC eluting with H_2O–MeOH (20%, 40%, 60%, 80%, 100% MeOH) to yield 5 sub-fractions (VRC241–VRC245). VRC243 was identified as **4** (34 mg), while the VRC244 was further purified by semi-preparative-HPLC (40% to 76% MeCN) to afford **8** (3.1 mg), **9** (27 mg), and **15** (6 mg), respectively. VRC25 and VRC26 were combined and recrystallized with CH_2Cl_2–MeOH (1:1) to give **24** (0.8 g). VRC3 (6 g) was chromatographed over silica gel CC eluting with *n*-hexane–EtOAc (10:1 to 1:1) to give 7 sub-fractions (VRC31–VRC-37). VRC33 (0.9 g) was further chromatographed on silica gel CC eluting with *n*-hexane-acetone (8:1 to 1:1) to afford 5 sub-fractions (VRC331–VRC335). VRC332 (40 mg) was further purified by semi-preparative HPLC (50% to 70% MeCN) to give **10** (4 mg) and **12** (3.2 mg). VRC333 (0.2 g) was further chromatographed over MPLC (MCI gel, 70% to 90% MeOH) to yield 4 sub-fractions (VRC3331–VRC3334). VRC3333 (0.12 g) was purified by semi-preparative HPLC (45% to 75% MeCN) to afford **2** (18 mg), **3** (24 mg), **5** (7 mg), **6** (12 mg), and **11** (2.4 mg), respectively. VRC334 was identified as **1** (112 mg). VRC4 (8 g) was chromatographed over MPLC (RP-18, 20% to 90% MeOH) to yield 9 sub-fractions (VRC41–VRC49). VRC43 (0.2 g) and VRC44 (0.1 g) were combined and purified by semi-preparative HPLC (20% to 50% MeCN) to give **19** (8 mg), **20** (6.2 mg), and **16** (12 mg). VRC46 (0.3 g) and VRC49 (0.6 g) were combined and further purified by semi-preparative HPLC (33% to 70% MeCN) to yield **7** (7 mg), **13** (4.5 mg), **14** (6.4 mg), and **17** (7 mg). VRC5 (12 g) was chromatographed over MPLC (RP-18, 20% to 60% MeCN, step gradient) to give **9** sub-fractions (VRC51–VRC59). VRC51 was identified as **18** (1.8 g), and VRC53 (1.1 g) was purified by semi-preparative HPLC (45% to 75% MeCN) to yield **21** (15 mg), **22** (8 mg), and **23** (7 mg).
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24. *Compound* **7**: Colorless syrup; $[\alpha]_D^{25}$ +19.3 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3624, 2934, 1734, 1370, 1250, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see. Tables 1 and 2; HRESIMS *m*/*z* 359.2554 (calcd for $C_{21}H_{36}O_3Na$, 359.2556).
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26. *Compound* ***15***: Colorless syrup; $[\alpha]_D^{25}$ +11.2 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3596, 2933, 1744, 1641, 1461, 1136 cm^{-1}; ^1H and ^{13}C NMR data, see. Tables 1 and 2; HRESIMS *m*/*z* 343.2245 (calcd for $C_{20}H_{32}O_3Na$, 343.2243).
27. *Compound* ***17***: Colorless syrup; $[\alpha]_D^{25}$ +9.9 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3624, 2930, 1738, 1626, 1463, 1370 cm^{-1}; ^1H and ^{13}C NMR data, see. Tables 1 and 2; HRESIMS *m*/*z* 401.2297 (calcd for $C_{22}H_{34}O_5Na$, 401.2298).
28. *Compound* ***19***: Yellow oil; $[\alpha]_D^{25}$ +14.7 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 221 (3.73), 280 (3.56) nm; IR (KBr) ν_{max} 3731, 2928, 1601, 1508, 1459, 1332 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 212 (+0.43), 234 (−0.38), 260 (+0.55) nm; ^1H and ^{13}C NMR data, see. Tables 1 and 2; HRESIMS *m*/*z* 411.1418 (calcd for $C_{21}H_{24}O_7Na$, 411.1414).
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33. *Measurement of NO production and cytotoxicity assay*: The nitrite concentration was measured as an indicator of NO production according to the Griess reaction. Briefly, RAW264.7 cells were seeded in 96-well plates at a density of 2×10^5 cells/mL, and stimulated with or without 1 μg/mL of LPS in the presence or absence of compounds. After incubation for 24 h, supernatant (100 μL) of was then reacted with 100 μL of Griess reagent to determine nitrite production. Absorbance at 550 nm was measured in a microplate reader, and the absorption coefficient was calibrated using a $NaNO_2$ solution standardand. The remaining cells after Griess assay were used for cell viability with the CCK (Cell Counting Kit, Dojindo, Tokyo, Japan)-based colorimetric assay.
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