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 constituent; *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 $\text{C}_{15}\text{H}_{24}\text{O}_2$ by high-resolution chemical ionization mass spectrum (HR-CI-MS m/z : 237.1862 $[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_2$: 237.1854). The ^1H -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 ^1H - 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-10 α -ol.⁵⁾ Thus the structure of 1 was concluded to be 10 α -hydroperoxy-guaia-1,11-diene.

Compound 2 was obtained as a colorless oil. This compound has the same molecular formula ($\text{C}_{15}\text{H}_{24}\text{O}_2$) with that of 1 (HR-CI-MS m/z : 237.1851 $[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_2$: 237.1854). The ^1H -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 ^1H - 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-1 α -ol.⁵⁾ Thus the structure of 2 was concluded to be 1 α -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 $\text{C}_{15}\text{H}_{24}\text{O}_2$ (HR-CI-MS m/z : 237.1860 $[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_2$: 237.1854). The ^1H - 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 ^1H - 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-15 α -ol.⁶⁾ Thus the structure of 3 was concluded to be 15 α -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

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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 μ M). 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. ¹H- and ¹³C-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 permeation 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 $\times$ 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 (μ g/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 μ g/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, $[\alpha]_D^{25} +24.5^\circ$ ($c=0.35$, EtOH). IR (KBr) cm^{-1} : 3325, 2931, 2874, 1709, 1643, 1447, 1373. HR-CI-MS m/z : 237.1862 ($[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_2$: 237.1854). CI-MS m/z (%): 237 ($[\text{M}+\text{H}]^+$, 36), 219 (60), 203 (100). ¹H-NMR (C_6D_6 , 500 MHz) δ : 6.84 (1H, brs, OOH), 5.65 (1H, brs, H-2), 4.73 and 4.72 (each 1H, brs, 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, brt, $J=11.6$ Hz, H-7), 1.86–1.79 (3H, m, H-3,9), 1.72 (1H, brd, $J=12.5$ Hz, H-6), 1.62 (1H, m, H-8), 1.60 (3H, s, H-13), 1.40 (3H, s, H-15), 1.12 (1H, brq, $J=11.6$ Hz, H-8), 1.03 (1H, q, $J=12.5$ Hz, H-6), 0.87 (3H, d, $J=7.0$ Hz, H-14). ¹³C-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). ¹H-NMR (CDCl_3 , 500 MHz) δ : 7.13 (1H, brs, OOH), 5.89 (1H, brs), 4.67 and 4.64 (each 1H, brs), 2.36 (2H, 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, brq, $J=11.3$ Hz), 1.04 (1H, q, $J=12.8$ Hz), 1.00 (3H, d, $J=6.7$ Hz). ¹³C-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), 46.4 (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-10 α -ol (**1a**): White solid, mp 117–118 °C (lit.⁵⁾ 118.5 °C), $[\alpha]_D^{25} -66.1^\circ$ ($c=0.40$, CHCl_3) (lit.⁵⁾ -79.2° , $c=0.71$, CHCl_3).

Compound 2: Colorless oil, $[\alpha]_D^{25} +71.8^\circ$ ($c=0.58$, EtOH). IR (KBr) cm^{-1} : 3418, 2932, 2870, 1643, 1450, 1377. HR-CI-MS m/z : 237.1851 ($[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_2$: 237.1854). CI-MS m/z (%): 237 ($[\text{M}+\text{H}]^+$, 18), 219 (61), 203 (100). ¹H-NMR (C_6D_6 , 500 MHz) δ : 6.71 (1H, brs, OOH), 4.97 and 4.96 (each 1H, brs, H-15), 4.73 and 4.70 (each 1H, brs, 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, brt, $J=11.1$ Hz, H-7), 1.90–1.83 (4H, m, H-3,5,6,8), 1.60 (3H, s, H-13), 1.57 (1H, brd, $J=14.7$ 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). ¹³C-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-1 α -ol (**2a**): White solid, mp 64–65 °C (lit.⁵⁾ 69.5 °C), $[\alpha]_D^{25} +67.6^\circ$ ($c=0.23$, CHCl_3) (lit.⁵⁾ $+67.1^\circ$, $c=0.73$, CHCl_3).

Compound 3: Colorless oil, $[\alpha]_D^{25} +7.3^\circ$ ($c=0.23$, EtOH). IR (KBr) cm^{-1} : 3356, 2928, 2870, 1439, 1342. HR-CI-MS m/z : 237.1860 ($[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{15}\text{H}_{25}\text{O}_2$: 237.1854). CI-MS m/z (%): 237 ($[\text{M}+\text{H}]^+$, 3), 219 (100), 203 (64). ¹H-NMR (CDCl_3 , 500 MHz) δ : 7.86 (1H, s, OOH), 4.65 and 4.64 (each 1H, brs, 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, brd, $J=12.0$ 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, brq, $J=12.0$ Hz, H-8), 1.05 (1H, q, $J=12.2$ Hz, H-6), 0.92 (3H, d, $J=7.1$ Hz, H-14). ¹³C-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-15 α -ol (**3a**): Colorless oil, $[\alpha]_D^{25} -8.3^\circ$ ($c=0.15$, 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), $[\alpha]_D^{25} -119^\circ$ ($c=1.0$, CHCl_3) (lit.⁷⁾ -124° , $c=0.22$, CHCl_3).

Trypanocidal Assay⁸⁾ Epimastigotes of *Trypanosoma cruzi* (Tulahuen strain) were kept in GIT medium (Wako) supplemented with hemin (12.4 μ M, Wako). The epimastigotes in GIT medium (10 μ l) were incubated with a test sample dissolved in EtOH (5 μ l) and autoclaved saline (185 μ l). After 24 h of incubation, the movement of epimastigotes was observed under a microscope ($\times 100$). 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 μ M.

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