Indonesian Medicinal Plants. VII.[1] Seven New Clerodane-Type Diterpenoids, Peronemins A_2, A_3, B_1, B_2, B_3, C_1, and D_1, from the Leaves of *Peronema canescens* (Verbenaceae)

Isao KITAGAWA,*[,a] Partomuan SIMANJUNTAK,[a] Kazuyuki HORI,[a] Nobumasa NAGAMI,[a] Taifo MAHMUD,[a] Hirotaka SHIBUYA,[b] and Motomasa KOBAYASHI[a]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1–6, Yamada-oka, Suita, Osaka 565, Japan, and Faculty of Pharmacy and Pharmaceutical Sciences, Fukuyama University,[b] 1-Gakuen-cho, Fukuyama, Hiroshima 729–02, Japan.
Received December 6, 1993; accepted January 18, 1994

Seven new clerodane-type diterpenoids, named peronemins B_2 (1), A_2 (2), B_1 (3), C_1 (4), B_3 (5), A_3 (6), and D_1 (7), were isolated from the leaves of *Peronema canescens* (Verbenaceae), an Indonesian medicinal plant collected in Bengkulu, Sumatera Island, Indonesia. The chemical structures of 1—7 have been elucidated on the basis of their chemical and physicochemical properties.

Keywords Indonesian medicinal plant; *Peronema canescens*; Verbenaceae; peronemin; clerodane diterpene

During the course of our investigations in search of new biologically active compounds from Indonesian medicinal plants,[1,2] we have been investigating the chemical constituents of the leaves of *Peronema canescens* JACK. (Verbenaceae), which was collected in the Curup area of Bengkulu, Sumatera Island, during our third expedition to Indonesia in August 1990. This medicinal plant *Peronema canescens* is a large tree growing in secondary forests and is locally called "sungkei." The decoction of the leaves has been traditionally prescribed for treatment of malaria. In this paper, we describe the chemical characterization of new clerodane-type diterpenoid constituents which were isolated from the acetone-soluble portion of the leaves.

The acetone extract of the air-dried leaves of *Peronema canescens* was subjected to silica gel column chromatography to give several diterpenoid fractions, which colored pink with the Ehrlich reagent on their thin-layer chromatograms (TLC). The chromatographic separation also provided some known compounds such as β-sitosterol, phytol, and β-amyrin. The respective diterpenoid fractions were further purified by means of high-performance liquid chromatography (HPLC) to provide seven new clerodane-type diterpenoids, named peronemins B_2 (**1**, 0.04% from the dried leaves), A_2 (**2**, 0.005%), B_1 (**3**, 0.01%), C_1 (**4**, 0.04%), B_3 (**5**, 0.03%), A_3 (**6**, 0.01%), and D_1 (**7**, 0.003%).

Peronemins B_2 (1) and A_2 (2) The major diterpenoid constituent peronemin B_2 (**1**) showed the molecular ion peak at *m/z* 344 in its electron impact mass spectrum (EI-MS), the composition being defined as $C_{20}H_{24}O_5$ from the high-resolution EI-MS analysis. The infrared (IR) spectrum of **1** showed absorption bands assignable to a γ-lactone carbonyl group (1770 cm^{-1}) and a furan moiety (1450, 875 cm^{-1}), whereas the ultraviolet (UV) spectrum of **1** showed an absorption maximum at 204 nm (ε=2700) attributable to a furan ring, the presence of which in **1** was also supported by its positive coloration with the Ehrlich reagent.

The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of peronemin B_2 (**1**) (taken in $CDCl_3$) showed

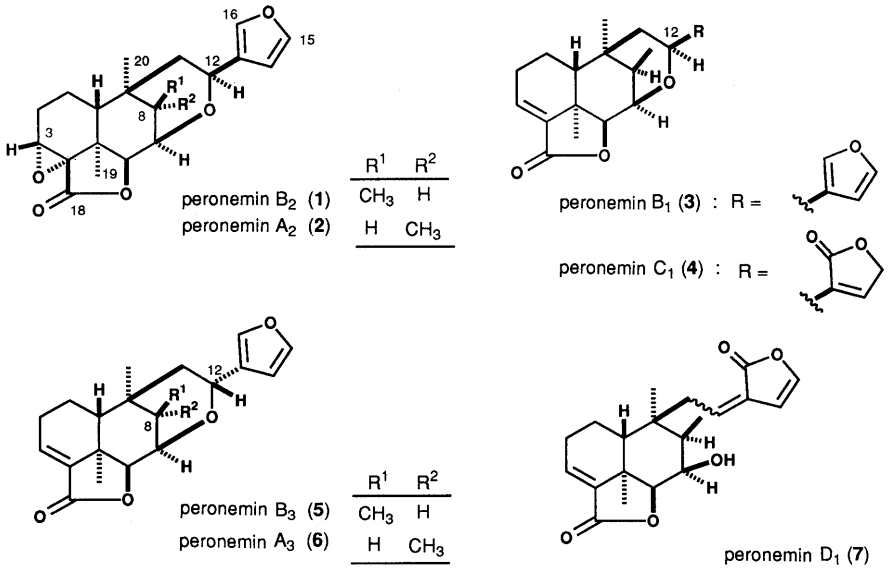


Fig. 1. Structures of Peronemins

the signals due to two tertiary methyl groups at δ 0.97, 1.18 (both 3H, s), a secondary methyl group at δ 1.32 (3H, d, $J=7$ Hz), and a proton attached to an epoxide ring at δ 3.39 (1H, d, $J=4.5$ Hz). Furthermore, characteristic signals assignable to protons on a furan ring were observed at δ 7.37 (1H, dd, $J=1$, 2 Hz), δ 7.30 (1H, d, $J=2$ Hz), and δ 6.26 (1H, d, $J=1$ Hz) together with the signals due to three methine protons, respectively attached to a carbon having an oxygen function, at δ 4.39 (2H, s) and δ 4.98 (1H, t, $J=8$ Hz). Among the latter signals, the two-proton singlet observed at δ 4.39 in $CDCl_3$ was separated into two one-proton signals at δ 3.70 (1H, d, $J=4$ Hz) and δ 3.98 (1H, d, $J=4$ Hz) in a spectrum taken in benzene-d_6.

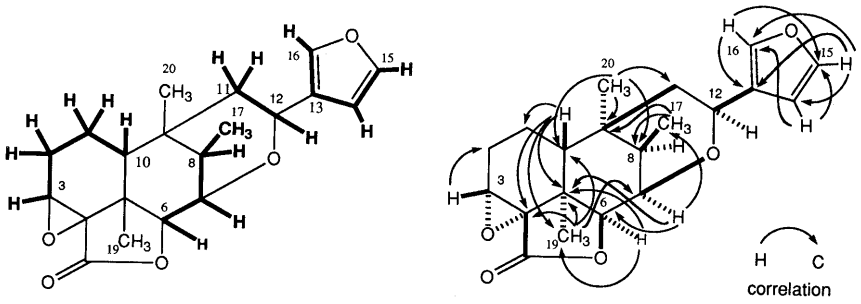
A distortionless enhancement by polarization transfer (DEPT) experiment on **1** by carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy disclosed the presence of three methyl carbons, three methylene carbons, six methine carbons, three olefinic carbons, and five quaternary carbons which included an olefinic carbon and a carbonyl carbon (Tables I and II). Furthermore, the ^{13}C–^{1}H correlation spectroscopy (C–H COSY) experiment of **1** revealed the connectivities between respective protons and carbons.

The plane structure of peronemin B_2 (**1**) was constructed on the basis of ^{1}H–^{1}H correlation spectroscopy (H–H COSY) and correlation spectroscopy carried out through the long-range coupling (COLOC) NMR experiments. Thus, the H–H COSY of **1** indicated the presence of four partial structures: from C-10 to C-3, from C-6 to C-17, from C-11 to C-12, and from C-14 to C-16. In the COLOC experiment, **1** was shown to have ^{1}H–^{13}C correlations between methyl protons at C-17 (δ 1.32) and carbons at C-8 (δ_C 33.8) and C-9 (δ_C 33.7) and also between methine protons at C-6 and C-7 (δ 4.39) and carbons at C-17 (δ_C 16.0), C-19 (δ_C 19.2), C-5 (δ_C 35.2), and C-6 (δ_C 87.2). Further correlations were observed between methyl protons at C-19 (δ 1.18) and carbons at C-4 (δ_C 64.2), C-5, C-7 (δ_C 74.7), and C-10 (δ_C 45.8), between methyl protons at C-20 (δ 0.97) and carbons at C-9, C-10, C-11 (δ_C 42.2), and C-8, and also between a methine proton at C-10 (δ 2.45) and carbons at C-1 (δ_C 14.8), C-4, C-5, and C-19 (Fig. 2).

The above-mentioned correlations demonstrate that peronemin B_2 (**1**) is a clerodane-type diterpene having a furan residue. The EI-MS of **1** provided supporting evidence by a base ion peak (**ii**) observed at m/z 94, which was assumed to be generated *via* cleavage of the pyran ring liberating a vinylfuran ion (**ii**) and a fragment ion peak (**i**) at m/z 250 presumably derived from the residual clerodane skeleton. Next, the relative stereostructure of peronemin B_2 (**1**) was deduced on the basis of the following nuclear Overhauser effect (NOE) correlations (2D-NOESY): *i.e.* cross peaks occurring between methyl protons at C-17 and 11-H_a (δ 1.75) and 12-H; between methyl protons at C-19 and 1-H_a (δ 1.51), 6-H, and 8-H; between 12-H and 11-H_a; between 10-H and 11-H_b (δ 1.77) and 2-H_a (δ 1.84) (Fig. 3). The conformation of **1** as depicted in Fig. 3 is further supported by the coupling constants observed for 7-H (d, $J=4$ Hz, in benzene-d_6), 10-H (dd, $J=2.5$, 13 Hz), and 12-H (t, $J=8$ Hz). Furthermore, the relative configuration around the 3,4-epoxide moiety was determined unequivocally from its correlation with the neighboring 4β,6β-γ-lactone moiety. Thus, the coupling patterns observed for the signals of the 2-H_a β-axial proton (dddd, $J=4.5$, 9.5, 9.5, 16 Hz), 2-H_b α-equatorial proton (dd, $J=7$, 16 Hz), and 3-H epoxide proton (d, $J=4.5$ Hz) were consistent with the α-orientation of the epoxide ring. Consequently, the relative stereostructure of peronemin B_2 has been clarified as **1**.

The EI-MS of peronemin A_2 (**2**) gave the molecular ion peak at m/z 344 and the base ion peak at m/z 94, which were identical with those of peronemin B_2 (**1**). In the ^{1}H- and ^{13}C-NMR spectra of **2** (Tables I and II), most proton and carbon signals were observed with similar chemical shifts to those of **1**. These observations have led us to presume that peronemin A_2 (**2**) is a stereoisomer of peronemin B_2 (**1**), and the relative stereostructure of **2** was demonstrated to be as shown on the basis of the NOESY. Thus, the NOE correlations depicted in Fig. 4 were observed for **2**: *i.e.* between methyl protons at C-17 and 7-H, 19-H_3, 20-H_3; between 8-H and 7-H, 11-H_a (δ 1.79), 12-H, 17-H_3; between 10-H and 2-H_a (δ 1.89, dddd, $J=4.5$, 9.5, 9.5, 11 Hz). Consequently, peronemin A_2 (**2**) is the C-8 stereoisomer of peronemin B_2 (**1**).

Peronemin B_1 (3) Peronemin B_1 (**3**) gave the molecular ion peak at m/z 328, the composition of which was $C_{20}H_{24}O_4$ from the high-resolution EI-MS analysis. The IR spectrum of **3** showed absorption bands assignable to α,β-unsaturated γ-lactone (1740, 1660 cm^{-1}) and furan (1450, 870 cm^{-1}) moieties. The UV absorption maximum of **3** observed at 213 nm ($\varepsilon=14500$) also supported the presence of an α,β-unsaturated γ-lactone moiety. In the ^{1}H-NMR spectrum of **3**, most of the proton signals were observed with similar chemical shifts to those of peronemin



peronemin B_2 (**1**)

Fig. 2

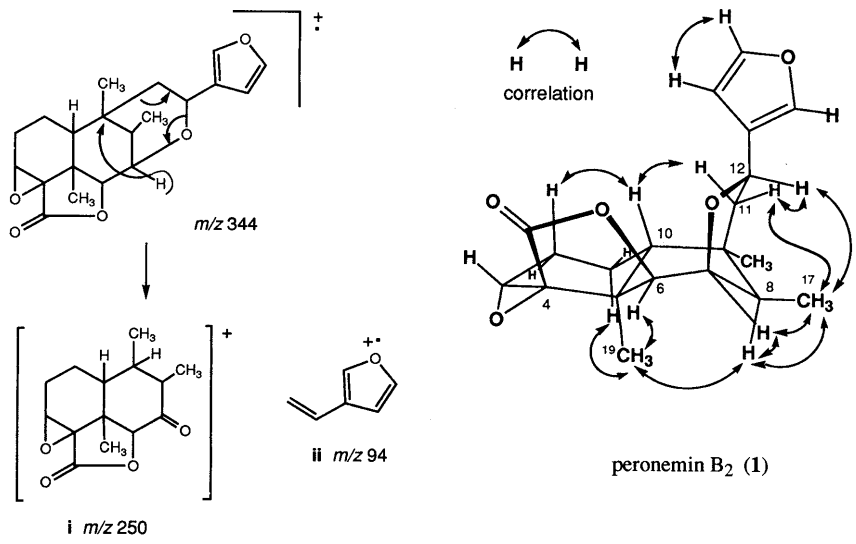


Fig. 3

TABLE I. ^{1}H-NMR Data for Peronemins B_2 (**1**), A_2 (**2**), B_1 (**3**), C_1 (**4**), B_3 (**5**), A_3 (**6**), and D_1 (**7**)

Proton(s)	1[a]	2[b]	3[b]	4[b]	5[b]	6[b]	7[a]
1	1.21 (m) 1.51 (dt-like, J=2.5, 11)	1.12 (m) 1.47 (dt-like, J=2.5, 11)	1.61 (m)	1.69 (m)	1.80 (m)	1.76 (m)	1.62 (m) 1.78 (m)
2	2.23 (dd, J=7, 16) 1.84 (dddd, J=4.5, 9.5, 9.5, 16)	2.19 (d, J=7.5, 11) 1.89 (dddd, J=4.5, 9.5, 9.5, 11)	2.49 (m)	2.49 (m)	2.56 (m)	2.56 (m)	2.33 (m)
3	3.39 (d, J=4.5)	3.41 (d, J=4.5)	6.64 (dd, J=2.5, 5.5)	6.65 (dd, J=3, 5.5)	6.69 (dd, J=4, 5)	6.61 (dd, J=3.5, 5)	6.66 (dd, J=2.5, 6)
6	4.39 (br s)	4.63 (d, J=4)	4.25 (d, J=4)	4.28 (br s)	4.31 (br s)	4.46 (d, J=3.5)	4.15 (d, J=4)
7	4.39 (br s)	4.66 (dd, J=4, 4)	4.28 (d, J=4)	4.28 (br s)	4.31 (br s)	4.50 (dd J=3.5, 4.5)	4.06 (d, J=4)
8	1.71 (q, J=7)	2.51 (dq, J=4, 7.5)	1.59 (q, J=7)	1.66 (m, J=7.5)	2.58 (q, J=7)	2.24 (dq, J=4.5, 7.5)	1.50 (q, J=7)
10	2.45 (dd, J=2.5, 13)	2.45 (dd, J=2.5, 13)	2.36 (t, J=10)	2.14 (t, J=10)	2.14 (t, J=10)	2.37 (m)	2.04 (dd, J=4, 10)
11	1.75 (d, J=8) 1.77 (d, J=8)	1.79 (d, J=8) 1.83 (d, J=8)	1.61 (m) 1.80 (m)	1.54 (dd, J=8, 13.5) 1.99 (dd, J=8, 13.5)	1.59 (m) 1.81 (m)	1.51 (m) 2.37 (m)	2.45 (dd, J=8, 15) 2.60 (dd, J=8, 15)
12	4.98 (t, J=8)	5.05 (t, J=8)	4.92 (t, J=8)	4.69 (t-like, J=8)	4.96 (t, J=8)	5.00 (br d, J=10)	7.01 (t, J=8)
14	6.26 (d, J=1)	6.24 (d, J=1)	6.20 (d, J=1.5)	7.16 (dd, J=1.5, 3)	6.33 (d, J=1.5)	6.36 (br s)	6.14 (d, J=3.5)
15	7.37 (dd, J=1, 2)	7.36 (t-like, J=1.5)	7.32 (dd, J=1.5, 1.5)	4.77 (m)	7.36 (dd, J=1.5, 1.5)	7.38 (br s)	6.97 (d, J=3.5)
16	7.30 (d, J=2)	7.30 (d, J=1.5)	7.24 (br s)	—	7.35 (d, J=1.5)	7.30 (br s)	—
17	1.32 (d, J=7)	1.15 (d, J=7.5)	1.29 (d, J=7)	1.28 (d, J=7.5)	1.03 (d, J=7)	0.95 (d, J=7.5)	1.20 (d, J=7)
19	1.18 (s)	1.24 (s)	1.18 (s)	1.19 (s)	1.21 (s)	1.26 (s)	1.19 (s)
20	0.97 (s)	0.95 (s)	1.00 (s)	1.00 (s)	0.99 (s)	0.99 (s)	1.10 (s)

a) At 500 MHz or *b*) at 270 MHz in $CDCl_3$, J values in Hz.

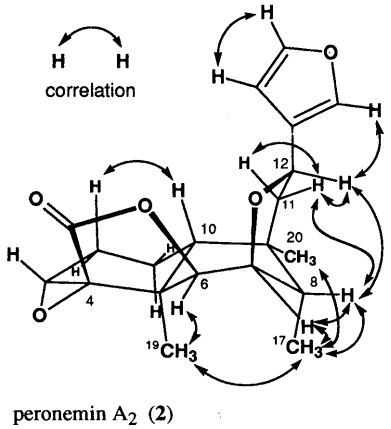


Fig. 4

B_2 (**1**). However, the ^{1}H-NMR spectrum of **3** lacked the signal due to an epoxide proton but instead showed an additional signal assignable to an olefinic proton at C-3 (δ 6.64, 1H, dd, J=2.5, 5.5 Hz) (Table I). Similarly, the ^{13}C-NMR spectrum of **3** showed the signals of olefinic carbons at C-3 and C-4, which were distinct from the signals of C-3 and C-4 of **1** (Table II). These findings led us to presume that **3** has a double bond at C-3 and C-4, in place of the 3,4-epoxide moiety of **1**.

In order to verify this presumption, peronemin B_1 (**3**) was treated in methanol with 35% aqueous hydrogen peroxide and 1 N aqueous sodium hydroxide solution to afford peronemin B_2 (**1**). Consequently, the relative stereostructure of peronemin B_1 is as shown in **3**.

Peronemins B_3 (5) and A_3 (6) The EI-MS of peronemin B_3 (**5**) and also that of peronemin A_3 (**6**) showed the molecular ion peak at m/z 328 of $C_{20}H_{24}O_4$, identical with that of peronemin B_1 (**3**). The IR and UV spectra of **5** and **6** also showed similar absorption patterns to those of **3**. Furthermore, in the ^{1}H- and ^{13}C-NMR spectra of both **5** and **6**, most of the proton and carbon signals were observed with similar chemical shifts to those of **3** (Tables

I and II). Consequently, it appears that **5** and **6** are both stereoisomers of **3**.

The relative stereostructures of peronemins B_3 (**5**) and A_3 (**6**) have been elucidated on the basis of the following NOE examinations. Thus, **5** showed NOE correlations (in $CDCl_3$) between the signals of 8-H and 6-H, 17-H_3, 19-H_3, 20-H_3; 10-H and 12-H; 19-H_3 and 6-H, 7-H, 8-H, 2-H_a; 7-H and 6-H, 17-H_3, 19-H_3 as shown in Fig. 5. The coupling constants observed for the signals of 7-H (d, $J=5.5$ Hz in benzene-d_6), 10-H (t, $J=10$ Hz), and 12-H (t, $J=8$ Hz) can be well explained if the conformation of **5** is as shown in Fig. 5. On the other hand, **6** showed NOE correlations (in benzene-d_6) between the signals of 17-H_3 and 6-H, 7-H, 8-H, 20-H_3; 10-H and 12-H, 2-H_a (δ 1.90, m); 19-H_3 and 6-H, 1-H as depicted in Fig. 5. The relative conformation of **6** depicted in Fig. 5 is further supported by the coupling constants (in benzene-d_6) observed for the signals of 7-H (δ 4.35, dd, $J=3.5$, 5.5 Hz), 10-H (δ 2.25, dd, $J=6$, 13 Hz), and 12-H (δ 4.80, dd-like, $J=2$, 9 Hz). Consequently, peronemin B_3 (**5**) is a C-12 stereoisomer of peronemin B_1 (**3**), while peronemin A_3 (**6**) is a C-8,12 stereoisomer of **3**.

TABLE II. ^{13}C-NMR Data for Peronemins B_2 (**1**), A_2 (**2**), B_1 (**3**), C_1 (**4**), B_3 (**5**), A_3 (**6**), and D_1 (**7**)

C No.	1	2	3	4	5	6	7
C-1	14.8	14.4	16.5	16.4	17.3	16.4	18.1
C-2	24.5	24.3	24.5	24.4	24.6	24.5	24.9
C-3	56.0	55.7	135.7	136.1	136.3	135.0	136.7
C-4	64.2	65.0	140.8	140.7	141.2	142.5	139.3
C-5	35.2	34.1	39.8	39.8	38.7	38.2	39.5
C-6	87.2	85.5	86.7	86.4	86.9	84.7	86.0
C-7	74.7	73.6	75.5	75.4	73.5	72.5	72.3
C-8	33.8	36.8	34.8	34.6	33.9	37.0	40.6
C-9	33.7	33.1	34.0	33.8	31.9	32.2	39.0
C-10	45.8	44.8	49.5	49.7	46.7	47.3	44.1
C-11	42.2	51.1	42.9	40.3	39.4	49.3	40.3
C-12	65.3	65.3	65.5	65.8	65.5	65.9	143.1
C-13	128.8	128.0	128.9	137.3	129.3	131.2	126.2
C-14	108.5	108.5	108.4	143.7	108.6	109.1	105.1
C-15	143.4	143.4	143.2	70.8	143.1	143.2	145.4
C-16	138.8	138.5	138.3	172.1	138.6	137.8	167.8
C-17	16.0	11.9	16.1	15.8	14.0	12.2	14.7
C-18	173.2	173.0	168.9	168.9	168.9	168.9	169.0
C-19	19.2	18.9	22.9	22.7	22.7	22.9	23.4
C-20	20.8	21.1	21.0	20.9	21.7	21.0	22.2

At 67.5 MHz in $CDCl_3$.

Peronemin C_1 (4) The EI-MS of peronemin C_1 (**4**) exhibited the molecular ion peak at *m/z* 344, the composition of which was $C_{20}H_{24}O_5$ by high-resolution EI-MS analysis. The IR and UV spectra of **4** showed strong absorptions of an α,β-unsaturated γ-lactone moiety [1760, 1660 cm^{-1}; 205 nm ($\varepsilon=17000$)]. The clerodane structure of **4** was deduced from the similarities of the ^{1}H- and ^{13}C-NMR spectra of **4** and those of peronemin B_1 (**3**). Thus, the ^{1}H-NMR spectrum of **4** was very similar to that of **3**, except for the signals assigned to the protons in the furan moiety of **3**. In the case of **4**, those furanoid proton signals were not observed but the corresponding signals were observed at δ 7.16 (1H, dd, $J=1.5$, 3 Hz) and at δ 4.77 (2H, m), which were respectively assignable to an olefinic proton and methylene protons in an α-substituted butenolide ring of **4**. In a benzene-d_6 solution of **4**, long-range couplings between 12-H and 15-H_2 were observed [*e.g.* δ 4.56 (ddt-like, $J=1.5$, 3, 8 Hz, 12-H), 3.72 (ddd, $J=1.5$, 1.5, 18 Hz, 15-H_a), and 3.85 (ddd, $J=1.5$, 3, 18 Hz, 15-H_b)]. Thus, **4** possesses the same relative stereostructure as that of **3**. Detailed examinations of the COLOC and NOESY spectra of **4** have led us to conclude that peronemin C_1 (**4**) is a C-16 oxygenated analogue of peronemin B_1 (**3**).[3]

Peronemin D_1 (7) Peronemin D_1 (**7**) was shown to have the same molecular formula, $C_{20}H_{24}O_5$, as that of peronemin C_1 (**4**) by the EI-MS and the high resolution EI-MS analyses. The clerodane structure of **7**, related to the structures of the above-elucidated peronemins (**1**—**6**), was presumed from the close similarity of its ^{1}H- and ^{13}C-NMR data to those for **1**—**6** (Tables I and II). Thus, the H–H COSY experiments on **7** showed the presence of four partial structures: from C-10 to C-3; from C-6 to C-17; from C-11 to C-12; from C-14 to C-15. Furthermore, NOE correlations were observed between the signals of 8-H and 6-H, 19-H_3. Consequently, the relative stereostructure of peronemin D_1 is as illustrated in **7**. The conclusive evidence to determine the geometry at C-12 of **7** has not yet been provided.

Absolute Configurations of Peronemins Finally, the absolute configurations of the above-described clerodane-type diterpenoids (**1**—**7**) have been determined from the chirality of the intrinsic α,β-unsaturated γ-lactone moiety.[4] Thus, the circular dichroism (CD) spectra of

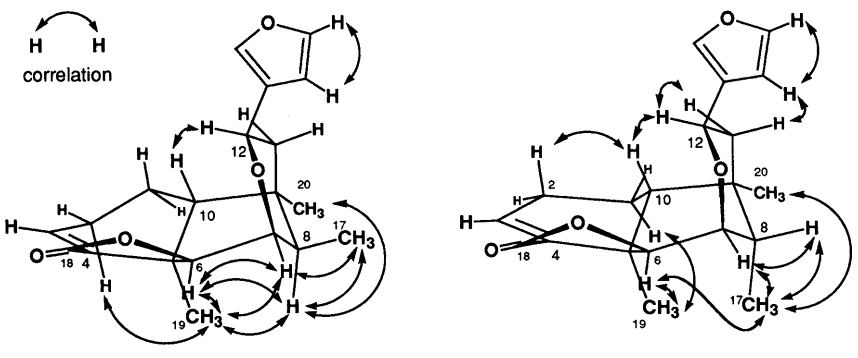


Fig. 5

peronemins B_1 (**3**), C_1 (**4**), B_3 (**5**), A_3 (**6**), and D_1 (**7**) each showed a positive maximum ($[\theta]_{246}$ +31000 for **3**, $[\theta]_{247}$ +35000 for **4**, $[\theta]_{248}$ +39000 for **5**, $[\theta]_{250}$ +34000 for **6**, and $[\theta]_{240}$ +39000 for **7**), which substantiated their absolute configurations as shown.

In conclusion, we have isolated seven new clerodane-type diterpenoids, named peronemins A_2 (**2**), A_3 (**6**), B_1 (**3**), B_2 (**1**), B_3 (**5**), C_1 (**4**), and D_1 (**7**), from the leaves of *Peronema canescens*, an Indonesian medicinal plant belonging to Verbenaceae. Among these diterpenoids, we have submitted **1**, **2**, **3**, **4**, and **6** to examinations of *in vitro* anti-malarial activity. Among these compounds, **4** and **6** were found to exhibit some inhibitory activity (IC_{50} 13.1 μM for **4** and 83% inhibition at 118 μM for **6**) against the proliferation of the malarial pathogen *Plasmodium falciparum* (K1 strain; a chloroquine-resistant strain in human erythrocytes).[2h,5] Further examination will be needed to identify the major constituents responsible for the anti-malarial use of the leaves of "sungkei."

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are recorded as read. The UV spectra were obtained with a Hitachi 330 spectrophotometer, and the IR spectra were taken with Hitachi 260-30 and JASCO FT/IR-5300 spectrophotometers. The EI-MS were taken on a JEOL JMS-D300 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL GX-500 spectrometer (500 MHz) or a JEOL EX-270 spectrometer (270 MHz). For HPLC, a Shimadzu LC-6A chromatograph was used. Column chromatography was carried out by using Kieselgel 60 (70—230 mesh, Merck) as the adsorbent. Thin-layer chromatography (TLC) was conducted on precoated Kieselgel 60 F_{254} plates (0.2 mm, Merck). Spots on the TLC plates were detected by spraying 1% $Ce(SO_4)_2$/10% H_2SO_4 (followed by heating at 110 °C) or the Ehrlich reagent.

Isolation of Peronemins B_2 (1), A_2 (2), B_1 (3), B_3 (5), A_3 (6), C_1 (4), and D_1 (7) The air-dried leaves (1 kg) of *Peronema canescens* JACK. (Verbenaceae) which were collected in the Curup area of Bengkulu, Sumatera Island, Indonesia in August 1990, were extracted with acetone under reflux and the solvent was evaporated off under reduced pressure to give the acetone extract (60 g, 6.0% from the dried leaves). The residual leaves were further extracted with methanol under reflux to give the methanol extract (37 g). The acetone extract (30 g) was then subjected to column chromatography [SiO_2 2 kg, elution with *n*-hexane–EtOAc (10 : 1→1 : 1), EtOAc, and acetone successively] to provide frs. 1—22 (9.2 g), fr. 23 (2.1 g), fr. 24—26 (1.72 g), fr. 27 (1.0 g), fr. 28 (2.53 g) and fr. 29 (1.77 g).

Fraction 23 (200 mg) was then separated by HPLC [YMC-Pack SIL, *n*-hexane–EtOAc (4 : 1)] to afford peronemins B_2 (**1**, 19 mg, 0.04%), A_2 (**2**, 3 mg, 0.005%), B_1 (**3**, 5 mg, 0.01%), A_3 (**6**, 5 mg, 0.01%) and B_3 (**5**, 15 mg, 0.03%), in the order of elution. Fraction 27 (0.5 g) was also separated by HPLC [Zorbax SIL, *n*-hexane–$CHCl_3$–isopropanol (8 : 1 : 1)] to furnish peronemin C_1 (**4**, 100 mg, 0.04%) and peronemin D_1 (**7**, 8 mg, 0.003%). Separation of fr. 15 (400 mg) by column chromatography [SiO_2, *n*-hexane– AcOEt (2 : 1)] furnished β-amyrin (225 mg, 0.05%), while fr. 17 (190 mg) and fr. 18 (290 mg) were separated by HPLC [Zorbax SIL, *n*-hexane–AcOEt (8 : 1)] to afford phytol (85 mg, 0.02%) and β-sitosterol (160 mg, 0.03%), respectively.

Peronemin B_2 (**1**): Colorless needles, mp 194—195 °C (*n*-hexane–EtOAc), $[\alpha]_D$ −34° (c=0.14, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1450, 1250, 930, 875. UV λ_{max}^{MeOH} nm (ε): 204 (2700). ^{1}H-NMR: as given in Table I (270 MHz, benzene-d_6), δ: 7.49 (1H, br s, 16-H), 7.09 (1H, dd, J=1.5, 1.5 Hz, 15-H), 6.20 (1H, d, J=1.5 Hz, 14-H), 4.70 (1H, t, J=8 Hz, 12-H), 3.98 (1H, d, J=4 Hz, 7-H), 3.70 (1H, d, J=4 Hz, 6-H), 3.16 (1H, d, J=2.5 Hz, 3-H), 2.29 (1H, dd, J=2.5, 13 Hz, 10-H), 0.77 (3H, d, J=7 Hz, 17-H_3), 0.74 (3H, s, 19-H_3), 0.48 (3H, s, 20-H_3). ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 344 (M^+, 1), 250 (15), 95 (32), 94 (100). High-resolution EI-MS m/z: Found : 344.1613. Calcd for $C_{20}H_{24}O_5$: 344.1621. CD (c=0.017, MeOH, 20 °C): $[\theta]_{260}$ 0, $[\theta]_{230}$ −20000 (neg. max.), $[\theta]_{214}$ 0.

Peronemin A_2 (**2**): Colorless needles, mp 227—228 °C (MeOH–ether), $[\alpha]_D$ −40° (c=0.12, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1470, 1250, 920, 870. UV λ_{max}^{MeOH} nm (ε): 207 (3200). ^{1}H-NMR: as given in Table I. ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 344 (M^+, 0.4), 250 (13), 95 (15), 94 (100). High-resolution EI-MS m/z: Found: 344.1603. Calcd for $C_{20}H_{24}O_5$: 344.1621. CD (c=0.014, MeOH, 20 °C): $[\theta]_{255}$ 0, $[\theta]_{230}$ −15000 (neg. max.), $[\theta]_{215}$ 0.

Peronemin B_1 (**3**): Colorless needles, mp 172—173 °C (MeOH–ether), $[\alpha]_D$ +36° (c=1.13, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1660, 1450, 910, 870. UV λ_{max}^{MeOH} nm (ε): 213 (14500). ^{1}H-NMR: as given in Table I. ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 328 (M^+, 4), 234 (100), 94 (95). High-resolution EI-MS m/z: Found: 328.1695. Calcd for $C_{20}H_{24}O_4$: 328.1674. CD (c=0.013, MeOH, 20 °C): $[\theta]_{273}$ 0, $[\theta]_{246}$ +31000 (pos. max.), $[\theta]_{230}$ +23000 (pos. min.), $[\theta]_{208}$ +45000 (pos. max.).

Peronemin B_3 (**5**): Colorless needles, mp 151—152 °C (*n*-hexane–EtOAc), $[\alpha]_D$ +98° (c=0.4, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1760, 1660, 1450, 910, 870. UV λ_{max}^{MeOH} nm (ε): 215 (13800). ^{1}H-NMR: as given in Table I (270 MHz, benzene-d_6) δ: 7.14 (2H, br s, 15,16-H), 6.53 (1H, dd, J=3, 6 Hz, 3-H), 6.22 (1H, br s, 14-H), 4.82 (1H, t, J=8 Hz, 12-H), 4.07 (1H, d, J=5.5 Hz, 7-H), 3.78 (1H, d, J=5.5 Hz, 6-H), 0.75 (3H, d, J=7 Hz, 17-H_3), 0.67, 0.60 (both 3H, s, 19,20-H_3). ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 328 (M^+, 30), 234 (50), 94 (68). High-resolution EI-MS m/z: Found: 328.1666. Calcd for $C_{20}H_{24}O_4$: 328.1674. CD (c=0.02, MeOH, 20 °C): $[\theta]_{290}$ 0, $[\theta]_{248}$ +39000 (pos. max.), $[\theta]_{230}$ +29000 (pos. min.), $[\theta]_{214}$ +32000 (pos. max.).

Peronemin A_3 (**6**): Colorless needles, mp 142—143 °C (MeOH–ether), $[\alpha]_D$ +35° (c=0.91, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750, 1660, 1450, 900, 870. UV λ_{max}^{MeOH} nm (ε): 214 (17800). ^{1}H-NMR: as given in Table I (270 MHz, benzene-d_6) δ: 7.12 (2H, br s, 15,16-H), 6.45 (1H, dd, J=3, 6 Hz, 3-H), 6.22 (1H, br s, 14-H), 4.80 (1H, dd-like, J=2, 9 Hz, 12-H), 4.35 (1H, dd, J=3.5, 5.5 Hz, 7-H), 4.09 (1H, d, J=3.5 Hz, 6-H), 2.25 (1H, dd, J=6, 13 Hz, 10-H), 0.78, 0.59 (both 3H, s, 19,20-H_3), 0.43 (3H, d, J=7.5 Hz, 17-H_3). ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 328 (M^+, 2), 234 (55), 94 (100). High-resolution EI-MS m/z: Found: 328.1674. Calcd for $C_{20}H_{24}O_4$: 328.1674. CD (c=0.008, MeOH, 20 °C): $[\theta]_{285}$ 0, $[\theta]_{250}$ +34000 (pos. max.), $[\theta]_{228}$ +13000 (pos. min.), $[\theta]_{208}$ +34000 (pos. max.).

Peronemin C_1 (**4**): Colorless needles, mp 186—187 °C (*n*-hexane–EtOAc), $[\alpha]_D$ +4° (c=1.52, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 1760, 1660, 1450, 880. UV λ_{max}^{MeOH} nm (ε): 205 (17000). ^{1}H-NMR ($CDCl_3$): as given in Table I (270 MHz, benzene-d_6) δ: 6.56 (1H, overlapped, 3-H), 6.54 (1H, overlapped, 14-H), 4.56 (1H, ddt-like, J=1.5, 3, 8 Hz, 12-H), 3.94 (1H, d, J=4 Hz, 7-H), 3.85 (1H, ddd, J=1.5, 3, 18 Hz, 15-H_b), 3.76 (1H, d, J=4 Hz, 6-H), 3.72 (1H, ddd, J=1.5, 1.5, 18 Hz, 15-H_a), 0.79 (3H, d, J=7 Hz, 17-H_3), 0.64, 0.59 (both 3H, s, 19,20-H_3). ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 344 (M^+, 18), 261 (1), 91 (100), 83 (25). High-resolution EI-MS m/z: Found: 344.1624. Calcd for $C_{20}H_{24}O_5$: 344.1622. CD (c=0.016, MeOH, 20 °C): $[\theta]_{283}$ 0, $[\theta]_{247}$ +35000 (pos. max.), $[\theta]_{222}$ +22000 (pos. min.), $[\theta]_{208}$ +28000 (pos. max.).

Peronemin D_1 (**7**): Colorless needles, mp 231—232 °C (*n*-hexane–EtOAc), $[\alpha]_D$ −13° (c=0.20, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3450, 1760, 1670, 980. UV λ_{max}^{MeOH} nm (ε): 214 (9600), 300 (4000). ^{1}H-NMR: as given in Table I. ^{13}C-NMR: as given in Table II. EI-MS m/z (%): 344 (M^+, 3), 215 (3), 109 (11), 83 (100). High-resolution EI-MS m/z: Found: 344.1617. Calcd for $C_{20}H_{24}O_5$: 344.1622. CD (c=0.0023, MeOH, 20 °C): $[\theta]_{310}$ 0, $[\theta]_{240}$ +39000 (pos. max.), $[\theta]_{223}$ 0.

Epoxidation of Peronemin B_1 (3) Giving Peronemin B_2 (1) A solution of **3** (5 mg) in MeOH (2 ml) was treated with 35% aqueous H_2O_2 (7 μl) and 1 N aqueous NaOH (5 μl) and the mixture was stirred at 15 °C for 10 min. After addition of water (2 ml) to the reaction mixture, the whole was extracted with ether and the ether solution was taken and dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was purified by column chromatography [SiO_2, benzene–EtOAc (4 : 1)] to yield **1** (1.5 mg), which was shown to be identical with an authentic sample by $[\alpha]_D$, IR, and ^{1}H-NMR data comparisons.

Acknowledgement The authors are grateful to Drs. Tahan Uji and Dr. Harry Wiriadinata, Herbarium Bogoriense, Research and Development Centre for Biologi-LIPI, Indonesia for collection and identification of the plant material, and to Dr. Masatsugu Kimura, Osaka City University for the bioassay of the anti-malarial activity. This work was financially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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