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STRUCTURAL ELUCIDATION AND SYNTHESIS OF NEW COMPONENTS ISOLATED FROM PIPER SARMENTOSUM (PIPERACEAE)[1]

Kittisak Likhitwitayawuid and Nijsiri Ruangrungsi
Department of Pharmacognosy, Faculty of Pharmaceutical Sciences
Chulalongkorn University, Bangkok 10500, Thailand

Gordon L. Lange* and Carl P. Decicco
Guelph-Waterloo Centre for Graduate Work in Chemistry
Department of Chemistry and Biochemistry, University of Guelph
Guelph, Ontario, N1G 2W1, Canada.

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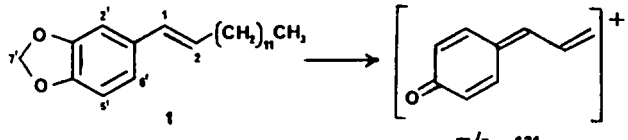
Abstract. Six components were isolated from the fruit of Piper sarmentosum. Two of the components are the ubiquitous β-sitosterol and the known unsaturated amide, pellitorine, **3**. The other four components, which are new natural products, consist of the aromatic alkene **1**, the pyrrole amide **2** and two unsaturated pyrrolidine amides which have been given the names sarmentine **4** and sarmentosine **5**. Components **1** and **2** were synthesized to establish unambiguously their structures.

The genus Piper in the Piperaceae family is composed of approximately 2000 species distributed primarily in tropical regions.[2] A number of Piper species are noted for their ethnomedical properties, of which the reputed stimulant, carminative, diuretic and diaphoretic activities of P. nigrum are probably the best known.[3,4] The species of interest in the present study, Piper sarmentosum Roxb., also known as "Cha-plu", has exhibited in vitro activity in the reduction of blood sugar in alloxan diabetic rabbits[5] and in Thailand the plant and fruit are used as an expectorant.[3] In the Malay and Indonesian Archipelago, the leaves and roots of this species have been reported to provide an effective remedy for toothache, fungoid dermatitis on the feet, coughing, asthma and pleurisy.[6] A previous investigation of P. sarmentosum yielded β-sitosterol and dihydrocinnamic acid as two constituents of the leaves.[6] Herein, we report the isolation and structural elucidation of six components isolated from the dried fruit of P. sarmentosum. Four of these components are previously unreported natural products and two of these have been synthesized to confirm their structures.

Results and Discussion

Six of the compounds extracted from the dried fruit of P. sarmentosum were isolated as described in the Experimental section. The structural elucidation of these compounds will be described in the order in which they were eluted from the chromatography column.

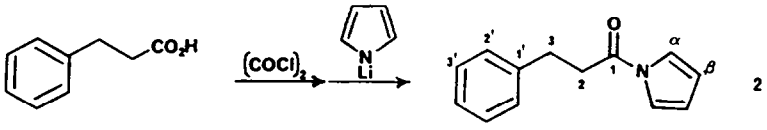
Compound **1**, the least polar component, was a low-melting solid which exhibited a parent peak in its electron impact mass spectrum (eims) at m/z 316 and an accurate mass consistent with the molecular formula $C_{21}H_{32}O_2$. The mass spectral fragment at m/z 131[7] (see proposed structure below), the ultraviolet[8] and ^{1}H NMR spectra[8] all suggested the presence of a 3,4-methylenedioxyphenyl moiety conjugated to an alkenyl side chain. The ^{1}H NMR also indicated that



1 m/z 131

the configuration of the 1,2-double bond is trans (J = 15.6 Hz) and both the ^{1}H and the ^{13}C NMR spectra (see Experimental) showed that the side chain is linear. The structure proposed for this first component, **1**, has not been reported previously in the literature although related natural products with C_{11} (isolated from P. longum[9]) and C_{12} trans-alkenyl side chains (pipataline, isolated from P. peepuloides[9]) have been reported. To establish unambiguously the strucure of **1** it was synthesized by reaction of piperonal with the Grignard reagent of 1-bromotridecane followed by dehydration of the resultant alcohol using a procedure similar to that employed previously for the preparation of pipataline.[10]

The second compound eluted from the column was a crystalline solid which exhibited an eims parent ion at m/z 199 and an accurate mass consistent with the formula $C_{13}H_{13}NO$. The infrared absorption at 1725 cm^{-1} (unusual position for an amide)[11] and the strong ultraviolet absorption at 240 nm[12] both were consistent with the presence of an N-acylpyrrole moiety. Detailed analysis of the ^1H and ^{13}C NMR spectra (for assignments see the Experimental) indicated this component possesses structure 2. Because of the lower barrier to rotation about the carbonyl-nitrogen bond in acylpyrroles,[11] the protons and carbons at the two α-positions of the pyrrole ring appear at the same position in the ^1H and ^{13}C NMR spectra, respectively, unlike the situation with saturated amides (to be discussed later). To our knowledge, 2 has neither been isolated from natural sources nor synthesized previously but it is worth noting that 3-phenylpropanoic acid, the parent acid of amide 2, was isolated previously from the leaves of P. sarmentosum.[6] To confirm our identification, 2 was prepared in 77% yield by reaction of 3-phenylpropanoyl chloride with the anion of pyrrole.



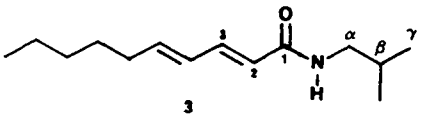
The third component was the ubiquitous β-sitosterol, which was found to be identical in all respects with a sample isolated previously from Typha elephantina.[13] β-Sitosterol had been isolated previously from the leaves of P. sarmentosum.[6]

The eims (M^+, 223), infrared spectrum (C=O, 1673 cm^{-1}), melting point, and nmr spectra all supported the conclusion that the fourth component is pellitorine, 3. A detailed analysis of the 400 MHz ^1H NMR spectrum of 3[14] is presented in Table 1 for comparison with 4 and 5 and in Table 2 the 100 MHz ^{13}C NMR data for 3 is reported for comparison with 4 and 5 and also because the chemical shifts for several carbons differ from those reported previously.[15] Pellitorine has been found in a number of Piper species as well as in the Compositae and the Rutaceae families.[16] 3 has also been the target of numerous synthetic investigations[17] presumably because of its significant biological activity (to be discussed later).

The fifth component, 4, was an oil which exhibited a parent ion at m/z 221 in its eims and an accurate mass consistent with the formula $C_{14}H_{23}NO$. The infrared (C=O, 1650 cm^{-1}) and ultraviolet spectra (λ_{max} 274 nm) suggested the presence of a dienamide moiety and the ^1H NMR spectrum indicated that both double bonds in the diene system were of the trans-configuration ($J_{2',3'}$ = 15.2, $J_{4',5'}$ = 14.1 Hz). The ^1H spectrum (Table 1) also suggested that the nitrogen atom was present in a pyrrolidine ring and because of the higher barrier to rotation about this carbonyl-nitrogen bond [18] (as compared with the barrier in 2[11]), the α- or syn-protons resonate upfield from the α'- or anti-protons[18] (anti to the carbonyl group). Conversely, it was discovered by a ^1H-^1H 2D-COSY experiment that the β syn protons resonate downfield from the β' anti protons. The ^{13}C NMR spectrum of component 4, which is recorded in Table 2, shows that the carbon chain beyond the diene moiety is linear and also exhibits four resolved resonances for the carbons of the pyrrolidine ring, with the α- or syn-carbon being assigned the more upfield resonance at 46.0 ppm.[18] The evidence described above indicates that this fifth component is the previously unreported N-acylpyrrolidine, 4, for which we propose the name sarmentine.

The sixth and final component in this study, 5, exhibited in its eims a parent ion at m/z 299 and an accurate mass appropriate for a molecular formula of $C_{18}H_{21}NO_3$. The IR and UV spectra suggested the presence of an unsaturated amide function and the base peak in the eims at m/z 131 indicated a partial structure consisting of a double bond conjugated to a 3,4-methylenedioxyphenyl substitutuent (i.e. aryl-CH=CH-CH_2-, see discussion of eims of 1). Analysis of the ^1H (Table 1) and ^{13}C NMR spectra (Table 2) for this compound indicated that the two double bonds present in 5 were of the trans-configuration ($J_{2',3'}$ = 15.2, $J_{6',7'}$ = 15.6 Hz) and that the nitrogen atom was again present in a pyrrolidine ring. All of this spectroscopic data supports the conclusion that this compound is another unsaturated N-acylpyrrolidine, 5, for which we propose the name sarmentosine.

Extracts of Piper sarmentosum have been used for medicinal purposes and thus some comment on the biological activity of the components isolated in this study is warranted. We have not assayed the activity of the four new components but the activity of structurally related compounds as well as the two known compounds isolated should be relevant. β-Sitosterol exhibits significant anti-tumor activity[20] and its use in the treatment of hypercholesterolemia has been investigated.[21] The in vivo and in vitro antitubercular activity of pellitorine, 3, has been reported[22] as well as its potent insecticidal activity[14] (e.g. paralyzing effect on houseflies[23]). Compounds similar to 5 are responsible for the pungent taste of black pepper[24,25] and pepper extracts containing such compounds have exhibited anti-tumor activity.[7] Clearly, investigation of the biological activity of some of these new natural products such as the pyrrolidine amides sarmentine (4) and sarmentosine (5) would be appropriate.



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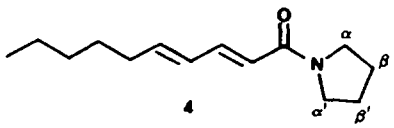

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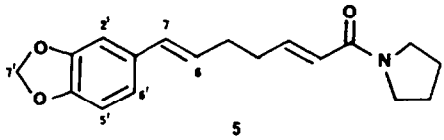

5

Table 1. ^{1}H NMR Data for 3, 4 and 5[a]

H	3	4	5
2	5.76(d,15.0)	6.01(d,15.2)	6.13(d,15.2)
3	7.19(dd,15.0,10.0)	7.21(dd,15.2,9.5)	6.92(dt,15.2,6.2)
4	6.10(dd,13.1,10.0)	6.08(dd,14.1,9.5)	2.36(br s)
5	6.12(dt,13.1,7.0)	6.10(dt,14.1,7.0)	2.36(br s)
6	2.14(dd,6.8,7.3)	2.08(dd,6.8,7.0)	6.02(dt,15.6,6.2)
7	1.42(quint,7.1)	1.35(quint,6.9)	6.32(d,15.6)
8	1.30(m)	1.23(m)	b
9	1.30(m)	1.23(m)	-
10	0.89(t,6.9)	0.81(t,4.3)	-
α	3.16(t,6.5)	3.44(t,6.9)	3.50(t,7.0)
β	1.80(m)	1.89(quint,7.0)	1.95(quint,6.4)
β'	-	1.79(quint,7.0)	1.86(quint,6.4)
α'	-	3.47(t,6.9)	3.53(t,7.0)
γ	0.92(d,6.7)	-	-
N-H	5.60(br,s)	-	-

[a]Chemical shifts are in ppm from TMS, multiplicities and coupling constants in Hertz are in parentheses and the samples were dissolved in $CDCl_3$.
[b]Assignments of aryl protons: δ 6.88 (br s, H-2'), 6.74 (m, H-5' and 6'), 5.94 (s, H-7')

Table 2. ^{13}C NMR Data for 3, 4 and 5[a]

Carbon	3	4	5
1	166.4(+)	165.2(+)	164.7(+)
2	121.8(-)	119.8(-)	120.3(-)
3	143.2(-)	143.1(-)	144.4(-)
4	128.2(-)	128.6(-)	32.2(+)
5	141.3(-)	142.2(-)	31.6(+)
6	32.9(+)	32.9(+)	130.2(-)
7	28.5(+)	28.5(+)	127.5(-)
8	31.4(+)	31.4(+)	b
9	22.5(+)	22.5(+)	-
10	14.0(-)	14.0(-)	-
α	46.9(+)	45.9(+)	45.7(+)
β	28.6(-)	26.1(+)	26.0(+)
β'	-	24.3(+)	24.2(+)
α'	-	46.5(+)	46.4(+)
γ	20.1(-)	-	-

[a]Chemical shifts are in ppm from TMS with $CDCl_3$ as solvent.
[b]Chemical shifts for carbons 1' to 7' are: δ 132.0(+), 105.3(-), 147.9(+), 146.7(+), 108.1(-), 122.2(-), 100.9(+), respectively. See reference (26) for assignments of related aromatic compounds.

Experimental

Instruments

IR spectra were obtained on a Perkin-Elmer Model 1330 or Nicolet Model 20 SX/C FTIR spectrometer, UV spectra on a Varian DMS 90 spectrometer in 95% ethanol and mass spectra on a VG Micromass 7070F or a ZAB-E spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker WH-400 or an AM-250 spectrometer with TMS (δ=0) as internal standard. The interpretation of some ^1H NMR spectra was facilitated by 2D-COSY experiments.[27] The multiplicities for ^{13}C spectra were determined by the attached proton test (APT)[28] with τ=0.01 s, which produced positive (+) quaternary C and CH_2 signals and negative (-) CH and CH_3 signals. TLC analyses were performed on silica gel GF 254 plates of thickness 0.25 mm. Petrol is the 30-60°C petroleum ether fraction.

Plant Material

The fruit of Piper sarmentosum (Piperaceae) was collected in February, 1986, from Kanchanaburi Province in Thailand. The plant material was authenticated by comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture and Cooperative, Bangkok, Thailand.

Extraction and Isolation

The dried, powdered fruit from P. sarmentosum (450 g) was extracted with 2 L of petroleum ether (40-60°C) for 8 h using a soxhlet apparatus. Removal of the solvent in vacuo yielded 21 g of a dark brown syrupy mass, of which 3 g was chromatographed on silica gel. The components were eluted using a solvent gradient of chloroform up to 50% MeOH/$CHCl_3$ to afford 19 fractions (25 mL each) which were examined by TLC. Portions containing components of similar polarity were combined as follows: fractions 2-5 were designated as residue A (1.5 g), 9-15 residue B (700 mg), and 16-19 residue C (684 mg).

Residue A was rechromatographed on silica gel with benzene as solvent to provide 50 mg of 1 and 215 mg of 2. Residue B was eluted with 15% EtOAc/benzene to afford 10 mg of β-sitosterol and 82 mg of pellitorine, 3. Residue C was eluted with 30% EtOAc/benzene to give 200 mg of 4 and 80 mg of 5.

Identification of Components 1 - 5

1-(3,4-methylenedioxyphenyl)-1E-tetradecene, 1

TLC (1% EtOAc/petrol) R_f 0.70; MP 33.5-36.5°C; UV (EtOH) λ_{max} 216 nm (ε 20,000), 260 (12,000), 268 (11,500), 312 (5,300); IR (CCl_4) 3010, 2920, 1500, 1480, 1245, 1040 cm^{-1}; ^1H NMR, 400 MHz ($CDCl_3$) δ 6.91 (1,s,H-2'), 6.75 (2,m,H-5' and 6'), 6.29 (1,d,J=15.6Hz,H-1), 6.06 (1,dt,J=15.6,6.5Hz,H-2), 5.94 (2,s,H-7'), 2.17 (2,dt,J=7.2,7.0Hz,H-3), 1.45 (2,m,H-4), 1.28 (18,m,H-5 to H-13), 0.89 (3,t,J=6.4Hz), assignments confirmed by 2D-COSY experiment; ^{13}C NMR, 100 MHz ($CDCl_3$) δ 147.9 (+,C-3'), 146.5 (+,C-4'), 132.5 (+,C-1'), 129.5(-,C-1), 129.2 (-,C-2), 120.1 (+,C-6'), 108.2 (-,C-5'), 105.3(-,C-2'), 100.9 (+,C-7'), 32.9(+), 31.9(+) 29.6(+), 29.5(+), 29.3(+), 29.2(+), 22.6(+), 14.1(-,C-14); MS (rel. int.) m/z 316 (M^+,4), 288 (82), 161 (69), 131 (100); exact masses calcd. for $C_{21}H_{32}O_2$ 316.2402 and C_9H_7O fragment 131.0494, found 316.2400 and 131.0496, respectively.

N-(3-phenylpropanoyl)pyrrole, 2

TLC (5% EtOAc/petrol) R_f 0.75; MP 48.5-50.0°C; UV (EtOH) λ_{max} 205 nm (ε 2500), 240 (8000); IR (CCl_4) 3060, 3030, 2940, 1725, 1470, 1370, 1325, 700 cm^{-1}; ^1H NMR, ($CDCl_3$) δ 7.27 (7,m,H-2',-3',-4' and -α) 6.27 (2,t,J=2.5Hz,H-β), 3.12 (2,t), 3.11(2,t,H-2 or -3); ^1H NMR (C_6D_6) δ 7.09 (5,m,H-3',-4', and -α), 6.94 (2,d,J=7.3Hz,H-2'); 6.07 (2,t,J=2.3Hz,H-β), 2.78(2,t,J=7.6Hz), 2.37 (2,t,J=7.6,Hz,H-2 or -3); ^{13}C NMR ($CDCl_3$) δ 164.6 (C-1), 140.2 (C-1'), 128.7 (C-2'), 128.4 (C-3'), 126.5 (C-4'), 118.9 (2xC-α), 113.2 (2xC-β); 36.4, 30.4 (C-2 or -3) MS (rel. int.) m/z 199 (M^+,58), 105 (55), 91 (100), 77 (21), 65 (17); exact masses calcd. for $C_{13}H_{13}NO$ 199.0997, found 199.1005.

β-Sitosterol

TLC (5% ether/petrol) R_f 0.27; MP 134-136°C (lit.[29] 136-137°C). This sample was identical in all respects (TLC, IR, ^1H NMR and MS) with the same compound isolated in a previous study.[13]

N-isobutyl-2E,4E-decadienamide (pellitorine), 3

TLC (5% EtOAc/petrol) R_f 0.10; MP 66-68°C (lit.[16] 69°C); IR and MS the same as those reported previously[30]; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively.

N-(2E,4E-decadienoyl)pyrrolidine (sarmentine), 4

TLC (30% EtOAc/petrol) R_f 0.42; a yellow oil; UV (EtOH) λ_{max} 274 nm (ε 16,9000; IR (CCl_4) 2940, 2920, 2860, 1650, 1635, 1610, 1420 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; MS (rel. int.) 221 (M^+,39), 192 (6), 178 (14), 150 (100), 81 (74); exact masses calcd. for $C_{14}H_{23}NO$ 221.1780 and $C_9H_{12}NO$ fragment 150.0920, found 221.1778 and 150.0917, respectively.

N-[7-(3,4-methylenedioxyphenyl)-2E,6E-heptadienoyl]pyrrolidine (sarmentosine), 5

TLC (30% EtOAc/petrol) R_f 0.32; MP 77.5-79.5°C; UV (EtOH) λ_{max} 217 nm (ε 36,700), 267 (18,700), 309 (8,900); IR (CCl_4) 2865, 1660, 1615, 1480, 1245, 1040 cm^{-1}; ^1H and ^{13}C NMR, see Tables 1 and 2, respectively; MS (rel. int.) 299 (M^+,16), 201 (18), 131 (100), 100 (57); exact mass calcd. for $C_{18}H_{21}NO_3$ 299.1521, found 299.1526.

Synthesis of 1

A solution of tridecylmagnesium bromide was prepared from 92 mg (3.8 mmol) of Mg turnings and 1.0 g (3.8 mmol) of 1-bromotridecane in 25 mL of anhydrous ether. To this solution at 0°C was added over 15 min. a solution of 570 mg (3.8 mmol) of piperonal in 10 mL of ether. The mixture was heated to reflux for 2 h, then cooled and 20 mL of 20% aqueous H_2SO_4 was added cautiously. The layers were separated, the aqueous layer was extracted with ether (2x10 mL) and the combined ether layers were washed with saturated $NaHCO_3$ solution (1x30 mL), with brine (1x20 mL) and then dried (anhydrous $MgSO_4$). Removal of the solvent *in vacuo* gave 868 mg (68%) of crude alcohol as a white solid.

A sample of 452 mg (1.4 mmol) of this crude alcohol and 450 mg of neutral alumina were heated under N_2 at 180-200°C for 30 min. The alumina was washed with ether and the solution dried ($MgSO_4$). Removal of the solvent gave, after recystallization from petrol/ethanol, 386 mg (87%) of 1 as a white solid, m.p. 33.5-36.5°C.

Synthesis of 2

To a solution of 800 mg (5.3 mmol) of 3-phenylpropanoic acid in 5 mL of dry CH_2Cl_2 was added dropwise 1.34 g (0.92 mL, 10.6 mmol) of oxalyl chloride and the reaction was stirred under N_2 at room temperature for 3 h. The solvent was removed *in vacuo* and the residue washed four successive times (4x20 mL) to remove unreacted oxalyl chloride. The yellow residue of crude acid chloride was used in the next step.

The pyrrole anion was prepared by reacting 355 mg (0.367 mL, 5.3 mmol) of freshly distilled pyrrole in 10 mL of dry THF under N_2 at -78°C with 2.1 mL (5.3 mmol) of 2.5 M n-butyl lithium in hexane and stirring for 15 min. The crude 3-phenylpropanoyl chloride was then added dropwise to the pyrrole anion solution at -78°C and the temperature brought to room temperture gradually. After stirring for one-half hour at room temperature, water was added and the product was extracted with ether. The combined organic phases were dried ($MgSO_4$) and the solvent removed to give one major UV positive spot on TLC at R_f 0.45 (10% EtOAc/petrol). Purification of the crude product by MPLC gave 815 mg (77%) of N-(3-phenylpropanoyl)pyrrole, 2, which was identical in all respects with the second component isolated from *P. sarmentosum*.

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