New Sesquiterpene Hydroperoxides with Trypanocidal Activity from *Pogostemon cablin*

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Trypanocidal constituents of *Pogostemon cablin* were investigated. Activity guided isolation of the acetone extract resulted in isolation of three new sesquiterpene hydroperoxides 1—3, together with a known sesquiterpene, patchouli alcohol (4). *In vitro* minimum lethal concentrations of the hydroperoxides 1—3 against epimastigotes of *Trypanosoma cruzi* were 0.84 μM (1), 1.7 μM (2) and 1.7 μM (3). The activity of the corresponding alcohols and patchouli alcohol was very weak (MLC>200 μM).

Key words *Pogostemon cablin*; Labiatae; sesquiterpene hydroperoxide; trypanocidal constitutent; *Trypanosoma cruzi*

Pogostemon cablin (BLANCO) BENTH. (Labiatae) is a perennial herb, which has been used as stomachic for indigestion, vomiting and diarrhea, and also for headache and fever in Asian countries.[1)] In our search for anti-trypanosomal compounds from natural medicines used in Vietnam,[2)] an acetone extract of this plant showed potent trypanocidal activity against the epimastigotes of *Trypanosoma cruzi*, the etiologic agent of American trypanosomiasis (Chaga's disease).[3)] In this paper, we report the isolation and characterization of three new sesquiterpene hydroperoxides (**1**—**3**) together with a known sesquiterpene, patchouli alcohol (**4**), as the trypanocidal constituents of this plant.

Results and Discussion

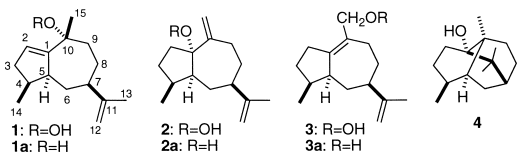
Dried herb of *P. cablin* was extracted with acetone under reflux and the extract, which showed strong *in vitro* trypanocidal activity,[2)] was separated by repeated column chromatography under the guidance of trypanocidal activity to give compounds **1**—**3** as the main trypanocidal constituents together with a known sesquiterpene which was identified with patchouli alcohol (**4**)[4)] by comparison of the spectral data to those reported.

Compound **1** was obtained as a colorless oil. The molecular formula was determined as $C_{15}H_{24}O_2$ by high-resolution chemical ionization mass spectrum (HR-CI-MS *m/z*: 237.1862 $[M+H]^+$, Calcd for $C_{15}H_{25}O_2$: 237.1854). The ^{1}H-NMR spectrum showed the presence of three methyls (δ_H 0.87, d, J=7.0 Hz; δ_H 1.40, s; δ_H 1.60, s), an *exo*-methylene (δ_H 4.72 and 4.73, each br s) and an olefine (δ_H 5.65, br s). The ^{13}C-NMR spectrum showed that it had fifteen carbons with two double bonds (δ_C 151.4, 151.2, 129.7, 108.9). These data indicated that **1** is a sesquiterpene with two double bonds and two ring structures. As the ^{13}C-NMR spectrum showed only one oxygenated carbon signal and the chemical shift (δ_C 84.2) was relatively large, the oxygen function was estimated to be a hydroperoxy group. This was confirmed by a reduction of **1** with triphenylphosphine. Treatment of **1** with triphenylphosphine gave a sesquiterpene alcohol (**1a**), whose ^{1}H- and ^{13}C-NMR spectra, together with its optical rotation, were in good agreement with those of a known sesquiterpene alcohol, (−)-guaia-1,11-dien-10α-ol.[5)] Thus the structure of **1** was concluded to be 10α-hydroperoxy-guaia-1,11-diene.

Compound **2** was obtained as a colorless oil. This compound has the same molecular formula ($C_{15}H_{24}O_2$) with that of **1** (HR-CI-MS *m/z*: 237.1851 $[M+H]^+$, Calcd for $C_{15}H_{25}O_2$: 237.1854). The ^{1}H-NMR spectrum showed the presence of two methyls (δ_H 0.76, d, J=7.4 Hz; δ_H 1.60, s) and two *exo*-methylenes (δ_H 4.70 and 4.73; 4.96 and 4.97, each br s). The ^{13}C-NMR spectrum showed that it had fifteen carbons with two double bonds (δ_C 151.6, 151.2, 115.6, 108.8). These data indicated that **2** is also a sesquiterpene with two double bonds and two ring structures. Since the ^{13}C-NMR spectrum showed only one oxygenated carbon signal and its chemical shift (δ_C 99.2) was very large, it was concluded to be a sesquiterpene hydroperoxide. Treatment of **2** with triphenylphosphine confirmed this conclusion giving a sesquiterpene alcohol (**2a**), whose ^{1}H- and ^{13}C-NMR spectra, together with its optical rotation, were in good agreement with those of a known sesquiterpene alcohol, (+)-guaia-10(15),11-dien-1α-ol.[5)] Thus the structure of **2** was concluded to be 1α-hydroperoxy-guaia-10(15),11-diene. A small amount of **2a** was also obtained from the extract.

Compound **3** was obtained as a colorless oil. This compound also has the molecular formula of $C_{15}H_{24}O_2$ (HR-CI-MS *m/z*: 237.1860 $[M+H]^+$, Calcd for $C_{15}H_{25}O_2$: 237.1854). The ^{1}H- and ^{13}C-NMR spectra indicated that it is a sesquiterpene hydroperoxide with two double bonds and two ring structures. Treatment of **3** with triphenylphosphine gave a sesquiterpene alcohol (**3a**), whose ^{1}H- and ^{13}C-NMR spectra, together with its optical rotation, were in good agreement with those of a known sesquiterpene alcohol, (−)-guaia-1(10),11-dien-15α-ol.[6)] Thus the structure of **3** was concluded to be 15α-hydroperoxy-guaia-1(10),11-diene.

The minimum lethal concentrations (MLCs) of the hydroperoxides **1**—**3** against epimastigotes of *Trypanosoma cruzi* were 0.84 μM (**1**), 1.7 μM (**2**), 1.7 μM (**3**). We have reported the isolation and trypanocidal activity of monoterpene hydroperoxides from *Laurus nobilis*[7)] and *Chenopodium ambrosioides*.[8)] In these cases, the corresponding monoterpene



1: R=OH **1a**: R=H **2**: R=OH **2a**: R=H **3**: R=OH **3a**: R=H **4**

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alcohols did not show trypanocidal activity. In agreement with these results, the activity of the alcohols **1a**—**3a** and patchouli alcohol (**4**) were very weak (MLC>200 μM). Thus the trypanocidal activity of the sesquiterpene hydroperoxides is ascribable to the hydroperoxy function. Hydroperoxides are unstable to steam-distillation,[8] and it may be one of the reasons that hydroperoxides **1**—**3** have not been found in the essential oils of this plant.

Experimental

General Experimental Procedures Optical rotations were determined on a JASCO DIP-370 polarimeter. IR spectra were measured on a Shimadzu FTIR-8700 spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-LA500 spectrometer with tetramethylsilane (TMS) as an internal standard, and chemical shifts are given in δ values. Mass spectra were measured on a JEOL JMS-HX/HX110A spectrometer. Gel permiation chromatography (GPC) was performed on JAI-508 system with JAIGEL-H1 and H2 columns.

Plant Materials Dried herb of *P. calbin* (hoac huong) was purchased from a market in Ho Chi Min city in June 2000. A voucher specimen (ESM-C225) was deposited at the Experimental Station of Medicinal Plants, Graduate School of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation Dried herb of *P. calbin* (2 kg) was extracted with acetone under reflux (3 h×3 times) and the extract was concentrated under reduced pressure to give 149.9 g of residue. A part of the residue (20 g) was applied to a silica gel column and eluted with hexane–AcOEt (19 : 1, 1 : 1) and MeOH to give eight fractions (fraction, weight (g), MLC (mg/ml): fr. 1, 4.90, 100; fr. 2, 0.28, 50; fr. 3, 0.21, 3.13; fr. 4, 4.42, 1.56; fr. 5, 0.59, 0.78; fr. 6, 0.97, 12.5; fr. 7, 5.01, 25; fr. 8, 3.40, >100). Fraction 5 was fractionated by silica gel column chromatography (CC) with hexane–acetone (8 : 1) to give seven fractions (fraction, weight (mg), MLC (μg/ml): fr. 5-1, 40, 25; fr. 5-2, 90, 50; fr. 5-3, 24, 12.5; fr. 5-4, 68, 0.78; fr. 5-5, 234, 0.20; fr. 5-6, 97, 1.56; fr. 5-7, 52, 12.5). The most active fraction (fr. 5-5) was purified with benzene–diethylether (30 : 1) to give compound **1** (64 mg). Fraction 5-4 was separated with Lobar® Si-60 (benzene–AcOEt=39 : 1) and Lobar® RP-18 (85% MeOH) column chromatography to give compound **2a** (10 mg). A fraction corresponding to fr. 4, which was prepared from 40 g of the extract, was separated by silica gel CC with benzene–diethylether (30 : 1) to give four fractions (fr. 4-1, 0.24 g; fr. 4-2, 0.57 g; fr. 4-3, 2.60 g; fr. 4-4, 2.08 g). Fraction 4-3 and fr. 4-4 contained patchouli alcohol (**4**) as the major constituent. The most active fraction (fr. 4-2, MLC 0.39 μg/ml) was separated by Lobar® Si-60 CC (benzene) and further fractionation of the major fraction (187 mg) by GPC (benzene) gave four fractions (fr. 4-2-1, 42 mg; fr. 4-2-2, 65 mg; fr. 4-2-3, 34 mg; fr. 4-2-4, 26 mg). Purification of a part of fr. 4-2-2 (30 mg) with Lobar® Si-60 CC (hexane–acetone=8 : 1) gave compound **3** (6 mg). Repeated separation of fr. 4-2-4 with Lobar® Si-60 CC (hexane–acetone=10 : 1 and 20 : 1) gave compound **2** (12 mg).

Compound **1**: Colorless oil, $[\alpha]_D$ +24.5° (c=0.35, EtOH). IR (KBr) cm^{-1}: 3325, 2931, 2874, 1709, 1643, 1447, 1373. HR-CI-MS *m/z*: 237.1862 ([M+H]$^+$, Calcd for $C_{15}H_{25}O_2$: 237.1854). CI-MS *m/z* (%): 237 ([M+H]$^+$, 36), 219 (60), 203 (100). ^{1}H-NMR (C_6D_6, 500 MHz) δ: 6.84 (1H, br s, OOH), 5.65 (1H, br s, H-2), 4.73 and 4.72 (each 1H, br s, H-12), 2.47 (1H, m, H-5), 2.17 (1H, m, H-4), 2.11 (1H, ddd, J=15.6, 7.7, 2.8 Hz, H-3), 1.92 (1H, br t, J=11.6 Hz, H-7), 1.86—1.79 (3H, m, H-3,9), 1.72 (1H, br d, J=12.5 Hz, H-6), 1.62 (1H, m, H-8), 1.60 (3H, s, H-13), 1.40 (3H, s, H-15), 1.12 (1H, br q, J=11.6 Hz, H-8), 1.03 (1H, q, J=12.5 Hz, H-6), 0.87 (3H, d, J=7.0 Hz, H-14). ^{13}C-NMR (C_6D_6, 125 MHz) δ: 151.4 (C-11), 151.2 (C-1), 129.7 (C-2), 108.9 (C-12), 84.2 (C-10), 50.1 (C-7), 47.1 (C-5), 39.3 (C-4), 38.4 (C-3), 37.5 (C-9), 35.3 (C-6), 29.6 (C-8), 23.1 (C-15), 20.7 (C-13), 15.4 (C-14). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.13 (1H, br s, OOH), 5.89 (1H, br s), 4.67 and 4.64 (each 1H, br s), 2.56 (1H, m), 2.37 (2H, m), 2.02 (2H, m), 1.87—1.72 (4H, m), 1.70 (3H, s), 1.47 (3H, s), 1.19 (1H, br q, J=11.3 Hz), 1.04 (1H, q, J=12.8 Hz), 1.00 (3H, d, J=6.7 Hz). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 151.5 (C-11), 150.5 (C-1), 130.5 (C-2), 108.5 (C-12), 84.8 (C-10), 49.9 (C-7), 46.8 (C-5), 39.3 (C-4), 38.3 (C-3), 37.1 (C-9), 35.0 (C-6), 29.2 (C-8), 22.7 (C-15), 20.7 (C-13), 15.4 (C-14).

Reduction of 1 with Triphenylphosphine Compound **1** (11 mg) dissolved in diethylether (5 ml) was treated with triphenylphosphine at room temperature for 15 min. The mixture was concentrated and the residue was separated on silica gel (benzene–diethylether=30 : 1) to give **1a** (9 mg).

(−)-Guaia-1,11-dien-10α-ol (**1a**): White solid, mp 117—118 °C (lit.[5] 118.5 °C), $[\alpha]_D$ −66.1° (c=0.40, $CHCl_3$) (lit.[5] −79.2°, c=0.71, $CHCl_3$).

Compound **2**: Colorless oil, $[\alpha]_D$ +71.8° (c=0.58, EtOH). IR (KBr) cm^{-1}: 3418, 2932, 2870, 1643, 1450, 1377. HR-CI-MS *m/z*: 237.1851 ([M+H]$^+$, Calcd for $C_{15}H_{25}O_2$: 237.1854). CI-MS *m/z* (%): 237 ([M+H]$^+$, 18), 219 (61), 203 (100). ^{1}H-NMR (C_6D_6, 500 MHz) δ: 6.71 (1H, br s, OOH), 4.97 and 4.96 (each 1H, br s, H-15), 4.73 and 4.70 (each 1H, br s, H-12), 2.36 (1H, m, H-4), 2.33 (1H, dd, J=13.0, 2.0 Hz, H-9), 2.25 (1H, m, H-2), 2.14 (1H, ddd, J=13.0, 5.1, 2.9 Hz, H-9), 1.97 (1H, br t, J=11.1 Hz, H-7), 1.90—1.83 (4H, m, H-3,5,6,8), 1.60 (3H, s, H-13), 1.57 (1H, br d, J=14.7 Hz, H-2), 1.30 (1H, qd, J=12.5, 2.9 Hz, H-8), 1.18 (1H, m, H-3), 1.09 (1H, q, J=11.3 Hz, H-6), 0.76 (3H, d, J=7.4 Hz, H-14). ^{13}C-NMR (C_6D_6, 125 MHz) δ: 151.6 (C-11), 151.2 (C-10), 115.6 (C-15), 108.8 (C-12), 99.2 (C-1), 51.1 (C-5), 49.9 (C-7), 38.2 (C-8), 36.6 (C-4), 33.3 (C-2), 31.7 and 31.4 (C-3,9), 29.8 (C-6), 20.7 (C-13), 15.9 (C-14).

Reduction of 2 with Triphenylphosphine Compound **2** (7 mg) dissolved in diethylether (1.5 ml) was treated with triphenylphosphine at room temperature for 15 min. The mixture was concentrated and the residue was separated on silica gel (benzene–diethylether=30 : 1) to give **2a** (4 mg).

(+)-Guaia-10(15),11-dien-1α-ol (**2a**): White solid, mp 64—65 °C (lit.[5] 69.5 °C), $[\alpha]_D$ +67.6° (c=0.23, $CHCl_3$) (lit.[5] +67.1°, c=0.73, $CHCl_3$).

Compound **3**: Colorless oil, $[\alpha]_D$ +7.3° (c=0.23, EtOH). IR (KBr) cm^{-1}: 3356, 2928, 2870, 1439, 1342. HR-CI-MS *m/z* 237.1860 ([M+H]$^+$, Calcd for $C_{15}H_{25}O_2$: 237.1854). CI-MS *m/z* (%): 237 ([M+H]$^+$, 3), 219 (100), 203 (64). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.86 (1H, s, OOH), 4.65 and 4.64 (each 1H, br s, H-12), 4.51 and 4.48 (each 1H, d, J=10.7 Hz, H-15), 2.55 (1H, m, H-5), 2.50 (1H, m, H-2), 2.36—2.25 (2H, m, H-2,9), 2.22 (1H, br d, J=12.0 Hz, H-9), 2.18—2.11 (2H, m, H-4,7), 1.78—1.67 (2H, m, H-3,8), 1.70 (3H, s, H-13), 1.65 (1H, m, H-6), 1.39 (1H, m, H-3), 1.23 (1H, br q, J=12.0 Hz, H-8), 1.05 (1H, q, J=12.2 Hz, H-6), 0.92 (3H, d, J=7.1 Hz, H-14). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 152.4 (C-1), 152.0 (C-11), 127.4 (C-10), 108.3 (C-12), 80.0 (C-15), 50.9 (C-7), 46.7 (C-5), 38.2 (C-4), 33.0 (C-3), 32.2 (C-8), 32.0 (C-6), 31.1 (C-9), 29.9 (C-2), 20.8 (C-13), 15.3 (C-14).

Reduction of 3 with Triphenylphosphine Compound **3** (9 mg) dissolved in benzene (5 ml) was treated with triphenylphosphine at room temperature for 8 h. The mixture was concentrated and the residue was separated on silica gel (benzene–diethylether=19 : 1) to give **3a** (4 mg).

(−)-Guaia-1(10),11-dien-15α-ol (**3a**): Colorless oil, $[\alpha]_D$ −8.3° (c=0.15, $CHCl_3$) (lit.[6] −11.6°, c=1.0, $CHCl_3$).

Patchouli Alcohol (**4**): White solid, mp 37—38 °C (lit.[7] colorless needles, mp 54—56 °C), $[\alpha]_D$ −119° (c=1.0, $CHCl_3$) (lit.[7] −124°, c=0.22, $CHCl_3$).

Trypanocidal Assay[8] Epimastigotes of *Trypanosoma cruzi* (Tulahuen strain) were kept in GIT medium (Wako) supplemented with hemin (12.4 μM, Wako). The epimastigotes in GIT medium (10 μl) were incubated with a test sample dissolved in EtOH (5 μl) and autoclaved saline (185 μl). After 24 h of incubation, the movement of epimastigotes was observed under a microscope (×100). Each assay was performed in duplicate. Gentian violet, which is used to disinfect trypanosomes from transfusion blood in Latin America, is used as a positive control. MLC of gentian violet under this assay condition was 6.3 μM.

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