New Diterpenes and Norditerpenes from the Fruits of *Vitex rotundifolia*

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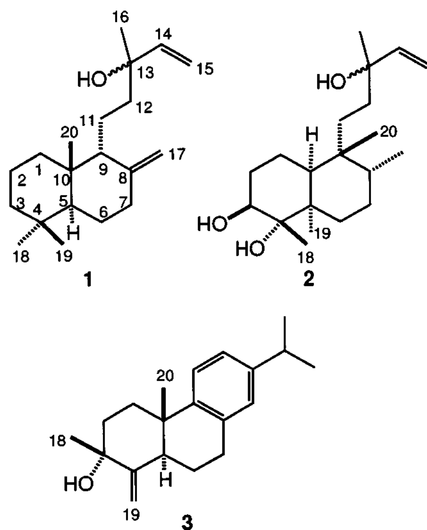
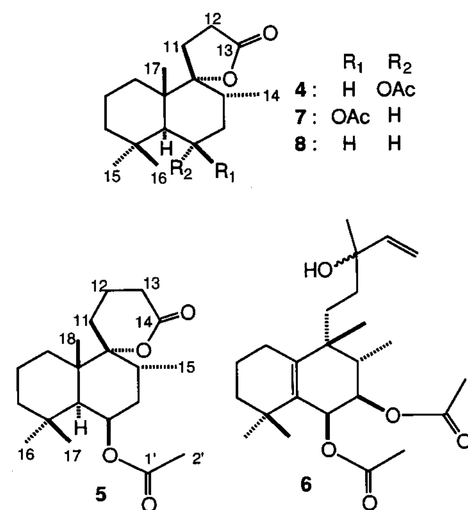
A new labdane-type diterpene, vitexifolin A (**1**), a new clerodane-type diterpene, vitexifolin B (**2**), a new abeoabietane-type diterpene, vitexifolin C (**3**), and two new norlabdane-type diterpenes, vitexifolin D (**4**) and vitexifolin E (**5**), were isolated from the fruits of *Vitex rotundifolia*, along with a known halimane-type diterpene, vitetrifolin D (**6**), two known norlabdane-type diterpenes, trisnor-γ-lactone (**7**) and *iso*-ambreinolide (**8**), and three known flavonoids, casticin (**9**), artemetin (**10**), and 5,3′-dihydroxy-6,7,4′-trimethoxyflavanone (**11**). Their chemical structures were determined on the basis of spectroscopic data. Casticin (**9**) exhibited considerable growth inhibitory activity against human lung cancer cells (PC-12) and human colon cancer cells (HCT116) using the MTT assay.

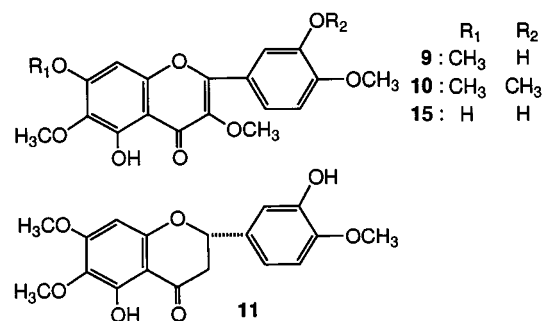
In preceding papers[1−5] we reported the isolation and structure elucidation of iridoids, phenylpropanoids, a flavanone, lignans, a diterpene glycoside, and diterpenes from the MeOH extract of the dried fruits of *Vitex rotundifolia* L. (Verbenaceae), which are called "Viticis Fructus", and are used as folk medicine for headaches, colds, migraine, eye-pain, etc.[6] Several of these compounds were found to have stronger antioxidative activity than the standard synthetic antioxidant, 3-*tert*-butylhydroxyanisole.[2] As part of a continuing study of *V. rotundifolia* fruit, we now describe the isolation and structure characterization of a new labdane-type diterpene (**1**), a new clerodane-type diterpene (**2**), a new abeoabietane-type diterpene (**3**) and



two new norlabdane-type diterpenes (**4**, **5**). A known halimane-type diterpene (**6**), two known norlabdane-type diterpenes (**7**, **8**), and three known flavonoids (**9**−**11**) were also isolated from the MeOH extract. Additionally, we report the in vitro cytotoxic activity of **7**, **9**−**11**, and (*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 (**12**) and vitexilactone (**13**),



which were previously isolated from the MeOH extract, against human lung cancer cells (PC-12)[7] and human colon



cancer cells (HCT116)[8] using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide (MTT) assay.[9]

Results and Discussion

Repeated chromatography of the MeOH extract of the dried fruits of *Vitex rotundifolia* led to the isolation of **1**−**11**. Compounds **6**−**11** were identified as vitetrifolin D,[10] trisnor-γ-lactone,[11] *iso*-ambreinolide,[12] casticin,[13] artemetin,[13] and 5,3′-dihydroxy-6,7,4′-trimethoxyflavanone,[14] respectively, although the detailed NMR spectral data of **7** and **8** have not been reported in the literature.

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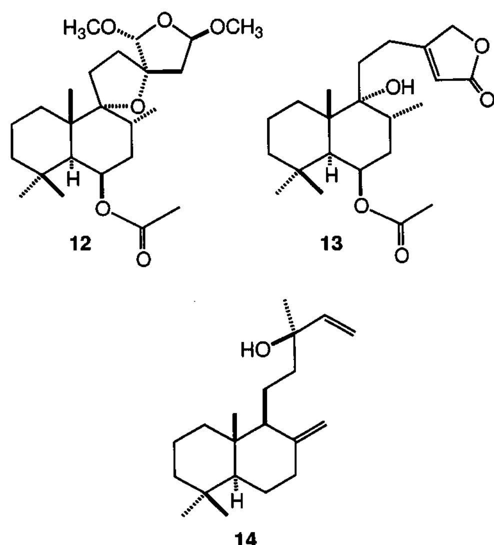

Compound **1**, trivially named vitexifolin A, was obtained as a colorless syrup. The molecular formula of **1** was determined as $C_{20}H_{34}O$ by HRFABMS (positive mode). The ^{1}H NMR spectrum of **1** indicated signals due to four tertiary methyl groups (δ 1.27, 0.91, 0.87, 0.81), one vinylic group (δ 5.91, 5.20, 5.05), and two *exo*-methylene protons (δ 4.67, 4.49). Since the ^{13}C NMR spectrum of **1** gave 20 carbon signals including four olefinic carbons (δ 149.2, 145.4, 111.5, 109.4) and one oxygenated quaternary carbon (δ 73.3), it was presumed to be a diterpene having a 3-hydroxy-3-methyl-1-propenyl group. These ^{1}H and ^{13}C NMR spectroscopic signals were assigned with the aid of ^{1}H−^{1}H COSY, HMQC, and HMBC techniques, as shown in Table 1 and Table 2, and suggested that **1** possessed the same planar structure as manool (**14**).[15] However, the ^{13}C NMR data of **1** differed from those of **14** and its *ent*-13-epimer.[16] To confirm the relative stereochemistry in **1**, the difference NOE spectra were measured in $CDCl_3$ and pyridine-d_5 solvents. Key NOE correlations between H_3-20 and H-9; H_3-18 and H-5; H_3-19 and H_3-20; and Ha-7 and H-11 were observed. Thus, **1** was elucidated as (*rel*-5*S*,9*R*,10*R*)-8(17),-14-labdadiene-13-ol. The configuration of the hydroxyl group at C-13 was not determined.

Compound **2**, trivially named vitexifolin B, was obtained as a colorless syrup and analyzed for the molecular formula $C_{20}H_{36}O_3$ by HRFABMS. The ^{1}H NMR spectrum of **2** gave signals analogous to those of **1**, except for the appearance of one secondary methyl group (δ 0.80) and one oxygenated methine proton (δ 3.59) and the lack of signals due to two *exo*-methylene protons. The ^{13}C NMR spectrum, which contained signals assignable to one oxygenated methine carbon (δ 76.3), two oxygenated quaternary carbons (δ 77.0, 73.7), and two olefinc carbons (δ 145.2, 111.7), displayed 20 carbon signals. In the same manner as for **1**, these ^{1}H and ^{13}C NMR signals were examined in detail, and the planar structure of **2**, a clerodane-type diterpene, was characterized as illustrated. The relative stereochemistry of **2** was defined on the basis of difference NOE spectra, which were mainly measured in pyridine-d_5; the ^{1}H NMR signals were rather congested in $CDCl_3$, but they were well separated in pyridine-d_5. Key NOE correlations were observed between Hα-2 and H-10; Hβ-6 and H-8; H-10 and H_3-19; H-10 and H_3-20; H_3-20 and Hβ-1; and H_3-20 and H-8, as illustrated in Figure 1, while no NOE correlations between Hα-2 and H_3-18 or between H_3-19 and H_3-20 were detected. Furthermore, in the ^{1}H NMR spectrum Hβ-1, Hα-2, Hβ-6, and H-10 were shifted downfield in pyridine-d_5[17] (Δδ = δpyridine-d_5 − δ$CDCl_3$; Hβ-1, Δδ + 0.50; Hα-2, Δδ + 0.61; Hβ-6, Δδ + 0.72; H-10, Δδ + ca. 0.62). Since the hydroxyl group forms a hydrogen bond with the nitrogen atom of pyridine, pyridine-induced deshielding effects are anticipated to be observed protons in the vicinity of the hydroxyl group.[17] These deshielding effects indicated that the hydroxyl groups at C-3 and C-4 have β and α configuration, respectively. The structure of **2** was therefore defined as (*rel*-3*S*,4*S*,5*R*,8*R*,9*R*,10*S*)-14-clerodene-3,4,13-triol. The configuration of the hydroxyl group at C-13 was not determined.

Compound **3**, trivially named vitexifolin C, and obtained as a colorless syrup, was concluded to have molecular formula $C_{20}H_{28}O$ by the NMR spectra and by EIMS, which showed an $[M]^+$ ion peak at *m*/*z* 284. The ^{1}H NMR spectrum of **3** indicated signals due to two tertiary methyl groups (δ 1.45, 0.95), two equivalent secondary methyl groups (δ 1.23 × 2), two *exo*-methylene protons (δ 5.12, 4.76), and a 1,2,4-trisubstituted benzene ring (δ 7.22, 7.01, 6.93). The ^{13}C NMR spectrum of **3** showed signals due to eight sp^2 carbons (δ 154.0, 145.8, 144.3, 134.7, 127.0, 125.4, 123.9, 106.9), one oxygenated quaternary carbon (δ 71.3), four methyl carbons (δ 27.9, 24.0 × 2, 21.6), four methylene carbons (δ 37.0, 34.0, 30.2, 21.8), two methine carbons (δ 42.7, 33.5), and one quaternary carbon (δ 38.9). The ^{1}H and ^{13}C NMR signals were assigned with the aid of NMR techniques similar to those of **1** and **2**, and the planer structure of **3**, an abeoabietane-type diterpene, was characterized as shown. In the difference NOE spectra, NOEs were observed between H_3-18 and Hβ-2; H_3-18 and Ha-19; H_3-20 and Hβ-1; H_3-20 and Hβ-6; H_3-20 and H-11; H_3-20 and Ha-19; and H_3-20 and Hb-19, as illustrated in Figure 1. These correlations suggested that the configurations of the methyl groups at C-3 and C-10 were both β. Furthermore, Hα-1 (Δδ + 0.38) and H-5 (Δδ + ca. 0.45) were shifted downfield in the ^{1}H NMR spectrum in pyridine-d_5.[17] Thus, **3** was concluded to be (*rel*-3*R*,5*R*,10*S*)-19(4→3)-abeo-4(18),8,-11,13-abietatetraen-3-ol.

Compound **4**, trivially named vitexifolin D, was obtained as colorless needles and analyzed for the molecular formula $C_{19}H_{30}O_4$ by HRFABMS. The EIMS of **4** indicated an $[M]^+$ ion peak at *m*/*z* 322 and an intense fragment ion peak at *m*/*z* 262 $[M - CH_3COOH]^+$. The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **7**, although the splitting patterns and/or chemical shifts of the signals due to H-5 (δ 1.91), H-6 (δ 5.13), H_3-15 (δ 1.04), and H_3-16 (δ 0.90) were different from those (H-5, δ 1.60; H-6, δ 5.44; H_3-15, δ 0.96; H_3-16, δ 1.00) of **7**. NOEs were observed between H_3-16 and H-6 and between H_3-17 and H-6, instead of the NOE correlation between H_3-15 and H-6 seen in **7**. Other NOE correlations between H_3-15 and H-5; H_3-16 and H_3-17; H_3-17 and H-8; and H_3-17 and H-11 were same as those of **7**. Thus, **4** was concluded to be (*rel*-5*S*,6*S*,8*R*,9*R*,10*S*)-14,15,-16-trinor-13,9-labdanolide, which is the C-6 epimer of **7**.

Compound **5**, trivially named vitexifolin E, was obtained as a colorless syrup. The EIMS gave a dominant fragment ion peak at *m*/*z* 276, which was 14 mass units $[CH_2]$ higher than the intense ion peak of **4** and **7**. The molecular formula of **5** was determined to be $C_{20}H_{32}O_4$ by HRFABMS. The ^{1}H and ^{13}C NMR spectra were similar to those of **7**, apart from the signal due to one more methylene group observed in the spectra of **5**. In comparing the chemical shifts of the ^{13}C NMR signals between **5** and **7**, the signals due to C-8, C-9, C-10, and the carbonyl carbon in **5** were shifted by +2.0, −3.5, +2.2, and −4.7 ppm, respectively. NOE correlations similar to those of **7** were observed in the difference NOE spectra of **5**. Thus, **5** was determined to be

Table 1. ^{1}H NMR Spectral Data for Compounds **1**−**3** in $CDCl_3$[a]

position	**1**	**2**	**3**
1a	ca. 1.53	ca. 1.77	2.06 ddd(2.5,4.5,14.0)
1b	1.04 dddd(1.0,2.5,2.5,12.0)	ca. 1.45	1.95 ddd(4.5,14.0,14.0)
2a	ca. 1.63	1.99 dddd(2.5,4.5,14.0,14.0)	1.84 ddd(2.5,4.5,14.0)
2b	ca. 1.44	ca. 1.62	1.75 ddd(4.5,14.0,14.0)
3a	ca. 1.40	3.59 dd(2.5,2.5)	
3b	1.17 ddd(3.5,12.5,12.5)		
5	ca. 1.30		ca. 2.84
6a	ca. 1.58	2.20 ddd(5.0,13.5,13.5)	ca. 1.77
6b	ca. 1.30	1.10 m	ca. 1.77
7a	2.15 m	ca. 1.45	2.92 dd like(2.5,10.0)
7b	2.03 m	ca. 1.29	ca. 2.84
8		ca. 1.64	
9	ca. 1.48		
10		ca. 1.61	
11a	ca. 1.58	ca. 1.73	7.22 d(8.0)
11b	ca. 1.34	ca. 1.17	
12a	ca. 1.46	ca. 1.66	7.01 dd(2.0,8.0)
12b	ca. 1.22	ca. 1.45	
14	5.91 dd(11.0,17.5)	5.89 dd(11.0,17.5)	6.93 d(2.0)
15a	5.20 dd(1.5,17.5)	5.19 dd(1.5,17.5)	ca. 2.84
15b	5.05 dd(1.5,11.0)	5.05 dd(1.5,11.0)	
16	1.27 s	1.29 s	1.23 d(7.0)
17a	4.67 dd(2.0,2.5)	0.80 d(7.5)	1.23 d(7.0)
17b	4.49 dd(2.0,2.5)		
18	0.87 s	1.24 s	1.45 s
19a	0.81 s	1.17 s	5.12 s
19b			4.76 s
20	0.91 s	0.91 s	0.95 s

[a] Chemical shifts (δ) are in ppm relative to TMS. Coupling constants (*J*) in Hz are given in parentheses. Values are recorded at 500 MHz.

Table 2. ^{13}C NMR Spectral Data for Compounds **1**−**5**, **7**, and **8** in $CDCl_3$[a]

position	**1**[b]	**2**[c]	**3**[b]	**4**[c]	**5**[c]	**7**[c]	**8**[b]
1	36.7	18.0	34.0	31.8	33.6	32.8	31.1[d]
2	19.1	29.1[d]	37.0	18.1	18.5	18.3	18.2
3	42.6	76.3	71.3	42.8	43.3	43.3	41.2
4	33.2	77.0	154.0	33.2	34.1	34.0	33.2
5	45.7	42.5	42.7	48.9	46.9	48.5	46.5
6	23.7	29.1[d]	21.8	71.5	70.1	69.7	21.2
7	31.6	27.9	30.2	37.0	35.5	36.0	30.9[d]
8	149.2	37.0	134.7	35.6	34.1	32.1	36.7
9	58.5	38.7	144.3	92.6	90.0	93.5	93.9
10	38.1	42.5	38.9	43.9	44.7	42.5	42.1
11	20.0	29.6[d]	125.4	24.5	26.1	24.4	24.6
12	41.0	36.8	123.9	29.3	19.4	30.0	29.4
13	73.3	73.7	145.8	177.3	30.6	177.5	177.8
14	145.4	145.2	127.0	15.1	172.8	15.2	15.6[e]
15	111.5	111.7	33.5	36.0	16.3	33.0	33.0
16	27.9	27.9	24.0	22.6	33.2	23.6	21.8
17	109.4	16.0	24.0	16.9	23.8	18.2	15.5[e]
18	33.5	21.9	27.9		18.4		
19	22.2[d]	21.0	106.9				
20	22.4[d]	29.3	21.6				
1′				170.5	170.3	170.3	
2′				21.9	21.9	21.9	

[a] Chemical shifts (δ) are in ppm relative to TMS. [b] Values are recorded at 100 MHz. [c] Values are recorded at 125 MHz. [d,e] Assignments in each column may be interchanged.

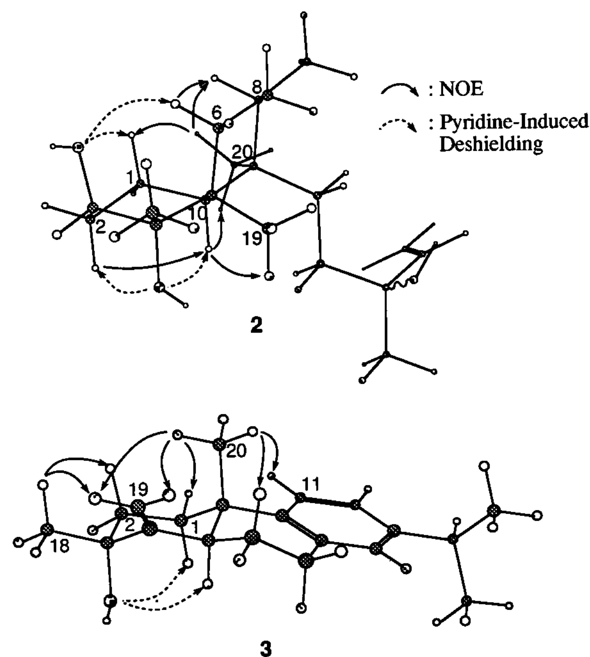

Figure 1. NOEs and pyridine-induced deshieldings for **2** and **3**.

(*rel*-5*S*,6*R*,8*R*,9*R*,10*S*)-15,16-dinor-14,9-labdanolide. The absolute configurations of **1**−**5** have not been confirmed.

The in vitro cytotoxic activity of **7**, **9**−**11**, and (*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 (**12**) and vitexilactone (**13**), which were previously isolated from the MeOH extract of *Vitex rotundifolia*,[4,5] was examined against two human cancer cells (PC-12, HCT116) using the MTT assay. Compound **9** showed potent cytotoxicities against both cells, with 50% growth inhibition (GI_{50}) values of 114 ng/mL (PC-12) and 119 ng/mL (HCT-116) (Table 4). Beutler et al. reported that the 3-hydroxy-4-methoxy group in the B-ring, a 3-methoxyl group, and a 5-hydroxyl group of flavonoid skeleton are important for the cytotoxic effects in the NCI 60 cells and associated inhibitory effects on tubulin polymerization, and further, centaureidin (**15**), which differs from **9** only by a hydroxy group in position 7 instead of a methoxyl group, showed the significant effects.[18] Therefore, the mechanism of cytotoxic action of **9** appears to be inhibition on tubulin polymerization.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting-point apparatus

Table 3. ^1H NMR Spectral Data for Compounds **4**, **5**, **7**, and **8** in $CDCl_3$[a]

position	4	5	7	8
1a	1.38 dddd(1.0,3.5,3.5,12.5)	ca. 1.51	ca. 1.36	
1b	ca. 1.30	1.12 ddd(3.5,12.5,12.5)	ca. 1.36	
2a	1.57 ddddd(3.5,3.5,12.5,12.5,12.5)	ca. 1.63	ca. 1.69	
2b	ca. 1.48	ca. 1.51	1.51 m	
3a	ca. 1.32	1.31 ddd(3.0,5.0,13.0)	ca. 1.33	1.18 ddd(3.5,13.5,13.5)
3b	ca. 1.26	1.19 ddd(3.5,13.0,13.0)	1.20 ddd(4.0,13.0,13.0)	
5	1.91 d(11.5)	1.75 d(2.5)	1.60 d(2.5)	1.51 br d(13.0)
6	5.13 ddd(5.0,11.5,11.5)	5.43 ddd(2.5,2.5,2.5)	5.44 ddd(2.5,2.5,2.5)	
7a	ca. 1.85	1.90 ddd(2.5,13.5,14.5)	ca. 1.67	
7b	ca. 1.48	ca. 1.51	ca. 1.64	
8	1.99 m	2.14 m	2.15 m	1.80 m
11a	2.19 ddd(7.5,11.5,13.5)	1.99 ddd(5.5,13.5,13.5)	2.28 ddd(8.5,11.5,14.5)	2.21 ddd(8.0,11.5,14.0)
11b	ca. 1.85	1.71 m	1.91 ddd(4.5,11.5,14.5)	1.84 ddd(5.0,11.5,14.0)
12a	2.56 ddd(7.5,11.5,19.0)	ca. 1.84	2.58 ddd(8.5,11.5,19.0)	2.54 ddd(8.0,11.5,19.0)
12b	2.49 ddd(5.5,11.5,19.0)	ca. 1.82	2.49 ddd(4.5,11.5,19.0)	2.46 ddd(5.0,11.5,19.0)
13a		2.54 m		
13b		2.18 ddd(5.5,12.5,17.0)		
14(15)	0.89 d(6.5)	0.94 d(6.5)	0.88 d(6.5)	0.85 d(6.5)
15(16)	1.04 s	0.98 s	0.96 s	0.87 s
16(17)	0.90 s	1.00 s	1.00 s	0.83 s
17(18)	1.00 s	1.28 s	1.26 s	0.92 s
2′	2.03 s	2.05 s	2.06 s	

[a] Chemical shifts (δ) are in ppm relative to TMS. Coupling constants (J) in Hz are given in parentheses. Values are recorded at 500 MHz.

Table 4. Cytotoxic Activities of Compounds **7**, **9**−**13**, and Cisplatin against PC-12 Cells and HCT116 Cells (GI_{50}, ng/mL)

compound	PC-12	HCT116
7	>5000	>5000
9	114	119
10	2270	2200
11	2270	2870
12	>5000	>5000
13	>5000	>5000
cisplatin	111	794

and are uncorrected. Optical rotations were measured with a JASCO DTP-1000 KUY digital polarimeter. ^1H NMR spectra were recorded in $CDCl_3$ or pyridine-d_5 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 $CDCl_3$ or pyridine-d_5 solution using JEOL JNM-GX-400 and JEOL alpha 500 spectrometers at 100 or 125 MHz, respectively. The MS were obtained on a JEOL JMS-DX-303HF instrument. Column chromatography was carried out with Diaion HP-20 (Mitsubishi Chemical Industries Co., Ltd.), silica gel 60 (Merck, 230−400 mesh and 70−230 mesh), Sephadex LH-20 (Pharmacia Fine Chemicals), and Chromatorex ODS (Fuji Silysia Chemical Co., Ltd.). HPLC separation was run using a Shimadzu Micro pump LC-10AS with a Shimadzu RI-Detector RID-10A. For HPLC column chromatography, YMC-pack ODS-5 (YMC Co., Ltd., 250 mm × 20 mm i.d.), YMC-pack SIL-06 (YMC Co., Ltd., 250 mm × 20 mm i.d.), Kusano C. I. G. prepacked Si-5 (Kusano Kagakukikai Co., 250 mm × 22 mm i.d.), and TSK-GEL ODS-120T (Tosoh Co., Ltd., 300 mm × 21.5 mm i.d.) were used.

Plant Material. Fruit of *Vitex rotundifolia* L. 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 Natural Products Chemistry, School of Agriculture, Kyushu Tokai University.

Extraction and Isolation. Powdered fruit of *Vitex rotundifolia* L. (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 Diaion HP-20 (40% MeOH, 70% MeOH, 90% MeOH, MeOH, acetone) to give fractions 1−4. Fraction 3 (36.1 g) was chromatographed over silica gel [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 15 (638 mg) was subjected to silica gel [hexane−acetone (20:1, 5:1, 3:1), acetone] to give fractions 22−30. Fraction 25 (100 mg) was subjected to HPLC (YMC pack SIL-06, hexane−EtOAc, 10:1) to give **8** (53 mg). Fraction 17 (1296 mg) was chromatographed over silica gel [hexane, hexane−acetone (20:1, 5:1, 3:1), acetone] to afford fractions 31−36. HPLC (TSK-GEL ODS-120T, 88% MeOH and YMC pack SIL-06, hexane−EtOAc, 10:1, in turn) of fraction 33 (118 mg) gave **3** (6 mg). Fraction 18 (687 mg) was subjected to HPLC (Kusano C. I. G. prepacked Si-5, hexane−EtOAc, 20:1, YMC pack ODS-5, 90% MeOH and YMC pack SIL-06, hexane−acetone, 20:1, in turn) to furnish **1** (7 mg). Fraction 7 (12.44 g) was subjected to Chromatorex ODS (60% MeOH, 70% MeOH, 75% MeOH, 80% MeOH, 90% MeOH, MeOH) to furnish fractions 37−53. Fraction 39 (183 mg), fraction 42 (592 mg), and fraction 44 (284 mg) were each recrystallized from MeOH to give **11** (50 mg) from fraction 39, **9** (326 mg) from fraction 42, and **10** (59 mg) from fraction 44. Fraction 45 (1248 mg) was subjected to Sephadex LH-20 (MeOH) to give fractions 54−57. Fraction 54 was chromatographed over silica gel [hexane−EtOAc (7:1, 5:1, 4:1, 3:1, 2:1, 3:2, 1:1), EtOAc)] to give **7** (87 mg), **2** (23 mg), **13** (190 mg), and fractions 58−61. Fraction 58 (39 mg) was subjected to HPLC (YMC pack ODS-5, 80% MeOH) to give **4** (10 mg). Fraction 47 (1145 mg) was chromatographed over Sephadex LH-20 (MeOH) to give fraction 62 and fraction 63. Fraction 63 (1015 mg) was subjected to silica gel [hexane−EtOAc (4:1, 5:2)] to give fractions 64−66. Fraction 64 (59 mg) and fraction 66 (115 mg) were each subjected to HPLC (TSK-GEL ODS-120T, 80%) to afford **6** (8 mg) from fraction 64 and **5** (7 mg) and fraction 67 (56 mg) from fraction 66.

Vitexifolin A (1): colorless syrup; $[\alpha]_D^{19}$ +5.2° (c 0.7, acetone); EIMS m/z $[M]^+$ absent, 272 (48) $[M - H_2O]^+$, 257 (100), 252 (73); ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS m/z 313.2474 $[M + Na]^+$ (calcd for $C_{20}H_{34}ONa$, 313.2507).

Vitexifolin B (2): colorless syrup; $[\alpha]_D^{21}$ +0.4° (c 2.3, acetone); EIMS m/z $[M]^+$ absent, 306 (44) $[M - H_2O]^+$, 288 (100) $[M - H_2O \times 2]^+$; ^1H NMR, see Table 1; ^{13}C NMR, see Table 2; HRFABMS m/z 347.2521 $[M + Na]^+$ (calcd for $C_{20}H_{36}O_3Na$, 347.2562).

Vitexifolin C (3): colorless syrup; $[\alpha]_D^{17}$ +168.1° (*c* 0.7, acetone); EIMS *m*/*z* 284 (43) $[M]^+$, 251 (100), 211 (67); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2.

Vitexifolin D (4): colorless needles (hexane−EtOAc); mp 100−101 °C; $[\alpha]_D^{17}$ −4.4° (*c* 2.8, acetone); EIMS *m*/*z* 322 (3) $[M]^+$, 262 (100) $[M - CH_3COOH]^+$; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 345.2015 $[M + Na]^+$ (calcd for $C_{19}H_{30}O_4Na$, 345.2042).

Vitexifolin E (5): colorless syrup, $[\alpha]_D^{28}$ −36.9° (*c* 0.8, acetone); EIMS *m*/*z* 276 $[M - CH_3COOH]^+$; ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; HRFABMS *m*/*z* 359.2151 $[M + Na]^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

Cell Culture and Cytotoxicity Assay. PC-12 and HCT116 cells were maintained at 37 °C in PRMI1640 medium (Gibco) containing 10% fetal bovine serum (Hyclone) in an atmosphere of humidified 5% CO_2. Growth inhibition experiments were carried out in quadruplicate in 96-well microplates, and the amount of viable cells at the end of the incubation was determined by using an MTT assay. Thus, PC-12 and HCT116 cells were inoculated at 1000 cells per each well, and they were continuously cultured without or with five concentrations (5000, 1000, 200, 40, and 8 ng/mL) of test compounds for 72 h from the next day. After addition of MTT (20 mL, 5 mg/mL in phosphate-buffered saline; Sigma), the medium was removed and the blue dye formed was dissolved in 150 mL of DMSO. The absorbance was measured at 540 nm using a Microplate Reader model 3550 (Bio-Rad). The GI_{50} value was defined as the concentration of sample necessary to inhibit the growth to 50% of the control. Cisplatin (Nippon Kayaku Co.) was used as a standard sample.

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