# Chemical Constituents of the Roots of *Piper Sarmentosum*

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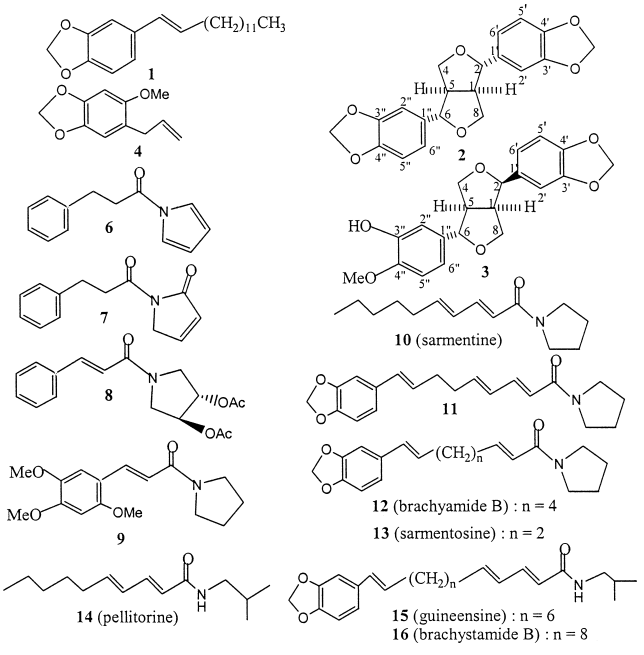
**Sixteen compounds were isolated from the fresh roots of *Piper sarmentosum*. Seven of these have been previously isolated from the fruits and leaves of this plant: the aromatic alkene (1), 1-allyl-2-methoxy-4,5-methylenedioxybenzene (4), β-sitosterol, pyrrole amide (6), sarmentine (10), sarmentosine (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; pyrolidone 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 alkamides[2,3)] and phenylpropanoids[4)] 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*-BuOH- 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**),[2)] (+)-sesamin (**2**),[5—7)] horsfieldin (**3**),[8)] 1-allyl-2-methoxy-4,5-methylenedioxybenzene (**4**),[4)] β-sitosterol and the pyrrole amide (**6**).[2)]




The $^1$H-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)-2*E*,4*E*,8*E*-nonatrienoyl]-pyrrolidine,[9)] *N*-[9-(3,4-methylenedioxyphenyl)-2*E*,8*E*-nonadienoyl]pyrrolidine (brachyamide B)[10)] and *N*-[7-(3,4-methylenedioxyphenyl)-2*E*,6*E*-heptadienoyl]pyrrolidine (sarmentosine),[2)] respectively. The amides **10** and **14** were identified from their spectral data to be *N*-pyrrolidyl-2*E*,4*E*-decadienamide (sarmentine) and *N*-isobutyl-2*E*,4*E*-decadienamide (pellitorine).[2)] The two unsaturated amides **15** and **16**, which had similar $^1$H-NMR spectra, were characterized by comparison with the literature data as *N*-isobutyl-13-(3,4-methylenedioxyphenyl)-2*E*,4*E*,12*E*-tridecatrienamide (guineensine)[11,12)] and *N*-isobutyl-15-(3,4-methylenedioxyphenyl)-2*E*,4*E*,14*E*-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 $C_{13}H_{13}O_2N$. The infrared spectrum showed strong absorption bands at 1725 and 1692 cm$^{-1}$ (amide). The $^1$H-NMR spectrum of **7** contained two triplets at $\delta$ 3.01 ($J$=7.8 Hz) and 3.30 ($J$=7.8 Hz) and five aromatic protons at $\delta$ 7.29 indicating the phenylpropanoyl moiety. The spectrum also contained signals from two methylene protons $\alpha$ to the nitrogen atom, at $\delta$ 4.41 (2H, t, $J$=2.1 Hz) and two olefinic protons at $\delta$ 6.16 (1H, dt, $J$=2.1, 6.0 Hz) and $\delta$ 7.29 (1H, overlapped with aromatic proton signals). Together with the $^{13}$C-NMR spectral data, which contained signals for a methylene carbon ($\alpha$ to the nitrogen atom) at $\delta$ 50.7, two methine olefinic carbons at $\delta$ 127.7 and 146.6 and a carbonyl carbon at $\delta$ 170.0, it appeared that **7** possessed a $\Delta^3$-2-pyrrolidone moiety. This was also supported by the long-range correlations observed between the carbonyl ($\delta$ 170.0) and C5 ($\delta$ 50.7) of $\Delta^3$-2-pyrrolidone ring and H-3 ($\delta$ 6.16) and H-4 ($\delta$ 7.29) (Fig. 1). $^2J$ and $^3J$ correlations were also shown between the carbonyl ($\delta$ 172.5) and C4′ ($\delta$ 141.8) of the phenylpropanoyl moiety and H-2′ ($\delta$ 3.30) and H-3′ ($\delta$ 3.01) (Fig. 1). Sarmentamide A was thus identified as *N*-(phenylpropanoyl)-$\Delta^3$-2-pyrrolidone (**7**). The $^{13}$C-NMR spectrum was assigned by the combination of DEPT, HMQC and HMBC experiments.

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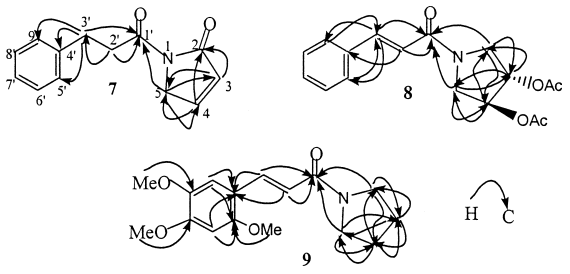


Fig. 1. 2D HMBC Correlations of **7**—**9**

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 $C_{17}H_{19}O_5N$. The infrared spectrum showed two strong absorption bands at 1743 $cm^{-1}$ (C=O, ester) and 1652 $cm^{-1}$ (C=O, amide). The $^1$H-NMR spectrum displayed an AB quartet at $\delta$ 6.68 (1H, d, $J$=15.5 Hz) and 7.77 (1H, d, $J$=15.5 Hz) and signals for five aromatic protons at $\delta$ 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, $\alpha$ to the nitrogen atom of the pyrrolidine ring, appeared at $\delta$ 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 $\beta$ protons of the pyrrolidine ring gave signals at $\delta$ 5.26 (1H, d, $J$=3.9 Hz) and 5.27 (1H, d, $J$=3.3 Hz); two acetoxy groups appeared at $\delta$ 2.11 (3H, s) and 2.12 (3H, s). The presence in **8** of a $\beta$,$\beta'$-diacetoxyl unit was indicated. The $^{13}$C-NMR spectrum was assigned by a combination of DEPT, HMQC and HMBC experiments. Important long-range correlations were observed between the cinnamoyl carbonyl ($\delta$ 165.1) and H-2′ ($\delta$ 6.68) and H-3′ ($\delta$ 7.77); C3 ($\delta$ 75.3) and C4 ($\delta$ 75.5) and H-2 ($\delta$ 3.80, 4.04) and H-5 ($\delta$ 3.89, 3.90); and C2 ($\delta$ 50.6) and C5 ($\delta$ 50.1) and H-3 ($\delta$ 5.26) and H-4 ($\delta$ 5.27) (Fig. 1). From the fact that **8** is optically active ($[\alpha]_D$=+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 $^{13}$C-NMR spectrum. Sarmentamide B was thus assigned to be *N*-cinnamoyl-*trans*-3,4-diacetoxypyrrolidine (**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 $C_{16}H_{21}O_4N$. 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 $\delta$ 6.64 (1H, d, $J$=15.4 Hz) and 7.64 (1H, d, $J$=15.4 Hz), and signals for two aromatic protons at $\delta$ 6.77 (2H, s) and three methoxy groups at $\delta$ 3.90 (3H, s) and 3.91 (6H, s). These data were consistent with the $^{13}$C-NMR spectral data which indicated signals for six aromatic carbons [$\delta$ 105.2 (2×), 130.9, 140.0, 153.4 (2×)], two olefinic carbons ($\delta$ 118.1, 141.8), one carbonyl carbon ($\delta$ 163.9) and three methoxyl carbons ($\delta$ 56.1, 56.2, 60.9). The $^1$H-NMR spectrum of **9** also showed bands of a pyrrolidine ring, again affected by amide isomerism. Two $\alpha$-methylenes appeared as two triplets at $\delta$ 3.62 ($J$=6.5 Hz) and 3.67 ($J$=6.5 Hz) and two $\beta$-methylenes as two quintets at $\delta$ 1.93 ($J$=6.5 Hz) and 2.03 ($J$=6.5 Hz). Four methylene carbons of the pyrrolidine ring appeared at $\delta$ 24.4, 26.2, 46.3 and 47.0 in the $^{13}$C-NMR spectrum of **9**. The $^{13}$C-NMR spectrum was assigned by a combination of DEPT, HMQC and HMBC experiments. The long-range correlations were observed between C1′ ($\delta$ 163.9) and H-2′ ($\delta$ 6.64) and H-3′ ($\delta$ 7.64); C4′ ($\delta$ 130.9) and H-2′ ($\delta$ 6.64), H-3′ ($\delta$ 7.64), H-6′ ($\delta$ 6.77) and H-9′ ($\delta$ 6.77); C2 ($\delta$ 46.3) and H-3 ($\delta$ 1.93); C5 ($\delta$ 47.0) and H-4 ($\delta$ 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 alkene **1**, phenylpropanoid **4**, $\beta$-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 $EC_{50}$ values ($\mu$g/ml) of 4.5 and 3.9, respectively, whereas **1**—**4**, **6**—**9**, **12** and **14**—**16** were inactive ($EC_{50}$>20 $\mu$g/ml). Compounds **1** and **11** exhibited antimycobacterial activity with MIC value ($\mu$g/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 $\mu$g/ml). Compounds **12** and **13** possessed antifungal activity with $IC_{50}$ ($\mu$g/ml) of 41.82 and 32.82, respectively, whereas compounds **1**—**4**, **6**—**11** and **14**—**16** were inactive ($IC_{50}$> 50 $\mu$g/ml).

**Experimental**

Melting points are uncorrected. Optical rotations were determined with a Jasco digital polarimeter. UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were recorded with a Jasco A-302 spectrophotometer. $^1$H- and $^{13}$C-NMR were measured in $CDCl_3$ or $CDCl_3$–DMSO-$d_6$ on a Bruker 300 (300 MHz for $^1$H-NMR and 75 MHz for $^{13}$C-NMR) spectrometer. Chemical shifts are in $\delta$ (ppm) with tetramethylsilane as an internal standard. MS were recored 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 out on Kieselgel 60 (Merck 70—230 mesh or 230—400 mesh) using gradient of hexane/EtOAc for elution. TLC and PLC were performed on precoated silica gel 60 $F_{254}$ plates (Merck); spots were detected by UV or by spraying with 1% $CeSO_4$ in 10% aq. $H_2SO_4$ followed 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*-BuOH (3×200 ml). Evaporation of the EtOAc-, *n*-BuOH- 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 alkene **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 $\beta$-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 **14** (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 (Merck, 0.040—0.063 mm) using MeCN–$H_2O$ (10 : 1) as the eluent to give **3** (9 mg), **7** (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 (Merck, 0.040—0.063 mm) using MeOH/$H_2O$ (4 : 1) as the eluent to give **8** (70 mg), **11** (14 mg), **12** (6 mg), **13** (28 mg) and **14** (11 mg). Fr. 14 was rechromatographed on Lichroprep RP-18 (Merck, 0.040—0.063 mm) using MeOH/$H_2O$ (4 : 1) as the eluent to give **9** (12 mg).

Aromatic Alkene **1**: A colorless solid, mp 35—36 °C.[2)]

(+)-Sesamin (**2**): Colorless needles, mp 117—119 °C.[5—7)]

Horsfieldin (**3**): Colorless needles, mp 156—159 °C.[8)]

1-Allyl-2-methoxy-4,5-methylenedioxybenzene (**4**): A colorless oil.[4)]

*N*-(3-Phenylpropanoyl)pyrrole (**6**): A pale yellow solid, mp 46—48 °C.[2)]

*N*-(3-Phenylpropanoyl)-$\Delta^3$-2-pyrrolidone (Sarmentamide A) (**7**): A colorless oil; UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$) nm: 209 (4.23), 230 (sh) (3.89); IR $\nu_{max}^{neat}$ cm$^{-1}$: 3027, 2923, 1725, 1692, 1601, 1441, 1380, 1271, 995, 805, 700; $^1$H-NMR ($CDCl_3$): $\delta$: 3.01 (2H, t, $J$=7.8 Hz, H-3′), 3.30 (2H, t, $J$=7.8 Hz, H-2′), 4.41 (2H, t, $J$=2.1 Hz, H-5), 6.16 (1H, dt, $J$=2.1, 6.0 Hz, H-3), 7.29 (1H, overlapped signal, H-4), 7.29 (5H, m, ArH×5); $^{13}$C-NMR ($CDCl_3$): 30.2 (C3′), 38.1 (C2′), 50.7 (C5), 126.1 (C7′), 127.7 (C3), 128.4 (C5′, C9′), 128.6 (C6′, C8′), 141.8 (C4′), 146.6 (C4), 170.0 (C2), 172.5 (C1′); MS *m*/*z* (rel. int.): 215 ($M^+$, 100%), 149 (3), 133 (40), 104 (47), 83 (27), 77 (19). HR-MS *m*/*z*: Calcd for $C_{13}H_{13}O_2N$: 215.0946. Found: 215.0946.

*N*-Cinnamoyl-*trans*-3,4-diacetoxypyrrolidine (Sarmentamide B) (**8**): A colorless wax; $[\alpha]_D^{25}$=+68.3° ($c$=0.12, MeOH); UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$) nm: 206 (4.27), 217 (4.21), 233 (sh) (4.08), 283 (4.25), 258 (3.78; IR $\nu_{max}^{neat}$ cm$^{-1}$: 2931, 1743, 1652, 1610, 1558, 1423, 1371, 1227, 1064, 764; $^1$H-NMR ($CDCl_3$): $\delta$: 2.11 (3H, s, $COCH_3$), 2.12 (3H, s, $COCH_3$), 3.80 (1H, d, $J$=12.0 Hz, Ha-2), 3.90 (2H, m, Hab-5), 4.04 (1H, dd, $J$=3.9, 12.0 Hz, Hb-2), 5.26 (1H, d, $J$=3.9 Hz, H-3), 5.27 (1H, d, $J$=3.3 Hz, H-4), 6.68 (1H, d, $J$=15.5 Hz, H-2′), 7.40 (3H, m, ArH×3), 7.55 (2H, m, ArH×2), 7.77 (1H, d, $J$=15.5 Hz, H-3′); $^{13}$C-NMR ($CDCl_3$): 20.8 ($CO\underline{CH}_3$×2), 50.1 (C5), 50.6 (C2), 73.5 (C4), 75.3 (C3), 117.6 (C2′), 128.0 (C5′, C9′), 128.9 (C6′, C8″), 130.0 (C7′), 134.9 (C4′), 143.1 (C3′), 165.1 (cinn. C=O), 170.2 ($\underline{CO}CH_3$×2); MS *m*/*z* (rel. int.): 317 ($M^+$, 2%), 257 (33), 197 (17), 131 (100), 103 (22), 77 (8). HR-MS *m*/*z*: Calcd for $C_{17}H_{19}O_5N$: 317.1262. Found: 317.1273.

*N*-(2,4,5-Trimethoxycinnamoyl)pyrrolidine (Sarmentamide C) (**9**): A colorless solid, mp 159—162 °C; UV $\lambda_{max}^{MeOH}$ (log $\varepsilon$) nm: 203 (4.25), 230 (4.29), 305 (4.26); IR $\nu_{max}^{nujol}$ cm$^{-1}$: 2939, 1649, 1603, 1582, 1506, 1416, 1330, 1243, 1125, 1004; $^1$H-NMR ($CDCl_3$): $\delta$: 1.93 (2H, quintet, $J$=6.5 Hz, H-3), 2.03 (2H, quintet, $J$=6.5 Hz, H-4), 3.62 (2H, t, $J$=6.5 Hz, H-2), 3.67 (2H, t, $J$=6.5 Hz, H-5), 3.90 (3H, s, $OCH_3$), 3.91 (6H, s, $OCH_3$×2), 6.61 (1H, d, $J$=15.4 Hz, H-2′), 6.77 (2H, s, H-6′, H-9′), 7.64 (1H, d, $J$=15.4 Hz, H-3′); $^{13}$C-NMR ($CDCl_3$): $\delta$: 24.4 (C3), 26.2 (C4), 46.3 (C2), 47.0 (C5), 56.1 ($OCH_3$), 56.2 ($OCH_3$), 60.9 ($OCH_3$), 105.2 (C6′, C9′), 118.1 (C2′), 130.9 (C4′), 140.0 (C5′), 141.8 (C3′), 153.4 (C7′, C8′), 163.9 (C=O); MS *m*/*z* (rel. int.): 291 ($M^+$, 64%), 221 (100), 191 (43), 179 (28), 163 (20), 147 (14), 105 (16), 70 (64); HR-MS *m*/*z*: Calcd for $C_{16}H_{21}O_4N$: 291.1469. Found: 291.1473.

*N*-(2*E*,4*E*-Decadienoyl)pyrrolidine (Sarmentine) (**10**): A pale yellow oil.[2)]

*N*-[9-(3,4-Methylenedioxyphenyl)-2*E*,4*E*,8*E*-nonatrienoyl]pyrrolidine (**11**): A pale yellow wax.[9)]

*N*-[9-(3,4-Methylenedioxyphenyl)-2*E*,8*E*-nonadienoyl]pyrrolidine (**12**): A pale yellow oil.[10)]

*N*-[7-(3,4-Methylenedioxyphenyl)-2*E*,6*E*-hepadienoyl]pyrrolidine (Sarmentosine) (**13**): Pale yellow needles, mp 77—79 °C.[2)]

*N*-Isobutyl-2*E*,4*E*-decadienamide (Pellitorine) (**14**): Colorless needles, mp 80—84 °C.[2)]

*N*-Isobutyl-13-(3,4-methylenedioxyphenyl)-2*E*,4*E*,12*E*-tridecatrienamide (Guineensine) (**15**): Colorless needles, mp 118—119 °C.[11,12)]

*N*-Isobutyl-15-(3,4-methylenedioxyphenyl)-2*E*,4*E*,14*E*-pentadecatrienamide (Brachystamide B) (**16**): Colorless needles, mp 115—116 °C.[13)]

**Antiplasmodial Assay** *Plasmodium falciparum* (K1, multidrug resistant strain) was cultured continuously according to the method of Trager and Jensen.[14)] The quantitative assessment of the antiplasmodial activity *in vitro* was performed by mean of the microculture radioisotope technique based upon the method described by Desjardins *et al*.[15)] Standard sample, chloroquine diphosphate ($IC_{50}$ value of 0.16 $\mu$g/ml, 0.31 $\mu$M) was used as reference compound for the assay.

**Antimycobacterial Assay** The antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H37Ra using the Microplate Alamar Blue Assay (MABA).[16)] Standard drugs, isoniazide (MIC of 0.04—0.09 $\mu$g/ml) and kanamycin sulfate (MIC of 2.0—5.0 $\mu$g/ml) were used as reference compounds for the assay.

**Antifungal Actvity Assay** The isolated compounds were tested for their antifungal activity against a clinical isolate of *Candida albicans* using a method modified from the soluble formazan assay.[17)] Briefly, 100 $\mu$l of $2\times10^6$ CFU/ml *C. albicans* in RPMI 1640 medium, containing 34.53 g/ml 3-[*N*-morpholino]propanesulfonic acid (MOP) was added to each well of 96-well microculture plates containing 100 $\mu$l of test compound diluted in 10% dimethylsulfoxide (DMSO). Plates were incubated at 37 °C for 4 h before adding 50 $\mu$l of a solution containing 1 mg/ml 2,3-bis-[2-methoxy-4-nitro-5-sulfonylphenyl]-5-[(phenylamino)carbonyl]-2*H*-tetrazolium hydroxide (XTT tetrazolium) and 0.025 mM *N*-methylphenazolium methosulfate (PMS). After an additional 4 h incubation at 37 °C, the number of living cells was determined by measuring the absorbance of XTT formazan at 450 nm. Amphotericin B and 10% DMSO were used as and negative controls, respectively. In our system, the $IC_{50}$ value of the standard drug, Amphotericin B, was 0.01 $\mu$g/ml.

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