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Bioactive Amides from *Glycosmis* species

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Besides the known imide ritigalin (**9**), six new phenethyl/styrylamine-derived amides isolated from lipophilic leaf extracts of *Glycosmis* cf. *mauritiana*, *Glycosmis* cf. *cyanocarpa*, and *Glycosmis crassifolia* displayed pronounced antifungal and/or insecticidal activity against *Cladosporium herbarum* and *Spodoptera littoralis*, respectively, the methylthiocarbonic acid derivatives niranin (**1**), dehydroniranin A (**2**), and dehydroniranin B (**3**) as well as the isovaleric and senecioic acid derivatives thalebanin B (**4**), dehydrothalebanin B (**5**), and dehydrothalebanin A (**6**).

Glycosmis represents a rather clear-cut genus within the tribe Clauseneae of the Aurantioideae subfamily of the Rutaceae comprising about 40 species.[1] Its range of distribution is centered in south and southeast Asia and extends to south China and Taiwan as well as to New Guinea and north Australia. The shrubs or small trees possess pinnate or simple leaves with translucent punctate glands emitting an aromatic odor when crushed. A good field and herbarium character of the genus is that the buds are usually covered by short rusty-red hairs. In spite of the good delimitation of *Glycosmis* from the nearest related genera *Clausena*, *Micromelum*, and *Murraya* and an already existing subrevisionary treatment by Stone,[1] there are still many taxonomic problems at the species level that remain to be solved. Consequently, phytochemical analyses have often been based on plant material that was not properly identified. Investigations have so far been focused mainly on the root and stem bark of *G. pentaphylla* (Retz.) DC., *G. parviflora* (Sims) Little [= *G. citrifolia* (Willd.) Lindley], and *G. mauritiana* (Lam.) Tanaka leading to the isolation of a number of different carbazole, acridone, and quinoline alkaloids, whereas quinazolines have been detected in the leaves.[2]

In more recent papers we have reported on a series of novel sulfur-containing amides from the leaves of different *Glycosmis* species, which obviously represent a typical chemical character of the genus. Until now 24 derivatives, which are mainly accumulated in the leaves, have already been isolated and identified.[3−8] Their acid moieties, most probably derived from the amino acid cysteine, additionally can be oxidized to sulfones[3,5] and sulfoxides[8] or shortened by β-oxidation, for example, ritigalin (**9**).[4] With the exception of the simple methylamides, for example, penangin (**11**),[6,8] the amine parts are characterized by phenethyl or styryl groups that additionally can be linked to different prenyloxy (dambullins) or geranyloxy groups in para position (gerambullins).[3] More recently a group of similar (methylsulfonyl)propenoic-acid amides has been detected where, by contrast, a dopamine part is linked to variously oxidized geranyl chains (sakerines).[5]

Apart from chemosystematic considerations, interest in this series of amides rests in their biological activity. In bioassays with different phytopathogenic fungi (e.g., *Cladosporium*, *Pyricularia*, *Alternaria*, *Botrytis*, *Fusarium*) and the well-known polyphagous pest insect *Spodoptera littoralis* (Lepidoptera, Noctuidae), some derivatives have displayed remarkable toxic effects (see Table 1). The most active antifungal amide has been shown to be methylillukumbin A (**8**) isolated from the leaves of some individuals of *G. mauritiana* collected in Sri Lanka. In this case comparative analyses of different provenances as well as of different individuals of the same population have exhibited a considerable chemical polymorphism towards different sulfur-containing amides.[7] In parallel tests against neonate larvae of *S. littoralis*, the recently described sulfur-containing imide ritigalin (**9**)[4] has been shown to possess pronounced contact toxicity.[9]

In the course of our continuing search for new bioactive compounds from different *Glycosmis* species collected in Thailand, we have now detected and isolated a series of novel amides with strong antifungal and insect-toxic properties (Table 1). Their structures were elucidated by spectroscopic methods and were shown to fall into two groups on the basis of different acid moieties: the group with sulfur-containing moieties designated as niranin (**1**), and dehydroniranins A (**2**) and B (**3**)[10] and the group derived either from isovaleric acid named thalebanin B (**4**) or from senecioic acid named dehydrothalebanins B (**5**) and A (**6**).[11]

The $CHCl_3$ fractions of the MeOH leaf extracts of *Glycosmis crassifolia* Ridley, *G.* cf. *mauritiana*, and *G.* cf. *cyanocarpa* (Bl.) Sprengel were compared by HPLC linked with diode array detection. According to our previous findings,[4,7] chemical polymorphism was observed in all three species. Especially *G.* cf. *mauritiana* showed considerable chemical variation between single

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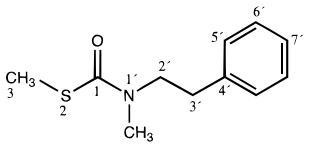
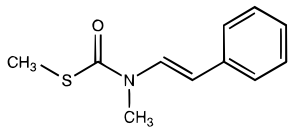
Table 1. Antifungal[a] and Insecticidal[b] Activities of Sulfur-Containing Amides from *Glycosmis* species

	Cladosporium herbarum	*Spodoptera littoralis*		
compd	germtube inhibition ED_{50} (μg/mL)	contact toxicity LC_{50} (μmol/dm^2)	growth inhibition EC_{50} (μmol/g)	survival rate LC_{50} (μmol/g)
dehydroniranin A (**2**)	100.0 (±3.0)	0.18 (±0.04)	0.41 (±0.09)	1.64 (±0.34)
dehydroniranin B (**3**)	8.4 (±1.2)	0.02 (±0.01)	0.37 (±0.06)	0.96 (±0.10)
methylillukumbin A (**8**)	5.5 (±0.6)	>6.50	0.94 (±0.34)	>5
methylsinharin (**7**)	17.6 (±0.6)	4.24 (±0.60)	0.47 (±0.05)	3.53 (±0.25)
niranin (**1**)	28.0 (±3.0)	0.03 (±0.01)	0.25 (±0.11)	0.81 (±0.14)
penangin (**11**)	>200	2.84 (±0.50)	0.84 (±0.08)	>10
ritigalin (**9**)	156.0 (±8.6)	0.05 (±0.01)	0.43 (±0.05)	1.34 (±0.22)

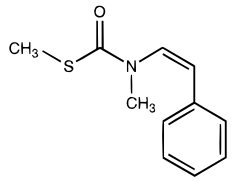
[a] Antifungal tests: germtube inhibition tests were carried out in micro wells with conidiospores of *Cladosporium herbarum*. Each compound was tested at seven different concentrations (200 μg−3.12 μg) in triplicate. Spore germination was stopped after 36 h with lactophenol blue and the reduced germtube growth was calculated from 10 spores per concentration and compared to controls. [b] Insecticidal bioassays[9,15]: Neonate larvae of *S. littoralis* (n = 20) were released on artificial diet (3.7 g) spiked with various concentrations of the analyzed compounds. After 5 days, survival rate and growth inhibition of the larvae were determined in triplicate and compared to controls treated with solvent (Me_2CO) only. For contact toxicity each compound was coated as an even film on the inside of glass vials in a dilution series. After evaporation of the solvent (Me_2CO) 20 neonate larvae were placed inside the vials, and after 48 h the mortality was monitored and compared with trials treated with the solvent only.



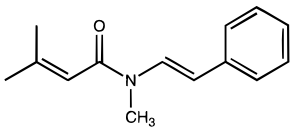
Niranin **1**



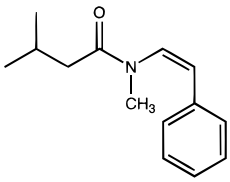
Dehydroniranin A **2**



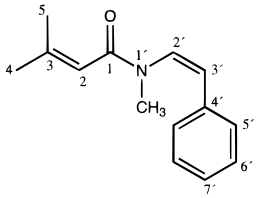
Dehydroniranin B **3**



Dehydrothalebanin A **6**



Thalebanin B **4**



Dehydrothalebanin B **5**

individuals of the same population. In fact each of the HPLC profiles from four different individuals collected near Prachuap Khiri Khan (southwest Thailand) showed a different composition. In addition to the already known sulfur-containing imide ritigalin (**9**),[4] retention times as well as simple UV spectra of the major compounds led us to expect a further series of related amides. Nearly identical patterns were also found in *G.* cf. *cyanocarpa* collected near Sangklaburi (west Thailand) where, by contrast, all individuals were characterized by a clear preponderance of compound **3**. Compounds with similar UV spectra but slightly different retention times were also found in the leaf extracts of different individuals of *G. crassifolia* collected in Thale Ban and near Phattalung (south Thailand). The amide nature of the compounds **1**−**6** was confirmed by IR data showing either two strong signals between 1672 and 1630 cm^{-1} for −C═C−N(CH_3)−CO−stretching (**2**−**6**) or only one strong signal at 1659 cm^{-1} for −CH_2−CH_2−N(CH_3)−CO− in compound **1**.

Comparative HPLC analyses within different collections of *G.* cf. *mauritiana* showed that compound **1**, named niranin,[10] was the only dominating component

Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **1**−**3** ($CDCl_3$, TMS)

	^{1}H NMR[a]			^{13}C NMR		
no.	**1**[b]	**2**	**3**	**1**	**2**	**3**
1				168.5 s	[c]	[c]
3	2.35 s	2.42 s	2.37 s	12.9 q	13.3 q	13.2 q
1′-Me	2.93 s	3.28 s	2.97 s	35.4 q	32.0 q	35.7 q
2′	3.60 t	∼7.25[d]	6.44 v br d	51.6 t	127.7 br.d[e]	[c]
3′	2.89 t	5.98 br d	6.16 v br d	34.1 t	[c,e]	[c]
4′				138.7 s	[c]	[c]
5′	7.18 −	7.20 −	7.25 −	128.5 d[f]	125.6 d[f]	128.5 d[f]
6′	7.34 m	7.35 m	7.35 m	128.7 d[f]	128.7 d[f]	128.7d[f]
7′	(5H)	(5H)	(5H)	126.4 d	126.6 d	127.9 d

[a] Coupling constants: **1**, J (2′,3′) = 7.6 Hz; **2**, J (2′,3′) = 14.4 Hz; **3**, J (2′,3′) ca. 8.5 Hz. [b] 320 K; the spectrum at 298 K shows partially very broad resonances, especially two groups of signals: δ = 3.45−3.65 (m, 2H, $W_{1/2}$ ca. 35 Hz, 2′-H_2) and 2.8−3.0 (m, 5H, $W_{1/2}$ ca. 30 Hz, 2′-H_2 + 1′-Me). [c] Quaternary and olefinic carbon atoms too broad for detection. [d] Partially obscured by the aromatic protons at δ = 7.20−7.35. [e] Either C-2′ or C-3′. [f] Interchangeable.

in the crude extract of individual no. 2, representing a good source for isolation. The UV spectrum of the isolate is inconspicuous due to the lack of a significant chromophoric system. At room temperature the ^{1}H-NMR spectrum of niranin (**1**) shows a narrow group of aromatic proton resonances (m, with a set of distinct lines for 5H), a sharp singlet at δ 2.35, and two further very broad and unresolved groups of signals (2H at δ ∼3.55 with $W_{1/2}$ ∼ 35 Hz and 5H at δ ∼ 2.9 with $W_{1/2}$ ∼ 30 Hz; cf. Table 2, footnote[b]). Based on our experience with previously isolated tertiary amides from *Glycosmis* species, this severe line broadening is caused by the hindered rotation about the amide N−CO bond. In comparison with the standard spectrum of 298 K, the ^{1}H-NMR spectrum at 320 K looks quite different (Table 2). The multiplet of 2H turns into a clear triplet at δ 3.60 (J = 7.6 Hz, 2′-H_2), the multiplet of 5H changes to a singlet of 3H at δ 2.93 (N−Me) and a triplet of 2H at δ 2.89 (J = 7.6 Hz, 3′-H_2). Together with the aromatic resonances of 5H (not influenced by the change in temperature), the chemical shifts and the dynamic behavior are typical for *N*-methyl-*N*-phenethylamides.[5,12,13] The ^{13}C-NMR data are also in full agreement with this amide; the resonances 1′-Me−C-7′ belong to the *N*-methyl-*N*-phenethylamide.[5,12,13] The acid part of the amide is obviously very small, because only one resonance is left in the ^{1}H-NMR spectrum (s, at δ 2.35) and two signals in the ^{13}C NMR (the carbonyl singlet at δ 168.5 and a quartet at δ 12.9). This means that

only one methyl group and the amide carbonyl can be detected by ^{1}H and ^{13}C NMR. The chemical shift of the methyl protons at δ 2.35 and the corresponding carbon resonance at δ 12.9 are in full agreement with the series of previously found methylthio derivatives. Most of these compounds were amides of methylthiopropenoic acid.[3,5−7,12,13] However, from *G. mauritiana* and *G. parviflora* we have also isolated the chain-shortened derivative ritigalin (**9**)[4] where CH_3S- is directly linked to $-CO-$, resulting in a *S*-methylthiocarbonic acid amide. So far, the imide ritigalin was the only derivative of this novel series of natural thiocarbonic acid amides. Compared to ritigalin, the new niranin (**1**) showed a simple phenethylamide group instead of the α-oxidized product (oxo group at C-2′ according to the numbering in the formula scheme; ritigalin therefore can be regarded as an imide of *S*-methylthiocarbonic acid and 2-phenylacetic acid). It is interesting to note that N-methylsinharine (**7**)[12,13] showed a similar dynamic behavior. However, the barrier to amide rotation is obviously higher in methylsinharine, because at room temperature both conformers were detected separately on the NMR time scale, whereas in the case of niranin (**1**) the two conformers are already averaged at that temperature. The mass spectral data (EIMS and HRMS) agree fully with the proposed structure.

Compounds **2** and **3** occurred regularly in all individuals of *G.* cf. *cyanocarpa*, with **3** as the dominating component. In contrast to the previous findings, where amide accumulation was always restricted to the leaves, compound **3** was found in large quantities also in the stem and root bark. As in niranin (**1**), the ^{1}H-NMR spectra of **2** and **3** also show a group of 5 aromatic phenyl protons. A characteristic feature of the proton spectra of both compounds is the AB system of two olefinic protons. In the case of **2**, one observes a slightly broadened doublet at δ 5.98 (J = 14.4 Hz, *trans*-coupling) and a broad signal at ca. 7.25 (partially covered by the unresolved but sharp aromatic resonances of 5 phenyl−H, cf. Table 2). In the case of **3** both parts of the AB system are very broad. The doublet at δ 6.16 still allows determination of the coupling constant of J ca. 8.5 Hz (*cis*-coupling), the doublet at δ 6.44 is so broad that it appears almost as a very broad, asymmetric singlet with a broad shoulder (merging of two lines of different intensity due to the AB roof effect). These broad lines are again the consequence of the hindered rotation about the amide bond. Matters are worse in the ^{13}C-NMR spectra, where no quaternary carbon atoms and few olefinic protons can be observed. The signals are obviously too broad and therefore too small for detection (Table 2). However, the ^{1}H-NMR data are rather conclusive, especially in comparison with the previously isolated (*E*)- and (*Z*)-(phenylethenyl)amides methylillukumbins A (**8**) and B.[7,13] These amides have an identical amide part, namely the *E*- and *Z*-isomers of (phenylethenyl)amide but a different acid part (methylthiopropenoic acid). The ^{1}H chemical shifts of the (phenylethenyl)amide part, the olefinic coupling constants, and the dynamics of the amide bond (line shapes) are almost the same for methylillukumbin A (**8**) and compound **2** [(*E*)-configuration] as for methylillukumbin B and compound **3** [(*Z*)-configuration]. The acid parts of **2** and **3** are methylthiocarbonic acid as indicated by the characteristic resonances in the ^{1}H and ^{13}C-NMR spectra (Table 2). The MS data confirm the structure. Compounds **2** and **3** represent, therefore, the chain-shortened analogues to methylillukumbin A (**8**) and methylillukumbin B (see Figure 1). In derivatization of niranin (**1**), the compounds were named dehydroniranin A (**2**) [(*E*)-isomer] and dehydroniranin B (**3**) [(*Z*)-isomer].

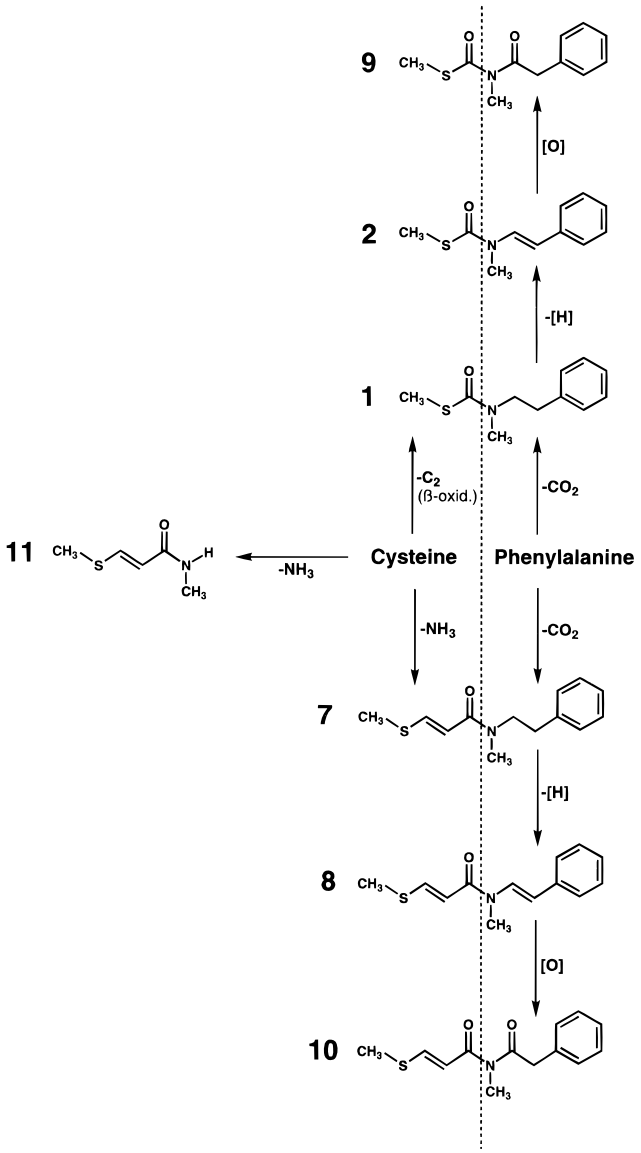


Figure 1. Supposed biosynthetic pathways of sulfur-containing amides characteristic of *Glycosmis* species.

All three individuals of *G. crassifolia* collected near Phattalung (south Thailand) are characterized by an accumulation of compound **5** accompanied by small amounts of compound **6**, whereas the numerous collections from Thale Ban (south Thailand) mainly showed a predominance of compound **4**. The ^{1}H- and ^{13}C-NMR spectra of **4** show all features of a (*Z*)-(phenylethenyl)-amide, namely two olefinic resonances at δ 6.40 and 6.07 with J = 8.8 Hz and five aromatic resonances for a phenyl group (cf. dehydroniranin B (**3**) or methylillukumbin B).[7] It is noteworthy that in **4** the hindered rotation about the amide bond is less pronounced than in dehydroniranin B (**3**), which can be seen from the relative line broadening of the olefinic resonances, especially in the ^{13}C-NMR spectrum (Table 3). The acid component of amide **4** is simply isovaleric acid giving the expected ^{1}H and ^{13}C resonances. M^+ with m/z =

Table 3. ^{1}H- and ^{13}C-NMR Data for Compounds **4**−**6** ($CDCl_3$, TMS)

	^{1}H NMR[a]			^{13}C NMR		
no.	**4**	**5**	**6**[b]	**4**	**5**	**6**[c]
1				172.9 s	[d]	166.7 v br s
2	2.24 d	5.96 br s	5.94 br s[b]	43.3 t	117.7 d	117.0 d
3	2.17 t hept			25.5 d	[d]	148.5 v br s
4	0.91 d	1.98 br s	1.93/1.97[b]	22.6 q	27.0 q	25.9 br q
5	0.91 d	1.79 br s	1.89 br s[b]	22.6 q	20.2 q	19.9 q
1′-Me	2.93 s	2.97 s	3.21/3.20[b]	34.3 q	34.1 q	28.7/32.4[c]
2′	6.40 d	6.43 d	7.44/8.15[b]	129.3 d	129.4 d	128.1 br d[e]
3′	6.07 d	6.05 d	5.92 d	123.5 d	122.2 d	109.9 br d
4′				136.7 s	[d]	136.4 br s
5′	7.23 −	7.18 −	7.10 −	128.6 d	128.4 d[f]	125.0 d[f]
6′	7.34 m	7.35 m	7.40 m	128.6 d	128.5 d[f]	128.1 d[f]
7′	(5H)	(5H)	(5H)	127.9 d	127.6 d	125.7 d

[a] Coupling constants: **4**, J (2,3) = J (3,4) = J (3,5) = 6.5 Hz, J (2′,3′) = 8.8; **5**, J (2′,3′) = 8.5 Hz; **6**, J (2′,3′) = 14.4 Hz. [b] Mixture of two conformers (ratio 2/1) due to the restricted rotation about the C−N amide bond at room temperature (298 K); changes in the spectrum at → 330 K: 5.94 (br s, 1H) → 5.93 (hept, 1H, J = 1.1 Hz, 2-H), 1.93/1.97 (two v br s, 3·0.67H/3·0.33H) → 1.94 (d, 3H, J = 1.1 Hz, 4-H_3 or 5-H_3), 1.89 (br s, 3H) → 1.89 (d, 3H, J = 1.1 Hz, 4-H_3 or 5-H_3), 3.21/3.20 (br s at 3.21 with shoulder at 3.20, approx. 2:1, together 3H) → 3.21 (s, 3H, N−CH_3), 7.44/8.15 (two v br d, 0.67H/0.33H) → 7.67 (br d, 1H, J = 14.4 Hz, 2′-H), the remaining signals do not change essentially. [c] Line broadening caused by restricted rotation about the C−N amide bond; only the resonance for 1′-Me (N-CH_3) shows two separate br s for the two rotamers. [d] Quaternary carbon atoms too broad for detection. [e] Partially obscured by the aromatic resonance at 128.1 (H-7′). [f] Interchangeable.

217 (62% rel int) is also compatible with the structure of an isovaleric acid *N*-methyl-*N*-(*Z*)-(phenylethenyl)-amide, named thalebanin B (**4**).[11] Analogous to other compounds, B was taken for the (*Z*)-configuration of the amine part, whereas A is reserved for the (*E*)-isomer.

Compounds **5** and **6** comprise a pair of (*Z*)- and (*E*)-*N*-methyl-*N*-(phenylethenyl)amides comparable to the dehydroniranins B (**3**) and A (**2**). The ^{1}H- and ^{13}C-NMR spectra for the (*Z*)-isomer **5** are simpler because there are no complications caused by hindered rotation about the amide bond. All NMR data of the amide part of **5** are very similar to the corresponding data for the (*Z*)-amides **3** and **4**. Especially the two olefinic protons (AB system) at δ 6.43 (d, 2′-H) and 6.05 (d, 3′-H) with J = 8.5 Hz are significant for (*Z*)-(phenylethenyl)amides (compare Tables 2 and 3). The remaining resonances are characteristic of the very common senecioic acid [broad, long-range coupled olefinic singlet at δ 5.96 (2-H) and two olefinic methyl groups at δ 1.98 and 1.79, which appear also as broad singlets (4-H_3 and 5-H_3); irradiation at the olefinic 2-H results in a clear line sharpening of the methyl groups]. The EIMS and HRMS confirmed the structure of a senecioic acid *N*-methyl-*N*-(*Z*)-(phenylethenyl)amide, named dehydrothalebanin B (**5**).

The ^{1}H- and ^{13}C-NMR spectra for compound **6** are much more complicated. Due to the hindered rotation about the amide C−N bond, the ^{1}H-NMR spectrum of the (*E*)-configured (phenylethenyl)amide **6** shows two different resonances for some of the protons. This dynamic behavior is especially clear for the olefinic proton next to the amide nitrogen (N−CH═, 2′-H). At room temperature (298 K) one observes a broad doublet at 7.44 and a second broad doublet at 8.15 (integration 0.67 and 0.33 for 1H, J = 14.4 Hz). The second olefinic proton (3′-H) appears as a single sharp doublet at δ 5.92 (integration 1.00 for 1H). At 330 K the former pair of broad doublets changes to one very broad doublet at δ 7.67 (integration for 1H), the doublet at 5.92 remains essentially unchanged (Table 3, footnote[b]). The integrations of signals in the slow exchange spectrum at 298 K (0.67:0.33) and the calculation of the chemical shift of the 1′-H resonance in the fast exchange spectrum at 330 K based on the slow exchange chemical shift data [7.67 = 8.15 × + 7.44 (1 − ×); × = 0.32] agree very well with a rotamer ratio of ca. 2:1. The coalescence temperature for the olefinic 2′-H is ca. 310 K, and the corresponding ΔG^* is therefore approximately 61 kJ mol^{-1}. The resonances for 2-H, 3′-H, and 5-H_3 are obviously very similar in both rotamers. Even the ^{1}H-NMR resonance for N−CH_3 is only slightly broadened at room temperature, showing a shoulder for the less favored rotamer at the highfield side (Table 3). Only one methyl group of the senecioic moiety also shows distinct lines for the two rotamers at δ 1.93 (v br s) and 1.97 (v br s) with relative integrations of ca. 2:1 at 298 K, and (after coalescence) a sharp singlet at δ 1.94 at 330 K. In the ^{13}C-NMR spectrum at room temperature all but one C atom give one average signal for the two possible rotamers. Due to the dynamic process of rotamer interconversion some of the signals are rather broad (cf. Table 3), however, only the resonances of the *N*-Me group show up separately for both rotamers due to the big difference in the chemical shift values (δ 28.7 and 32.4, $\Delta\delta$ 3.7 ppm, coalescence at higher temperature); all other C atoms are already in the fast exchange region at room temperature. The mass spectral data agree with the proposed structure of a senecioic acid *N*-methyl-*N*-(*E*)-(phenylethenyl)amide, named dehydrothalebanin A (**6**).

On the basis of our current HPLC screening within the Clauseneae genera *Glycosmis*, *Clausena*, *Micromelum*, *Murraya*, and *Bergera*, the accumulation of amides appears to be restricted to the leaves and seeds of the first two genera. Although the leaf extracts of *Clausena* are mainly characterized by amides derived from cinnamic, benzoic, and isovaleric acid together with large amounts of different coumarins,[2,14] those of *Glycosmis* clearly deviate by sulfur-containing and anthranilic-acid-derived amides[3,5] as well as by a lack of coumarin accumulation. The present investigation additionally informs about a new series of amides in *Glycosmis* species, which are derived from isovaleric and senecioic acid. Regarding the different biological activities of the sulfur-containing amides (Table 1), the pronounced infraspecific variation of the amide pattern, even differing between the individuals of the same population,[4,7] deserves special biological interest. This varying com-

position of amides could be interpreted as a survival strategy to prevent adaptation of herbivores or microorganisms to a uniform chemical profile.

Because the first active sulfur-containing amides [sinharine and methylsinharine (**7**)] were originally detected by a bioassay-monitored screening of crude extracts,[12] all extracts of later collections were also tested for antifungal and insect-toxic properties. From more detailed biotests with isolated pure compounds it became apparent that all amides derived from methylthiopropenoic acid linked with styryl (phenylethenyl)-amine moieties (e.g., **8**) show higher antifungal activity than those with phenethyl amine (e.g., **7**, see Table 1). The simple methylamides penangin (**11**) and isopenangin, by contrast, have shown no activity at all.[7] A pronounced fungitoxicity has now also been determined for the new series of methylthiocarbonic acid-derived amides, particularly for dehydroniranin B (**3**) (Table 1). Moreover, preliminary bioautographic tests with the new thalebanins (**4**−**6**), which are derived from isovaleric senecioic acid, also exhibited clear antifungal activity.

A significant ecological function may be attached to the very high contact toxicity of the methylthiocarbonic acid amides, especially of dehydroniranin B (**3**), against the test insect *S. littoralis*.[9] This finding is in accordance with the previously tested ritigalin (**9**) (Table 1). The niranins also showed a pronounced toxicity when incorporated into an artificial diet.[9] Very similar effects have also been observed in preliminary tests for the thalebanins, where especially thalebanin B (**4**) and dehydrothalebanin B (**5**) show high insect toxicity. By contrast, the corresponding methylthiopropenoic acid amides, for example, methylillukumbin A (**8**), have only moderate activity against *S. littoralis*.[9] Taking into account that the occurrence of amides is usually restricted to the leaves and seeds, the extraordinarily high accumulation of dehydroniranin B (**3**) and dehydrothalebanin B (**4**) not only in the leaves but also in the stembark and in the roots is exceptional. More detailed comparative investigations of samples from different habitats will have to show to what extent this accumulation can be interpreted as an ecological function.

Apart from the thalebanins (**4**−**6**) with a more restricted distribution, the many sulfur-containing amides obviously play an important systematic and ecological role in the genus *Glycosmis*. For systematic conclusions, however, chemical polymorphism should always be taken into consideration. From the many biotests available so far it has become clear that derivatives elongated with C_{10}-(geranyl-) chains as well as with higher oxidized sulfur (sulfones) have less insect and fungitoxic activity. As shown in a hypothetical scheme (Figure 1), biogenetically all sulfur-containing amides may be derived from the amino acid cysteine either by deamination and methylation (e.g., **7**, **8**, **10**, **11**) or by an additional chain shortening by β-oxidation and methylation (e.g., **1**, **2**, **9**). With the exception of penangin (**11**), the amine moieties most likely are formed by decarboxylation, dehydrogenation, and oxidation of phenylalanine. Because some *N*-methyl derivatives shown in Figure 1 were found to occur together with the corresponding N−H-derivatives, *N*-methylation presumably represents the latest step on this biosynthetic pathway.

Experimental Section

General Experimental Procedures. NMR, Bruker, AM 400 WB and AC 250; EIMS, Varian MAT-CH7; IR, Perkin-Elmer 398 and 16PC FT-IR; UV, Perkin-Elmer Lambda 5 and Hewlett-Packard 8452A diode array; HPLC, Hewlett-Packard HP 1090 II, UV diode array detection at 230 nm, column 290 × 4 mm Spherisorb ODS, 5 μm, mobile phase MeOH (gradient 60−100%) in aqueous buffer (0.015 M phosphoric acid, 0.0015 M tetrabutylammonium hydroxide, pH 3), flow rate 1 mL/min.

Plant Material. Leaves from different individuals of three *Glycosmis* species were collected separately: (a) *G.* cf. *mauritiana* from the Huai Yang Waterfall, southwest of Prachuap Khiri Khan (southwest Thailand); (b) *G.* cf. *cyanocarpa* from the trail to Laipa near the Karen village Sanepong, Thung Yai Naresuan Wildlife Sanctuary, Sangklaburi, Kanchanaburi Province (west Thailand); and (c) *G. crassifolia* from Thale Ban, National Park, near Satun (south Thailand) and from Mom Chui Waterfall near Ban Tramod, Phattalung (south Thailand). Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and Isolation. Dried leaves from *Glycosmis* species were ground and extracted with MeOH at room temperature for 7 days, filtered, and concentrated. The aqueous residue was then 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) and further by preparative MPLC (400 × 40 mm column, Merck LiChroprep silica 60, 25−40 μm, UV detection, 254 nm).

Compound 1: 605 mg of the $CHCl_3$ fraction from 27 g of dried leaves of individual no. 2 of *G.* cf. *mauritiana* was directly separated by MPLC with 10% EtOAc in hexane affording 74 mg of niranin.

Compounds 2 and 3: 800 mg of the $CHCl_3$ fraction from 101 g of dried leaves of individual no. 2/1 of *G.* cf. *cyanocarpa* was roughly separated by column chromatography (Si gel). The fractions eluted with 10% Et_2O in hexane, containing the amides (300 mg), were combined and separated further by cyclic MPLC (3 cycles) with 10% EtOAc in hexane to afford 137 mg of dehydroniranin B (**3**) and 31 mg of dehydroniranin A (**2**).

Compound 4: 1.2 g of the $CHCl_3$ fraction from 33 g of dried leaves of individuals nos. 5 + 9 of *G. crassifolia* from Thale Ban was directly separated by MPLC first with 15% EtOAc (114 mg) and further purified with 5% EtOAc in hexane to give 34 mg of thalebanin B.

Compounds 5 and 6: 632 mg of the $CHCl_3$ fraction from 11 g of dried leaves of individuals nos. 2 + 4 of *G. crassifolia* from Mom Chui Waterfall were roughly separated by column chromatography (Si gel). The amide-containing fractions (147 mg) were combined and separated further by MPLC with 10% EtOAc in hexane to give 40 mg of dehydrothalebanin B (**5**) and 10 mg of dehydrothalebanin A (**6**).

Biotests with Fungi and Insects. Antifungal tests were performed either by bioautography assays on TLC with *Cladosporium herbarum* (Pers.: Fr.) Link or by germtube inhibition tests with serial dilutions in micro wells to determine the ED_{50} values (see Table 1). Larvae of *Spodoptera littoralis* were reared on an artificial diet under controlled conditions. Crude ex-

tracts as well as pure compounds were incorporated into the artificial diet, and the survival and growth rates of neonate larvae were determined (Table 1). For contact toxicity neonate larvae were placed inside glass vials where the compounds were coated in a dilution series. The mortality of larvae was monitored (Table 1).[9,15]

Niranin (1) (*S*-methylthiocarbonic acid *N*-methyl-*N*-phenethylamide): IR (CCl_4) ν max 3090 w, 3070 w, 3033 w, 2934 m, 2861 w, *1659* vs, 1606 w, 1495 w, 1455 m, 1417 w, *1384* s, 1334 w, 1285 m, 1165 m, 1155 m, *1087* s, 1074 m, 1031 w, 935 w, 900 w, *698* s, 663 w cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 209 (62), 162 (28), 118 (100), 105 (67), 91 (52), 75 (35), 61 (45), 42 (49); $C_{11}H_{15}NOS$, M calcd 209.0874; exp, 209.087; ^1H and ^{13}C NMR, see Table 2.

Dehydroniranin A (2) [*S*-methylthiocarbonic acid (*E*)-*N*-methyl-*N*-phenylethenyl)amide: UV (Et_2O) λ max (log ϵ) 302 (sh, 4.07), 286 (4.23), 279 (sh, 4.22), 216 (4.11) nm; IR (CCl_4) ν max 3084 w, 3064 w, 3026 w, 2958 w, 2932 m, 2854 w, *1670* s, *1638* vs, 1608 w, 1496 w, 1468 m, 1448 w, 1430 w, 1412 w, 1368 m, 1334 w, *1304* s, *1240* s, 1200 w, 1156 w, *1072* s, 1030 w, 946 w, 924 w, 892 w, 862 w, 824 w, 692 m, 656 w cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 207 (58), 193 (13), 160 (8), 150 (100), 149 (40), 145 (29), 135 (30), 132 (31), 130 (18), 117 (56), 103 (16), 91 (64), 85 (21), 77 (29), 75 (41), 65 (25), 57 (35), 42 (39); $C_{11}H_{13}NOS$, M calcd 207.0718; exp, 207.072; ^1H and ^{13}C NMR, see Table 2.

Dehydroniranin B (3) [*S*-methylthiocarbonic acid (*Z*)-*N*-methyl-*N*-(phenylethenyl)amide]: UV (Et_2O) λ max (log ϵ) 271 (4.10), 216 (4.05) nm; IR (CCl_4) ν max 3086 w, 3064 w, 3030 w, 2956 w, 2930 m, 2860 w, *1670* vs, *1635* s, 1492 w, 1465 w, 1448 m, 1423 w, 1403 w, 1330 m, *1293* s, 1213 w, 1174 m, *1072* s, 1029 w, 970 w, 935 m, 927 m, 912 w, 844 w, 696 m, 664 w, 652 w cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 207 (52), 160 (10), 150 (100), 145 (7), 135 (37), 132 (32), 117 (66), 103 (15), 91 (79), 77 (31), 75 (40), 65 (30), 42 (74); ^1H and ^{13}C NMR, see Table 2.

Thalebanin B (4) [isovaleric acid (*Z*)-*N*-methyl-*N*-(phenylethenyl)amide]: UV (Et_2O) λ max (log ϵ) 270 (4.08), 216 (4.04) nm; IR (CCl_4) ν max 3082 w, 3060 w, 3026 w, 2958 m, 2930 m, 2870 w, *1672* vs, *1632* s, 1494 w, 1468 m, 1448 m, 1426 w, 1406 w, *1374* s, *1316* s, 1260 m, 1214 m, 1176 m, 1136 w, 1112 w, *1074* s, 1028 w, 982 w, 936 w, 920 w, 890 w, 834 w, *696* s, 668 w cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 217 (62), 133 (100), 117 (18), 104 (12), 91 (46), 85 (12), 77 (11), 57 (55), 42 (15); $C_{14}H_{19}NO$, M calcd 217.1467; exp, 217.147; ^1H and ^{13}C NMR, see Table 3.

Dehydrothalebanin B (5) [senecioic acid (*Z*)-*N*-methyl-*N*-(phenylethenyl)amide]: UV (Et_2O) λ max (log ϵ) 281 (3.92), 221 (4.06) nm; IR (CCl_4) ν max 3084 w, 3061 w, 3029 w, 2975 w, 2937 w, 2917 m, 2856 w, *1665* s, *1630* vs, 1491 w, *1448* s, 1427 w, 1405 w, 1378 w, *1361* s, *1296* s, 1217 w, 1176 m, 1121 w, 1100 m, *1059* s, 1029 w, 940 w, 922 w, 843 w, 695 m, 563 w cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 215 (25), 133 (100), 117 (12), 104 (11), 91 (14), 83 (66), 55 (24), 42 (15); $C_{14}H_{17}NO$, M calcd 215.1310; exp, 215.131; ^1H and ^{13}C NMR, see Table 3.

Dehydrothalebanin A (6) [senecioic acid (*E*)-*N*-methyl-*N*-(phenylethenyl)amide]: UV (Et_2O) λ max (log ϵ) 305 (sh, 4.15), 296 (4.18), 221 (4.08) nm; IR (CCl_4) ν max 3087 w, 3065 w, 3031 w, 2920 w, 2857 w, *1669* s, *1635* vs, 1602 w, 1469 w, 1449 m, 1429 w, 1415 w, 1381 m, 1371 m, 1319 m, 1306 m, *1248* s, 1190 w, *1112* s, *1061* s, 989 w, 953 w, 936 m, 841 w, 692 m, 581 w cm^{-1}; EIMS (70 eV) *m/z* $[M^+]$ 215 (69), 163 (10), 133 (100), 130 (18), 117 (17), 104 (12), 91 (44), 83 (99), 77 (10), 65 (12), 55 (81), 53 (11), 42 (18); $C_{14}H_{17}NO$, M calcd 215.1310; exp, 215.131; ^1H and ^{13}C NMR, see Table 3.

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