**Chemical Constituents of the Roots of *Piper Sarmentosum***

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Sixteen compounds were isolated from the fresh roots of *P. sarmentosum*. Seven of these have been previously isolated from the fruits and leaves of this plant: the aromatic alkene (1), 1-allyl-2-methoxy-4,5-methylene dioxybenzene (4), β-sitosterol, pyrrole amide (6), sarmentine (10), sarmentosine (13) and pellitorine (14). (+)-Sesamin (2), horsfieldin (3), two pyrrolidine amides 11 and 12, guineensine (15) and brachystamide B (16) are new for *P. sarmentosum*. Sarmentamide A, B, and C (7—9) are new natural products. Compounds 1—4 and 6—16 were tested for antiplasmodial, antimycobacterial and antifungal activities.

**Key words** *Piper sarmentosum*; Piperaceae; pyrrole amide; pyrrolidone amide; pyrrolidine amide; lignan

*Piper sarmentosum* Roxb. is known as “cha-plu” in Thailand. The roots are used as carminative and stomachic in Thailand. In Malaysia and the Indonesian Archipelago, the leaves and roots of this plant are used for the treatment of toothache, fungoid dermatitis on the feet, coughing, asthma and pleurisy.1) In previous investigations of *P. sarmentosum*, several alkamides2—3) and phenylpropanoids4) were isolated from the fruits and leaves. We now report the isolation and structural elucidation of sixteen components from the fresh roots of *P. sarmentosum*. Three of these components are new natural products, six are new for *P. sarmentosum* and seven have been previously isolated from the plant.2—4) Compounds 1—4 and 6—16 were tested for antiplasmodial, antimycobacterial and antifungal activities.

The ethanolic extract of fresh roots of *P. sarmentosum* was separated into three fractions: EtOAc-, n-ButOH- and water-soluble fractions. The EtOAc-soluble fraction was purified to give sixteen compounds by silica gel CC and preparative TLC (see Experimental). Compounds 1—6 were identified to be the aromatic alkene (1),21 (+)-sesamin (2),5—7) horsfieldin (3),8—10) 1-allyl-2-

methoxy-4,5-methylene dioxybenzene (4),4) β-sitosterol and the pyrrole amide (6).2,3)

The 1H-NMR spectral data of the unsaturated pyrrolidine amides 11—13 were very similar. Together with the MS data and comparison with the literature data, they were identified as N-[9-(3,4-methylenedioxyphenyl)-2E,4E,8E-nonatrienoyl]-pyrroline,9) N-[9-(3,4-methylenedioxyphenyl)-2E,8E-nonadienoyl]pyrrolidine (brachymide B)10) and N-[7-(3,4-methylenedioxyphenyl)-2E,6E-heptadienoyl]pyrrolidine (sarmentosine),2) respectively. The amides 10 and 14 were identified from their spectral data to be N-pyrrolylidyl-2E,4E-decadienamide (sarmentine) and N-isobutyl-2E,4E-decadienamide (perrontine).2) The two unsaturated amides 15 and 16, which had similar 1H-NMR spectra, were characterized by comparison with the literature data as N-isobutyl-13-(3,4-methylenedioxyphenyl)-2E,4E,12E-tridecatrienamide (guineensine)11,12) and N-isobutyl-15-(3,4-methylenedioxyphenyl)-2E,4E,14E-pentadecatrienamide (brachyst-amide B),13) respectively.

Sarmentamide A (7), a colorless oil, exhibited a parent ion at m/z 215 in the EI-MS and an accurate mass consistent with the formula C13H13O2N. The infrared spectrum showed strong absorption bands at 1725 and 1692 cm⁻¹ (amide). The 1H-NMR spectrum of 7 contained two triplets at δ 3.01 (J=7.8 Hz) and 3.30 (J=7.8 Hz) and five aromatic protons at δ 7.29 indicating the phenylpropanoyl moiety. The spectrum also contained signals from two methine protons α to the nitrogen atom, at δ 4.41 (2H, t, J=2.1 Hz) and two olefinic protons at δ 6.16 (1H, dt, J=2.1, 6.0 Hz) and δ 7.29 (1H, overlapped with aromatic proton signals). Together with the 13C-NMR spectral data, which contained signals for a methylene carbon (α to the nitrogen atom) at δ 50.7, two methine olefinic carbons at δ 127.7 and 146.6 and a carbonyl carbon at δ 170.0, it appeared that 7 possessed a Δ2,2-pyrrolidinemoiety. This was also supported by the long-range correlations observed between the carbonyl (δ 170.0) and C5 (δ 50.7) of a Δ2-2-pyrrolidinemoiety and H-3 (δ 6.16) and H-4 (δ 7.29) (Fig. 1). 2J and 3J correlations were also shown between the carbonyl (δ 172.5) and C4′ (δ 141.8) of the phenylpropanoyl moiety and H-2′ (δ 3.30) and H-3′ (δ 3.01) (Fig. 1). Sarmentamide A was thus identified as N-(phenylpropanoyl)-Δ2,2-pyrrolidinone (7). The 13C-NMR spectrum was assigned by the combination of DEPT, HMOC and HMBC experiments.

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2,4,5-trimethoxycinnamoyl moiety was indicated by an AB/H-1032 from (C2/C5) in the 13C-NMR spectrum. Sarmentamide B the other one is not, or is only slightly so, such that the signal being non-equivalent and give a first order spectrum whereas bond. This results in one of the C2/C5 methylene groups.

Fig. 1. 2D HMBC Correlations of 7—9

Sarmentamide B (8), a colorless wax, showed a parent ion in the EI-MS at m/z 317 and an accurate mass consistent with the molecular formula C_{17}H_{19}O_{5}N. The infrared spectrum showed two strong absorption bands at 1743 cm⁻¹ (C=O, ester) and 1652 cm⁻¹ (C=O, amide). The 1H-NMR spectrum displayed an AB quartet at δ 6.68 (1H, d, J=15.5 Hz) and 7.77 (1H, d, J=15.5 Hz) and signals for five aromatic protons at δ 7.40 (3H, m) and 7.55 (2H, m), indicating the presence of a cinnamoyl moiety in compound 8. Signals for two non-equivalent methylene groups, α to the nitrogen atom of the pyrrolidine ring, appeared at δ 3.80 (1H, d, J=12.0 Hz), 4.04 (1H, dd, J=3.9, 12.0 Hz) and 3.90 (2H, m) and two β protons of the pyrrolidine ring gave signals at δ 5.26 (1H, d, J=3.9 Hz) and 5.27 (1H, d, J=3.3 Hz); two acetoxy groups appeared at δ 2.11 (3H, s) and 2.12 (3H, s). The presence in 8 of a β,β'-diacetoxy unit was indicated. The 13C-NMR spectrum was assigned by a combination of DEPT, HMQC and HMBC experiments. Important long-range correlations were observed between the cinnamoyl carbonyl (δ 165.1) and H-2’ (δ 6.68) and H-3’ (δ 7.77); C3 (δ 75.3) and C4 (δ 75.5) and H-2 (δ 3.80, 4.04) and H-5 (δ 3.89, 3.90); and C2 (δ 50.6) and C5 (δ 50.1) and H-3 (δ 5.26) and H-4 (δ 5.27) (Fig. 1). From the fact that 8 is optically active ([α]_D = +68.3°), the 3,4-diacetoxy groups can be defined as being trans. The doubling of signals in the NMR spectra can be explained as being due to isomerism about the amide bond. This results in one of the C2/C5 methylene groups being non-equivalent and give a first order spectrum whereas the other one is not, or is only slightly so, such that the signal from this pair is strongly second order. There are two signals from (C2/C5) in the 13C-NMR spectrum. Sarmentamide B was thus assigned to be N-cinnamoyl-trans-3,4-diacetoxy-3,4-pyrrolidine (8), with the relative stereochemistry shown.

Sarmentamide C (9), a colorless solid, showed a parent ion at m/z 291 in the EI-MS and an accurate mass consistent with the molecular formula C_{17}H_{19}O_{5}N. The infrared spectrum had a strong absorption band at 1649 cm⁻¹ (C=O, amide). A 2,4,5-trimethoxycinnamoyl moiety was indicated by an AB quartet at δ 6.64 (1H, d, J=15.4 Hz) and 7.64 (1H, d, J=15.4 Hz), and signals for two aromatic protons at δ 6.77 (2H, s) and three methoxy groups at δ 3.90 (3H, s) and 3.91 (6H, s). These data were consistent with the 13C-NMR spectral data which indicated signals for six aromatic carbons [δ 105.2 (2×), 130.9, 140.0, 153.4 (2×)], two olefinic carbons (δ 118.1, 141.8), one carbonyl carbon (δ 163.9) and three methoxyl carbons (δ 56.1, 56.2, 60.9). The 1H-NMR spectrum of 9 also showed bands of a pyrrolidine ring, again affected by amide isomerism. Two α-methylene appeared as two triplets at δ 3.62 (J=6.5 Hz) and 3.67 (J=6.5 Hz) and two β-methylenees as two quintets at δ 1.93 (J=6.5 Hz) and 2.03 (J=6.5 Hz). Four methylene carbons of the pyrrolidine ring appeared at δ 24.4, 26.2, 46.3 and 47.0 in the 13C-NMR spectrum of 9. The 13C-NMR spectrum was assigned by a combination of DEPT, HMQC and HMBC experiments. The long-range correlations were observed between C1’ (δ 163.9) and H-2’ (δ 6.64) and H-3’ (δ 7.64); C4’ (δ 130.9) and H-2’ (δ 6.64), H-3’ (δ 7.64), H-6’ (δ 6.77) and H-9’ (δ 6.77); C2 (δ 46.3) and H-3 (δ 1.93); C5 (δ 47.0) and H-4 (δ 2.03) (Fig. 1). Sarmentamide C was thus identified as N-(2,4,5-trimethoxycinnamoyl)pyrrolidine (9).

In summary, sarmentamide A (7), B (8) and C (9) are new natural products. (+)- Sesamin (2), horsfieldin (3) and amides 11, 12, 15 and 16 are new constituents for *P. sarmentosum*. Aromatic alkenes 1, phenylpropanoid 4, β-sitosterol, amides 6, 10, 13 and 14 were previously isolated from the fruits and leaves of this plant.

Compounds 10 and 13 possessed *in vitro* antiplasmodial activity with EC₅₀ values (µg/ml) of 4.5 and 3.9, respectively, whereas 1—4, 6—9 12 and 14—16 were inactive (EC₅₀>20 µg/ml). Compounds 1 and 11 exhibited antinymbo-cobacterial activity with MIC value (µg/ml) of 25, compounds 10, 12—14 and 16 had MIC value of 50, while compounds 2—4, 6—9 and 15 were inactive (MIC>200 µg/ml). Compounds 12 and 13 possessed antifungal activity with IC₅₀ (µg/ml) of 41.82 and 34.82, respectively, whereas compounds 1—4, 6—11 and 14—16 were inactive (IC₅₀>50 µg/ml).

**Experimental**

Meltng points are uncorrected. Optical rotations were determined with a Jasco digital polarimeter. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. 1H- and 13C-NMR were measured in CDCl₃ or CDCl₃-DMSO-d₆ on a Bruker 300 (300 MHz for 1H-NMR and 75 MHz for 13C-NMR) spectrometer. Chemical shifts are in δ (ppm) with tetramethylsilane as an internal standard. MS were recorded on a VG 7070 mass spectrometer operating at 70 eV or with a VG Quattro triple quadrupole mass spectrometer for the electrospray mass spectra. Unless otherwise stated column chromatography was carried on Kieselgel 60 (Merck 70—230 mesh or 230—400 mesh) using gradient of hexane/EtOAc, EtOAc, EtOAc/MeOH (1:1) and MeOH to give 14 fractions. TLC and PLC were performed on precoated silica gel 60 F 254 plates (Merck); spots were visualized by heating. A voucher specimen of the plant material has been deposited at the National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Paholyothin Road, Klong 1, Klong Luang, Pathumthani 12120, Thailand. Known compounds were identified by comparison of mp’s and spectroscopic data with published data.

**Extraction and Isolation**

The fresh roots of *P. sarmentosum* (481 g) were ground and extracted with 95% EtOH at room temperature. After filtration and evaporation, the ethanolic extract was obtained as a brown viscous oil (19.4 g). The oil was partitioned between water (200 ml) and EtOAc (3×200 ml) and the water layer was further partitioned with n-ButOH (3×200 ml). Evaporation of the EtOAc-, n-ButOH- and water-soluble fractions gave a dark brown oil (6.3 g), a brown oil (1.8 g) and a light brown oil (10.5 g), respectively.

The EtOAc-soluble fraction (6.3 g) was separated by flash column chromatography using silica gel (Merck, 230—400 mesh, diameter×height: 10.0 cm×5.0 cm) and the column was eluted with 200 ml each fraction of hexane, gradient of hexane/EtOAc, EtOAc, EtOAc/MeOH (1:1) and MeOH to give 14 fractions.

Fr. 2, a colorless solid (56 mg), was identified as the aromatic alkenes 1. Fr. 4 was rechromatographed on silica gel and eluted with hexane–EtOAc (100:1 and 50:1) to give 1 (12 mg), 4 (97 mg) and 6 (288 mg). Fr. 7 was rechromatographed on silica gel and eluted with benzene–EtOAc (10:1) to give 2 (11 mg) and β-sitosterol (69 mg). Fr. 9 was separated by column chromatography and further purified by preparative TLC using benzene–EtOAc (5:1, 2 runs) to give 4 (33 mg) which was crystallized from benzene as...
colorless needles. Fr. 10 was undergone series of chromatographic separations on silica gel using a gradient of benzene–EtOAc as the eluent and Lichroprep RP-18 (Merek, 0.040—0.063 mm) using MeCN–H₂O (10: 1) as the eluent to give 3 (9 mg), 17 (33 mg), 13 (17 mg), 14 (25 mg), 15 (26 mg) and 16 (12 mg). Fr. 12 was separated on preparative TLC using benzene–EtOAc (2: 1) as the developing solvent to give 8 (8 mg) and 10 (18 mg). Fr. 13 was undergone series of chromatographic separations on silica gel using benzene–EtOAc (3: 1, 2: 1, 1: 1) as the eluent and Lichroprep RP-18 (Merek, 0.040—0.063 mm) using MeOH–H₂O (4: 1) as the eluent to give 7 (70 mg), 11 (14 mg), 12 (6 mg), 13 (28 mg) and 14 (11 mg). Fr. 14 was rechromatographed on Lichroprep RP-18 (Merek, 0.040—0.063 mm) using MeOH/H₂O (3: 1) as the eluent to give 9 (12 mg).

Aromatic Alkene I: A colorless solid, mp 35—36 °C. の
(+)-Suberin (2): Colorless needles, mp 117—119 °C.

Horsfieldin (3): Colorless needles, mp 156—159 °C.

1-Allyl-2-methoxy-4,5-methylenedioxycyclohexene (4): A colorless oil. の

N-(3-Phenylpropionaryl)pyrrole (6): A pale yellow solid, mp 46—48 °C. の

N-(3-Phenylpropionyl)-A₂-2-pyrrolidone (Sarmentamidine A) (7): A colorless oil; UV λₘₐₓ (log ε) nm: 209 (4.23), 230 (3.89); IR ν max cm⁻¹: 3027, 2923, 1725, 1692, 1601, 1441, 1380, 1271, 995, 805, 700; H-NMR (CDCl₃): 8: 3.01 (2H, t, J₇-H₈ = 7.8 Hz, H-3), 3.30 (2H, t, J₇-H₈ = 7.8 Hz, H-2), 4.41 (2H, t, J₅-H₆ = 2.1 Hz, H-5), 6.16 (1H, t, J₅-H₆ = 6.0, H-6), 7.29 (1H, overlapped signal, H-5), 7.29 (5H, m, ArH=5); 13C-NMR (CDCl₃): 76.8 (C₂), 55.0 (C₃), 144.1 (C₁₅), 144.6 (C₁₆), 170.0 (C₁₇), 172.5 (C₂₁); MS m/z (rel. int.): 215 (M⁺, 100%), 149 (33), 104 (47), 83 (27), 77 (19). HR-MS m/z: Caled for C₁₇H₁₅NO₂: 215.0946. Found: 215.0946.

N-Cinnamoyl-trans-3,4-diacetoxy-3,3-(2H, t, J₇-H₈ = 7.8 Hz, H-3), 6.16 (1H, t, J₅-H₆ = 6.0, H-6), 7.29 (1H, overlapped signal, H-5), 7.29 (5H, m, ArH=5); 13C-NMR (CDCl₃): 76.8 (C₂), 55.0 (C₃), 144.1 (C₁₅), 144.6 (C₁₆), 170.0 (C₁₇), 172.5 (C₂₁); MS m/z (rel. int.): 215 (M⁺, 100%), 149 (33), 104 (47), 83 (27), 77 (19). HR-MS m/z: Caled for C₁₇H₁₅NO₂: 215.0946. Found: 215.0946.

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References