Infraspecific Variation of Sulfur-Containing Bisamides from *Aglaia leptantha*

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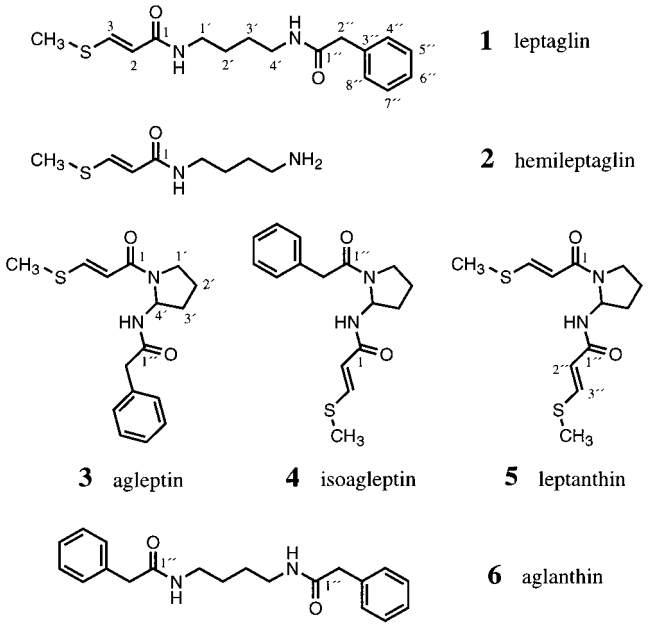
Six new amides, leptaglin (**1**), hemileptaglin (**2**), aglanthin (**3**), agleptin (**4**), isoagleptin (**5**), and leptanthin (**6**), together with known lignans yangambin, eudesmin, grandisin (**7**), epigrandisin (**8**), and dehydrodiconiferyl alcohol, were isolated and identified from the lipophilic leaf and stem bark extracts of *Aglaia leptantha*. The dominating sulfur-containing bisamides contained either putrescine or the corresponding pyrrolidine ring as the diamine part, linked to phenylacetic and/or methylthiopropenoic acid moieties showing a remarkable infraspecific variation in eight individuals from two different habitats. Structures were determined by MS and NMR, including lanthanide-induced shifts.

During the course of taking an inventory of the biogenetic capacity within the genus *Aglaia* (Meliaceae), we routinely compared lipophilic crude extracts from many different species collected in southeast Asia. Based on literature and unpublished results from our laboratory, the genus is mainly characterized by the occurrence of bisamides and flavaglines, including cyclopenta[*b*]benzofurans, cyclopenta[*bc*]benzopyrans, and benzo[*b*]oxepines.[1−5] Various lignans, triterpenoids, and flavonoids also contribute to the chemical profiles obtained from different species and organs. The biologically very active cyclopenta[*b*]-benzofurans mainly accumulated in the roots and stem bark, whereas bisamides occurred mainly in the leaves.[3] In those species where no flavaglines could be detected, lignans often accumulated in all three organs and were frequently accompanied by bisamides.

Based on HPLC profiles and typical UV spectra obtained by parallel diode array detection, the lipophilic extracts from different organs (leaves, stem bark, and root bark) of *A. leptantha* Miq. were characterized by bisamides and lignans from which the former mainly accumulated in the leaves and the latter in the roots. The stem bark, at least from young trees (2−4 m tall), showed an intermediate composition accumulating both bisamides and lignans. Collections from eight individuals from two different habitats in southeast Thailand revealed nearly identical lignan profiles, but showed remarkable infraspecific variation of different amides. Common to all, however, is the accumulation of sulfur-containing derivatives indicating a species specific biogenetic trend.

Five novel sulfur-containing amides (**1**−**5**) were isolated from the leaves and stem bark, together with a sulfur-free derivative known previously as a synthetic product (**6**).[6] A series of lignans was also isolated, which were classified into three groups: the widespread derivatives yangambin and eudesmin, two tetrahydrofurofuran (sesamin)-type lignans; the inseparable stereoisomers of grandisin,[7] **7** and **8**, a substituted furan-type lignan; and dehydrodiconiferyl alcohol,[8,9] a neolignan. Of special interest, however, was the formation of the sulfur-containing amides **1**−**5**, from which four were shown to be bisamides (**1**, **3**−**5**). According to our previous report on *Aglaia*,[3] all derivatives contained either putrescine or the corresponding pyrrolidine ring as the diamine part. These compounds, to which we have assigned the trivial names leptaglin (**1**), hemileptaglin (**2**), agleptin (**3**), isoagleptin (**4**), and leptanthin (**5**), represent the first sulfur-containing amides in the family Meliaceae. Amides with sulfur-containing acid moieties have been reported previously from the genus *Glycosmis* (Rutaceae)[10,11] and the legume *Entada phaseoloides* (L.) Merr.,[12] but in these cases no bisamides were detected. Because the non-sulfur-containing amide **6** was previously only prepared as a side product in a kinetic study,[6] and its structure only deduced from synthetic arguments and elemental analysis, we report here on this compound as a natural product on the basis of spectroscopic data and named it aglanthin (**6**).



Results and Discussion

Ten compounds were isolated from the chloroform fraction of the methanolic crude extracts of the leaves and stem bark of three different individuals of *A. leptantha* (HG505, HG506, HG574), collected in the same habitat (Khao Soi

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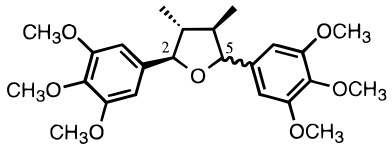
Dao, southeast Thailand), by preparative MPLC based on UV detection. The chemical profiles of five further individuals from different habitats were also analyzed by HPLC comparison with authentic samples. Aside from the known lignans eudesmin and yangambin from the leaves, and yangambin and dehydrodiconiferyl alcohol from the stem bark, an inseparable pair of stereoisomeric furan-type lignans (**7** and **8**) was also isolated from the bark with a 2:1 ratio of (+)-grandisin (**7**) to **8** on the basis of ^{1}H NMR integration. (+)-Grandisin (**7**) was already reported in the Piperaceae,[13] whereas the latter is only known so far as a synthetic product.[14]



7 5Hβ (+)-grandisin
8 5Hα epigrandisin

Comparing the amide patterns of eight different individuals, two of them, originating from Khao Soi Dao (HG505, HG508) clearly deviated by the accumulation of **1** and **2** in the leaves and stem bark, whereas the others were characterized by a preponderance of amides **3** and/or **4**, together with smaller amounts of **5**. Amide **6**, by contrast, could be detected in all individuals, characterized by an inconspicuous UV spectrum with a very weak maximum at 260 nm (MeOH). The UV spectra of **1** and **2** were very similar, with strong maxima at 270 (**1**) and 272 nm (**2**). However, **2** could be distinguished by an additional weak maximum at 225 nm, which was also observed in **5**, indicating the absence of an aromatic acid moiety. The two isomers **3** and **4** were discriminated by different maxima at 282 and 272 nm, respectively. IR spectra of the isolated amides were characterized by >N−H stretching at 3412−3442 cm^{-1} ($CHCl_3$), typical for secondary amides, and by dominating signals at 1620−1670 cm^{-1} and 1496−1582 cm^{-1} for the >N−C=O/C=C stretching region. The presence of a pyrrolidine ring in compounds **3**−**5** was indicated by a characteristic broad signal at 1410−1412 cm^{-1}.

The ^{1}H NMR spectra of compounds **1** and **2** showed several common features, from which the characteristic olefinic resonances at δ 7.54 and 5.80−5.81 (two doublets with J = 14.7−14.8 Hz) and the methyl signal at δ 2.34 (s) were already well-known from a series of (*E*)-3-(methylthio)-propenoic acid amides recently isolated in our laboratory from different *Glycosmis* species.[15] The ^{13}C NMR spectra also agreed perfectly with the known data of this acid moiety, and the EIMS of both compounds were dominated by a 100% peak for the stable CO−CH=CH−SMe ion (*m*/*z* 101). However, **1** showed a second carbonyl function in the ^{13}C NMR spectrum, and two amide NH resonances in the ^{1}H NMR (Table 1). In the ^{1}H NMR spectrum, the four methylene groups of the putrescine moiety appeared as two broad multiplets of 4 H each (δ 1.52 and 3.21), and in the ^{13}C NMR spectrum the characteristic four triplets were found in two narrow groups (δ 40.0/40.2 and 27.8/27.9). These data agreed perfectly with putrescine, known to be a typical component of many other bisamides of *Aglaia* species.[3,16] The second acid moiety of bisamide **1** was characterized by a phenyl residue (relatively narrow m of 5H and δ 7.28), and a benzylic CH_2 group α to an amide carbonyl function (singlet of 2H at δ 3.48 in the ^{1}H NMR spectrum, and a corresponding triplet at δ 43.9 in the ^{13}C NMR spectrum). In the EIMS of **1**, the benzyl unit of the phenylacetic amide moiety was indicated by an intense tropylium ion peak at *m*/*z* 91. The molecular mass of $C_{16}H_{22}N_2O_2S$ (HRMS) confirmed the structure of a methylthiopropenoic phenylacetic putrescine bisamide for **1**. Compound **2** was shown to be a simple 3-methylthiopropenoic putrescine amide containing one free amino group and named hemileptaglin. The ^{1}H and ^{13}C chemical shifts of the putrescine methylene groups and the (*E*)-3-(methylthio)propenoic unit were very similar to the corresponding data of **1**; however, a second acid moiety was missing. The proposed structure was also supported by the MS data, which were characterized by the molecular mass peak at *m*/*z* 188, a prominent M−NH_3 fragment at *m*/*z* 171, and the parent peak at *m*/*z* 101.

In compounds **3**−**5** the ^{1}H and ^{13}C NMR spectra again showed characteristic signals for (*E*)-3-(methylthio)propenoic amides.[15] In **6** these characteristic signals appeared twice, indicating a second methylthiopropenoic amide moiety in the molecule (two amide CO resonances at δ 166.2 and 165.7 in the ^{13}C NMR spectrum). The second acid components in **3** and **4** were phenylacetic acid moieties as in **1** and **6**. This was clear from the ^{1}H and ^{13}C resonances by comparison of **1** and **6** with compounds **3** and **4**. The amine parts of **3**−**5** were 2-aminopyrrolidine systems, which were derived from putrescine by cyclization. A series of 2-aminopyrrolidine bisamides has already been described from several *Aglaia* species.[16−21] The ^{1}H and ^{13}C NMR data of the pyrrolidine ring of these bisamides agreed very well with the corresponding data of compounds **3**−**5**.[3,16] Assignments of the NMR resonances for **3**−**5**, were derived by means of 2D NMR (C−H COSY and HMBC long-range C−H correlation) and lanthanide-induced shift (LIS) measurements (see below). The HRMS and EIMS peaks for M^+, CO−CH=CH−SMe (*m*/*z* 101), M^+ −101, and Ph−CH_2^+ (tropylium *m*/*z* 91, only for **3** and **4**) confirmed the structures of the obviously isomeric methylthiopropenoic-phenylacetic 2-aminopyrrolidine bisamides **3** and **4** and the structure of the bis(methylthiopropenoic) 2-aminopyrrolidine bisamide **5**.

The ^{1}H and ^{13}C NMR spectra of compound **6** were rather simple. The characteristic methylene signals of a symmetrically substituted putrescine unit and the resonances for two phenylacetic moieties were conclusive for the symmetrical structure of the bis(phenylacetic) putrescine bisamide. Although amide **6** was known as a synthetic compound,[6] this is the first report of **6** as a natural product.

In the case of the isomers agleptin (**3**) and isoagleptin (**4**), the question of which acid is connected to which nitrogen atom was still open. Neither NOESY nor HMBC was able to give an answer, because no cross-peaks between the pyrrolidine ring atoms and the atoms of any one of the two acid moieties could be detected. However, the results of LIS using Eu(fod)$_3$ as reagent gave a clear-cut answer. Complexation of the reagent to the tertiary amide carbonyl of the pyrrolidine ring is favored clearly over the coordination to the secondary amide carbonyl attached to the 2-amino position of pyrrolidine. This followed from the relatively high LIS values ($\Delta\delta$) of the protons H_2-1′ and H-4′ in the lanthanide-doped ^{1}H NMR spectra of **3** and **5**. The values of $\Delta\delta$ 2.10 and 2.00 ppm for H_2-1′ of **3** and **5** were actually the largest ones within the molecule. This is compatible only with highly preponderant coordination at the carbonyl oxygen next to the ring nitrogen. Because in **3** the LIS values for H-2 (1.08) and H-3 (1.98) of the methylthiopropenoic acid moiety were also large compared to the benzylic H_2-2″ (0.38) and the aromatic protons (0.10−

0.20), the methylthiopropenoic acid moiety is obviously attached to the ring nitrogen and the phenylacetic acid to the 2-amino group. As a consequence, the structure of isoagleptin (**4**) is characterized by reversely attached acid moieties: phenylacetic acid at the ring nitrogen and methylthiopropenoic acid at the 4′-amino group of the pyrrolidine ring. This was confirmed by the high LIS values of the phenylacetic acid protons and the low LIS values for the methylthiopropenoic moiety in **4**. The LIS data of **3** were already conclusive for the structure shown. However, additional support was derived from the LIS data of topologically related fatty acid pyrrolidides and pyrrolideides from *Achillea falcata* L. (Asteraceae−Anthemideae), with a double bond in conjugation to the amide carbonyl group.[22] The relative LIS values of topologically corresponding protons of the N-containing five-membered ring and acid side chain agreed very well. For all compounds (**3**, **5**, and the previously published amides from *Achillea*[22]), the LIS effects for comparable positions followed the same order: 1′ > 3 > 2 > 4′ > 2′/3′. The LIS data for **6**, with two identical methylthiopropenoic acid residues at both possible amino functions, agreed again with the results concerning the favored complexation at the tertiary amide carbonyl. This followed from the relative LIS values of the two identical acid moieties. The values were 4−5 times larger for H-2, H-3, and SCH_3-3 compared to the LIS values of H-2″, H-3″, and SCH_3-3″, allowing also a clear-cut assignment of all chemical shifts for the two sets of methylthiopropenoic resonances of **6**.

The lignans were identified on the basis of their ^1H and ^{13}C NMR spectra.[24,25] The epimeric mixture of **7** and **8** was composed of (+)-grandisin (**7**) with 33% of a second stereoisomeric form (**8**). Contrary to (−)-grandisin, the enantiomeric (+)-grandisin (**7**) was mentioned only once in the literature,[13] and an epimer of grandisin has never been isolated from natural sources. Comparison of the ^1H NMR resonances of component **8** with the data of a synthetic[14] and a semisynthetic product (methylation of the related eupobennetin[23]) proved that **8** was an epimer of grandisin, with a change in the configuration of the trimethoxyphenyl substituent at C-5. The absolute configurations of grandisin have not been determined; however, in analogy with the known absolute configurations of the closely related galbelgin (3,4-dimethoxyphenyl substituents at C-2 and C-5) and galbacin (3,4-methylenedioxy),[26] the absolute configurations of (+)-grandisin (**7**) are (2*R*,3*R*,4*R*,5*R*) and, consequently, (2*R*,3*R*,4*R*,5*S*) for the corresponding epimeric epigrandisin (**8**), as shown in the formula scheme. The optical rotation of epigrandisin was calculated from the optical rotation of the mixture of the known component **7** with **8** (see Experimental Section).

Based on the HPLC profiles it became apparent that the pyrrolidine-type bisamides agleptin (**3**) and isoagleptin (**4**) were the most widespread major components in *A. leptantha*. Although this pair of isomers could not be sufficiently separated by HPLC, either with MeOH−H_2O or acetonitrile−H_2O gradients, more detailed inspections of the UV spectra allowed us to distinguish between both components and show that **4** was more abundant. It dominated in three individuals (HG561, HG574, HG680), whereas in two others (HG575, HG681), mixtures of both isomers (**3**, **4**) were detected. Only one individual (HG506) deviated by the predominance of agleptin (**3**). All these samples were additionally characterized by the pyrrolidine-type leptanthin (**5**), always co-occurring as a minor component. In contrast to previous findings in the genus *Aglaia*, where bisamides were always shown to be linked to two different acid moieties,[3,16,18−21,30] this derivative was characterized by two identical acid groups, consisting of the rather rare methylthiopropenoic acid. With regard to the previously known bisamides of *Aglaia*, neither the more widespread pyrrolidine-type odorines[18,19] and piriferines[3,20] nor the frequently occurring putrescine-type pyramidatin[3,16] could be detected in the present investigation.

It is of some biogenetic interest that in two individuals (HG505, HG508), the dominating pyrrolidine-type amides **4**, **5**, and **6** were replaced by the putrescine-type amides, leptaglin (**1**) and hemileptaglin (**2**). In this case **1** was the dominating amide in the HPLC profiles of the leaves and may be regarded as the biosynthetic precursor for the major components isoagleptin (**5**) and agleptin (**4**), formed by different cyclization processes of the linear putrescine moiety. These biosynthetic connections were also suggested by the co-occurrence of both types in individual HG574, where, apart from the dominating pyrrolidine-type amides **5** and **6**, small amounts of the putrescine-type derivatives **1** and **2** could also be detected.

In contrast to many other Aglaia species, the crude extracts of *A. leptantha* did not show strong activities against the test insect *Spodoptera littoralis*; however, moderate antifungal activity was observed with pure **4**, using bioautographic tests on TLC plates with spore suspensions of the pathogenic microfungus *Cladosporium herbarum*. Apart from the already described cytotoxic[16,21] and antiviral potential,[31] bisamides may also play an important role as building blocks in the biosynthesis of the genus-specific flavaglines.[1,3−5] The chemical polymorphism of bisamide composition within *A. leptantha* has a strong resemblance to that already described for the sulfur-containing amides in *Glycosmis* (Rutaceae) species.[11,32] In this case, the varying composition of the biologically very active amides was discussed as a possible survival strategy to prevent adaptation of herbivors or microorganisms to a uniform chemical profile.[11]

Experimental Section

General Experimental Procedures. Optical rotation, Perkin-Elmer polarimeter 241; UV, Hewlett-Packard 8452A diode array spectrophotometer; IR, Perkin-Elmer 16PC FT−IR; NMR, Bruker AM 400 WB; MS, Finnigan MAT 900 S; HPLC, Hewlett-Packard 1090 II, UV diode array detection at 230 nm, column 250 × 4 mm, Hypersil BDS C_{18}, 5 μm, mobile phase MeOH (gradient 60−100%) and acetonitrile (gradient 20−60%) in aqueous buffer (0.015 M *o*-phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 mL/min.

Plant Material. Leaves, stem bark, and root bark from eight individuals of *A. leptantha* were collected separately from Khao Soi Dao (near Chantaburi, southeast Thailand), five individuals (HG505, HG506, HG508, February 1998; HG574, HG575, July 1998), and from Khao Khieo (near Chonburi, southeast Thailand), three individuals (HG561, July 1998; HG680, HG681, March 1999). Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and Isolation. Dried parts of *A. leptantha* were ground and extracted with MeOH at room temperature for 5 days, filtered, and concentrated. The aqueous residue was extracted with $CHCl_3$. The $CHCl_3$ fractions were evaporated to dryness and roughly separated by column chromatography (Merck Si gel 60, 35−70 mesh) using solvent mixtures of increasing polarity (EtOAc in hexane and MeOH), and further by preparative MPLC (400 × 40 mm column, Merck LiChroprep silca 60, 25−40 μm, UV detection, 254 nm), also using mixtures of EtOAc in hexane. Preparative TLC (Merck, Si gel 60, 0.5 mm) with mixtures of CH_2Cl_2−MeOH was used to finally purify the compounds.

Compounds 1, 2, 6, 7, 8, and Dehydrodiconiferyl Alcohol. A portion (710 mg) of the $CHCl_3$ fraction from 90 g of dried stem bark of individual HG505 was separated roughly by column chromatography (Si gel). The fractions eluted with 50% EtOAc in hexane and 100% EtOAc mainly contained lignans (160 mg), whereas bisamides were eluted with more polar MeOH fractions (350 mg). Further separation of the lignan fractions (160 mg) by MPLC with 30% EtOAc in hexane yielded 15 mg of **6** and 10 mg of impure **7** and **8**, which were further purified by TLC with 2% MeOH in CH_2Cl_2 to obtain 4 mg of material (**7** and **8**). The fractions eluted with MeOH (350 mg) were further separated by MPLC with 100% EtOAc to give 34 mg of **1**, 30 mg of **2**, and 11 mg of impure dehydrodiconiferyl alcohol, which was purified by TLC with 2% MeOH in CH_2Cl_2 to yield 3 mg of pure dehydrodiconiferyl alcohol.

Compounds 4−6, Yangambin, and Eudesmin. A portion (1200 mg) of the $CHCl_3$ fraction from 45 g of dried leaves of individual HG574 was separated roughly with Si gel chromatography. The fractions eluted with 50% EtOAc in hexane (110 mg) were further separated by MPLC with 30% EtOAc in hexane to yield 6 mg of yangambin and 8 mg of eudesmin. The fractions eluted with 100% EtOAc and 50% EtOAc in MeOH (435 mg), containing the bisamides, were further separated by MPLC with 100% EtOAc to afford 128 mg of **4** and a mixture (60 mg) of **5** and **6**, from which **6** was obtained by crystallization (5 mg) and preparative TLC of the mother liquor (3 mg), together with 35 mg of **5**.

Compounds 3 and 5. A portion (210 mg) of the $CHCl_3$ fraction from 21 g of dried stem bark of individual HG506 was separated roughly with Si gel chromatography. The bisamide-containing fractions (100% EtOAc and MeOH, 105 mg) were further separated by MPLC with 100% EtOAc to yield 19 mg of **3** and 13 mg of **5**.

Leptaglin, (*E*)-*N*$_1$-[3-(Methylthio)propenoyl]-*N*$_2$-(phenylacetyl)-1,4-butanediamide (1): UV (MeOH) λ_{max} (log ϵ) 270 (4.19) nm; IR ($CHCl_3$) ν_{max} 3436 m, 3338 w, 2926 m, 2854 w, 1654 s, 1580 s, 1518 s, 1454 w, 1436 w, 1328 w, 1254 m, 1190 w, 992 w, 942 m, 842 w cm^{-1}; ^{1}H NMR (MeOH-d_4) d 7.54 (d, 1H, J = 14.8 Hz, H-3), 7.25−7.30 (m, 5H, H-4″-H-8″), 5.80 (d, J = 14.8 Hz, H-2), 3.48 (s, 2H, H-2″), 3.21 (m, 4H, H-1′ and H-4′), 2.34 (s, 3H, SMe), 1.52 (m, 4H, H-2′ and H-3′); ^{13}C NMR (MeOH-d_4) δ 174.1 (s, C-1″), 167.3 (s, C-1), 143.4 (d, C-3), 137.1 (s, C-3″), 130.0 and 129.6 (2 × d, C-4″/-8″ and C-5″/-7″), 127.9 (d, C-6″), 116.8 (d, C-2), 43.9 (t, C-2″) 40.2 and 40.0 (2 × t, C-1′ and C-4′), 27.9 and 27.8 (2 × t, C-2′ and C-3′), 14.3 (q, SMe); HMBC, H-2 (C-1), H-3 (C-1, S*C*H$_3$), H-2″ (C-1″, C-3″, C-4″/-8″), H-1′ + H-4′ (C-1, C-2′, C-3′, C-1′), EIMS (70 eV) *m*/*z* 306 (10, M$^+$), 233 (9), 215 (9), 189 (20), 171 (10), 124 (15), 101 (100, $^+$CO−CH=CH−SMe), 91 (77), 70 (64); HREIMS *m*/*z* 306.1402 (calcd for $C_{16}H_{22}N_2O_2S$, 306.1402).

Hemileptaglin, (*E*)-*N*-[3-(Methylthio)propenoyl]-4-amino-1-butanamide (2): UV (MeOH) λ_{max} (log ϵ) 272 (3.96), 225 (3.55) nm; IR ($CHCl_3$) ν_{max} 3442 m, 3328w, 2925 m, 2853 w, 1652 s, 1580 s, 1515 m, 1456 w, 1437 w, 1327 w, 1254 m, 1189 w, 1126 w, 1080 w, 941 m, 844 w cm^{-1};^{1}H NMR (MeOH-d_4) δ 7.54 (d, 1H, J = 14.7 Hz, H-3), 5.81 (d, J = 14.7 Hz, H-2), 3.25 (m, 4H, H-1′ and H-4′), 2.34 (s, 3H, SMe), 1.55 (m, 4H, H-2′ and H-3′); ^{13}C NMR (MeOH-d_4) δ 167.4 (s, C-1), 143.4 (d, C-3), 116.7 (d, C-2), 43.9 (t, C-2″), 40.0 (t, C-1′ and C-4′), 27.9 (t, C-2′ and C-3′), 14.3 (q, SMe); EIMS (70 eV) *m*/*z* 188 (5, M$^+$), $C_8H_{16}N_2OS$, 171 (65, M$^+$ − NH$_3$), 124 (58), 101 (100, $^+$CO−CH=CH−SMe), 73 (56), 70 (67).

Agleptin, (*E*)-*N*-[3-(Methylthio)propenoyl]-2-(phenylacetylamino)pyrrolidine (3): mp 121−123 °C; $[\alpha]^{20}_D$ −3°, $[\alpha]^{20}_{436}$ −12° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 282 (4.08) nm; IR ($CHCl_3$) ν_{max} 3412 m, 3270 w, 2926 m, 2880 w, 1670 s, 1622 s, 1564 s, 1496 m, 1454 w, 1410 s, 1360 w, 1324 w, 1296 m, 1172 m, 1120 w, 1076 w, 1016 w, 978 w, 962 w, 944 m, 918 w, 882 m cm^{-1};^{1}H NMR (MeOH-d_4) δ 7.63 (d, 1H, J = 14.4 Hz, H-3), 7.23−7.30 (m, 5H, H-4″−H-8″), 5.95 (d, 1H, J = 14.4 Hz, H-2), 5.94 (br d, 1H, J = 6.8 Hz, H-4′), 3.66 (m, 1H, H-1′a), 3.52 (d, 1H, J = 13.7 Hz, H-2″a), 3.43 (d, 1H, J = 13.7 Hz, H-2″b), 3.40 (m, 1H, H-1′b), 2.20 (m, 1H, H-3′a), 2.00 (m, 2H, H-2′), 1.93 (s, 3H, SMe), 1.92 (m, 1H, H-3′b); ^{13}C NMR (MeOH-d_4) δ 146.5 (d, C-3), 130.0 and 129.7 (2 × d, C-4″/-8″ and C-5″/-7″), 128.0 (d, C-6″), 114.0 (d, C-2), 64.2 (d, C-4′), 47.0 (t, C-1′), 43.8 (t, C-2″), 35.0 (t, C-3′), 22.5 (t, C-2′), 14.0 (q, SMe); ^{1}H NMR ($CDCl_3$) δ 7.73 (d, 1H, J = 14.4 Hz, H-3), 7.20−7.38 (m, 5H, H-4″−H-8″), 6.02 (d, 1H, J = 14.4 Hz, H-2), 5.96 (br m, 1H, H-4′), 5.81 (br s, 1H, NH), 3.59 (m, 1H, H$_a$-1′), 3.54 (s, 2H, H-2″), 3.42 (m, 1H, H$_b$-1′), 2.20 (s, 3H, SMe-3), 2.15 (m, 1H, H$_a$-3′), 1.90 (m, 3H, H$_{a,b}$-2′ and H$_b$-3′), ^{1}H LIS NMR ($CDCl_3$) H-2 (1.08 ppm), H-3 (1.98), SMe-3 (0.32), H$_2$-1′ (2.10), H$_2$-2′ and -3′ (ca 0.36), H-4′ (1.20), H$_2$-2″(0.38), H-4″−H-8″ (ca. 0.10−0.20); the LIS were determined by adding increasing amounts of Eu(fod)$_3$ (Merck) to a solution of ca. 3 mg substrate in 0.5 mL $CDCl_3$ and extrapolation to a ratio substrate to reagent 1:1; due to the low substrate concentration ([S$_o$] < 0.01 M and the unfavorable complex binding constant, the LIS values were much smaller than for the true 1:1 complex[27−29]); EIMS (70 eV) *m*/*z* 304 (8, M$^+$), 257 (5), 203 (28, M$^+$ −101), 185 (8), 169 (12), 149 (16), 122 (7), 101 (100, $^+$CO−CH=CH−SMe), 91 (32), 85 (34), 70 (32), 57 (14); HREIMS *m*/*z* 304.1245 (calcd for $C_{16}H_{20}N_2O_2S$, 304.1246).

Isoagleptin, (*E*)-2-[3-(Methylthio)propenoylamino]-*N*-(phenylacetyl)pyrrolidine (4): mp 149−151 °C; $[\alpha]^{20}_D$ −2°, $[\alpha]^{20}_{436}$ −7° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 272 (4.26) nm; IR ($CHCl_3$) ν_{max} 3430 m, 3304 w, 2926 m, 2880 w, 1640 s, 1582 s, 1496 m, 1456 w, 1412 m, 1324 w, 1182 m, 1156 m, 1118 w, 1076 w, 1008 w, 964 w, 942 m, 918 w, 858 m cm^{-1};^{1}H NMR (MeOH-d_4) δ 7.61 (d, 1H, J = 14.6 Hz, H-3), 7.20−7.30 (m, 5H, H-4″−H-8″), 5.99 (br d, 1H, J = 5.5 Hz, H-4′), 5.70 (d, 1H, J = 14.6 Hz, H-2), 3.77 (d, 1H, J = 14.8 Hz, H-2″a), 3.67 (d, 1H, J = 14.8 Hz, H-2″b), 3.62 (m, 1H, H-1′a), 3.43 (m, 1H, H-1′b), 2.34 (s, 3H, SMe), 1.80−2.20 (m, 4H, H-2′ and H-3′); ^{13}C NMR (MeOH-d_4) δ 145.0 (d, C-3), 130.1 and 129.5 (2 × d, C-4″/-8″ and C-5″/-7″), 127.8 (d, C-6″), 116.0 (d, C-2), 65.2 (d, C-4′), 47.1 (t, C-1′), 41.7 (t, C-2″), 35.2 (t, C-3′), 22.3 (t, C-2′), 14.4 (q, SMe); ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1H, J = 14.6 Hz, H-3), 7.20−7.32 (m, 5H, H-4″−H-8″), 5.97 (br dd, 1H, J = 7.0, 7.0 Hz, H-4′), 5.70 (br s, 1H, NH), 5.43 (d, 1H, J = 14.6 Hz, H-2), 3.75 (d, 2H, J = 14.6 Hz, H$_a$-2″), 3.65 (d, 2H, J = 14.6 Hz, H$_b$-2″), 3.60 (m, 1H, H$_a$-1′), 3.40 (m, 1H, H$_b$-1′), 2.28 (s, 3H, SMe-3), 2.07 (m, 1H, H$_a$-3′), 1.92 (m, 3H, H$_{a,b}$-2′ and H$_b$-3′); ^{1}H LIS NMR ($CDCl_3$) H-2 ($\Delta\delta$ 0.63 ppm), H-3 (0.70), SMe-3 (0.11), H$_2$-1′ (1.61), H$_2$-2′ and 3′ (ca 0.49), H-4′ (1.89), NH (0.84), H$_2$-2″ (1.68), H-8″/-4″(ca 1.19), H-5″−H-7″ (ca 0.35); EIMS (70 eV) *m*/*z* 304 (9, M$^+$), 203 (16, M$^+$ − 101), 187 (32), 185 (15), 169 (6), 118 (29), 101 (100, $^+$CO−CH=CH−SMe), 91 (41), 85 (32), 70 (65); HREIMS *m*/*z* 304.1246 (calcd for $C_{16}H_{20}N_2O_2S$, 304.1246).

Leptanthin, (*E*,*E*)-*N*-(3-Methylthiopropenoyl)-2-(3-methylthiopropenoylamino)pyrrolidine (5): $[\alpha]^{20}_D$+9°, $[\alpha]^{20}_{436}$ +29° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 273 (4.26), 226 (3.81) nm; IR ($CHCl_3$) ν_{max} 3428 m, 3269 w, 2924 m, 2852 w, 1658 m, 1620, s, 1579 s, 1529 w, 1504 w, 1412 m, 1354 w, 1324 w, 1297 w, 1251 w, 1154 w, 1119 w, 1010 w, 943 m, 885 w, 855 w cm^{-1}; ^{1}H NMR (MeOH-d_4) δ 7.67 (d, 1H, J = 14.4 Hz, H-3), 7.66 (d, 1H, J = 14.6 Hz, H-3″), 6.16 (d, 1H, J = 14.4 Hz, H-2), 6.03 (br d, 1H, J = 6.0 Hz, H-4′), 5.79 (d, 1H, J = 14.6 Hz, H-2″), 3.62 (m, 1H, H-1′a), 3.42 (m, 1H, H-1′b), 2.35 (s, 3H, SMe-3″), 2.32 (s, 3H, SMe-3), 2.21 (m, 1H, H-3′a), 2.01 (m, 2H, H-2′), 1.92 (m, 1H, H-3′b); ^{13}C NMR (MeOH-d_4) δ 166.2 (s, C-1), 165.7 (s, C-1″), 146.4 (d, C-3), 145.2 (d, C-3″), 116.0 (d, C-2″), 114.3 (d, C-2), 64.1 (d, C-4′), 46.9 (t, C-1′), 35.1 (t, C-3′), 22.5 (t, C-2′), 14.5 and 14.4 (2 × q, 2 × SMe); HMBC, SCH$_3$-3 (C-3), SCH$_3$-3″ (C-3″), H-2 (C-1, C-3), H-2″ (C-1″, C-3″), H-4′ (C-3′, C-1′); ^{1}H NMR ($CDCl_3$) δ 7.75 (d, 1H, J = 14.4 Hz, H-3), 7.70 (d, 1H, J = 14.6 Hz, H-3″)′, 6.38 (br s, 1H, NH), 6.11 (d, 1H, J = 14.4 Hz, H-2), 6.05 (br m, 1H, H-4′), 5.67 (d, 1H, J = 14.6 Hz, H-2″), 3.57 (m, 1H, H$_a$-1′), 3.40 (m, 1H, H$_b$-1′), 2.33 (s, 3H, SMe-3″), 2.31 (s, 3H, SMe-3), 2.17 (m, 1H, H$_a$-3′), 1.93 (m, 3H, H$_{a,b}$-2′ and H$_b$-3′); ^{1}H LIS NMR ($CDCl_3$) H-2 (1.24 ppm), H-3 (1.72), SMe-3 (0.36), H$_2$-1′ (ca 2.0), H$_2$-2′ and −3′ (ca 0.42), H-4′ (1.50), H-2″ (0.25), H-3″ (0.42), SMe-3″ (0.07); EIMS (70 eV) *m*/*z* 286 (9, M$^+$), 239 (5), 185 (28) M$^+$ − 101, 169 (12), 122 (5), 101 (100) CO−CH=CH−SMe, 85 (49), 73 (22), 70 (22); HREIMS *m*/*z* 286.0810 (calcd for $C_{12}H_{18}N_2O_2S$, 286.0810).

Aglanthin, N_1,N_2-bis(phenylacetyl)-1,4-butanediamide (6): mp 175−178 °C; UV (MeOH) λ_{max} (log ϵ) 260 (2.75) nm; IR ($CHCl_3$) ν_{max} 3430 m, 3242 m, 2932 m, 2874 w, 1656 s, 1626 m, 1602 w, 1522 w, 1518 s, 1496 m, 1476 w, 1454 m, 1350 w, 1318 w, 1272 m, 1156 w, 1076 w, 994 w, 918 w cm^{-1}; ^1H NMR (MeOH-d_4) δ 7.22−7.30 (m, 10H, H-4″−H-8″), 3.47 (s, 4H, H-2″), 3.16 (m, 4H, H-1′ and H-4′), 1.48 (m, 4H, H-2′ and H-3′); ^{13}C NMR (MeOH-d_4) δ 174.1 (s, C-1″), 137.2 (s, C-3″), 130.0 and 129.6 (2 × d, C-4″/-8″ and C-5″/-7″), 127.9 (d, C-6″), 43.9 (t, C-2″), 40.2 (t, C-1′ and C-4′), 27.7 (t, C-2′ and C-3′); EIMS (70 eV) *m*/*z* 324 (55, M^+), 233 (76), 207 (31), 188 (40), 176 (38), 149 (22), 115 (74), 91 (100), 70 (68), 65 (64), 55 (65); HREIMS *m*/*z* 324.1836 (calcd for $C_{20}H_{24}N_2O_2$, 324.1838).

(+)-Grandisin[13] and Epigrandisin as a 2:1 Mixture [(2*R*,3*R*,4*R*,5*R*)- and (2*R*,3*R*,4*R*,5*S*)-3,4-Dimethyl-2,5-bis-(3,4,5-trimethoxyphenyl)tetrahydrofuran] (7 and 8): $[\alpha]^{20}_D$ +33°for the mixture (*c* 0.2, $CHCl_3$; for grandisin[13] = +57°; taking into account the usual deviations in the measurements of optical rotations, this allowed to estimate an $[\alpha]^{20}_D$ value of about −15 ± 10° for epigrandisin).

Grandisin (7): ^1H and ^{13}C NMR data, see Barbosa-Filho et al.[25]

Epigrandisin (8): ^1H NMR ($CDCl_3$) δ 6.75 (s, 2H, H-2′/-6′), 6.56 (s, 2H, H-2″/-6″), 5.11 (d, 1H, J = 8.7 Hz, H-5), 4.43 (d, 1H, J = 9.1 Hz, H-2), 3.88, 3.86, 3.84, and 3.835 (4 × s, 6 × 3H, 6 × OCH_3), 2.26 (m, 1H, H-4), 1.80 (m, 1H, H-3), 1.12 (d, 3H, J = 6.6 Hz, CH_3-3), 0.70 (d, 3H, J = 7.1 Hz, CH_3-4); ^{13}C NMR ($CDCl_3$) δ 104.2 (d, C-2″/-6″), 103.6 (d, C-2′/-6′), 87.4 (d, C-2), 83.3 (d, C-5), 60.9 (q, OCH_3-4′ and -4″), 56.1 (q, OCH_3-3′/-5′ and -3″/-5″), 47.9 and 46.1 (2 × d, C-3 and C-4), 14.9 and 14.4 (2 × q, CH_3-3 and CH_3-4).

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Note added in proof: During the processing of our manuscript for the *Journal of Natural Products*, compounds **1** and **6** were also published by Saifah et al. (*Phytochemistry* **1999**, *52*, 1085).

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