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Alcoholysis of Naturally Occurring Imides: Misleading Interpretation of Antifungal Activities[⊥]

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The frequent presence of the sulfur-containing amide penangin (**10**) in leaf extracts of *Glycosmis* species turned out to be the result of decomposition of imides generated by extraction and storage in MeOH. Reinvestigation of *Glycosmis mauritiana* and *G.* cf. *puberula* with acetone revealed the presence of six imides. In addition to penimides A (**1**) and B (**2**) and ritigalin (**6**), three new derivatives, krabin (**4**), isokrabin (**5**), and methoxypenimide B (**3**), were isolated and identified by spectroscopic methods. All six imides were shown to be susceptible to different rates of methanolic cleavage, leading to their corresponding methyl esters and sulfur-containing amides. Whereas the decomposition products penangin (**10**), isopenangin (**11**), and sinharin (**14**) are known, the corresponding cleavage of methyl *N*-methylthiocarbamate (**7**) from ritigalin (**6**), monitored *in situ* by ^{1}H NMR spectroscopy, is described here for the first time. Its structure was further confirmed by GC-MS coupling. HPLC-UV comparison of many different samples of *G. mauritiana*, extracted with MeOH, revealed considerable chemical variations in sulfur-containing amides, strongly correlated with different antifungal potency. The lack of activity of many methanolic crude extracts can be explained by a preponderance of the inactive decomposition product penangin (**10**), whereas the corresponding naturally occurring imides penimides A (**1**) and B (**2**) and methoxypenimide B (**3**), extracted with acetone, showed high fungitoxic properties.

The amides in the genus *Glycosmis* Corrêa of the family Rutaceae deserve attention. They are not derived from cinnamic acid and thus differ from other amides in the family, including those of the closely related genus *Clausena* Burm. f. Instead, sulfur-containing acid moieties, most likely derived from the amino acid cysteine, play a prominent role in the amide formation in many *Glycosmis* species. The amine parts are mostly represented by phenethyl or phenethenyl (styryl) groups that can be further linked to various prenyloxy and geranyloxy groups in the *para* position.[1,2] These amides can also be oxidized to sulfones and sulfoxides[1,3,4] or shortened by *β*-oxidation.[5] The accumulation of sulfur-containing amides, otherwise rarely reported in the plant kingdom,[6] was shown to represent an important chemical character for an infrageneric grouping in this genus.[7] Apart from their chemotaxonomic value,[8] many amides exhibited pronounced biological activities in various bioassays.[9,10]

In the course of our broad-based phytochemical comparison of many different samples of *Glycosmis mauritiana* (Lam.) Tanaka, collected in Sri Lanka and Thailand, a considerable chemical variation of accumulated sulfur-containing amides was observed, even among single individuals of the same population.[7,9] The numerous collections from Sri Lanka showed remarkable differences in amide patterns, which were strongly correlated with variations in fungitoxic properties. The highly active antifungal amides methyllillukumbins A (**8**) and B (**9**)[7] were detected only in a few collections, whereas the inactive penangin (**10**)[11] was shown to be more widespread as the major component. In view of the expected protective role of **8** and **9** against fungal attack, the predominance of the latter was surprising. This seemingly functional inconsistency can now be explained by using different solvents for extraction. Our findings demonstrated that the inactive penangin (**10**) was actually an artifact obtained by conventional MeOH extraction, but could not be detected in acetone. In acetone the
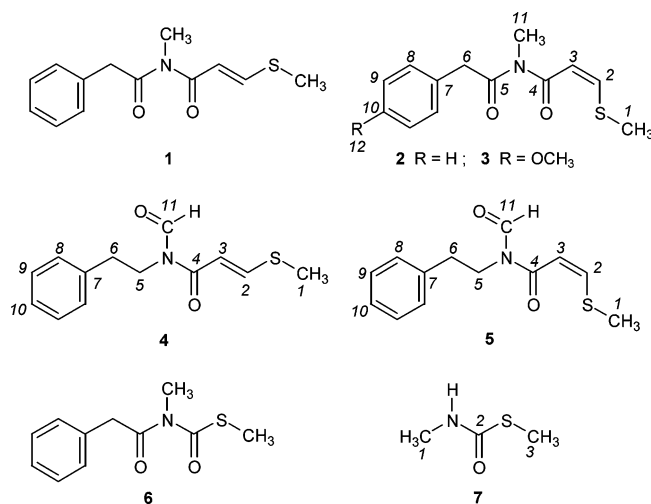
Figure 1. Structures of penimides A (**1**) and B (**2**), methoxypenimide B (**3**), krabin (**4**), isokrabin (**5**), ritigalin (**6**), and methyl *N*-methylthiocarbamate (**7**).

amide penangin (**10**) was replaced by the imide penimide A (**1**). In fact, subsequent tests with pure penimide A (**1**) dissolved in MeOH showed a complete transformation into penangin (**10**) after ~40 h at room temperature.[12] In contrast to the inactive penangin (**10**), penimide A (**1**) displayed pronounced antifungal activity.[12,13] However, due to the small amounts of penimide obtained in the original crude extract, this effect has not yet been determined.[11]

In order to get an overview about the possible occurrence of other transformation products, generated during MeOH extraction, freshly harvested leaves from two *Glycosmis* species, cultivated in the Botanical Garden of the University of Vienna, were dried and extracted separately with acetone and MeOH. The chemical profiles of both extracts were compared by HPLC-UV analyses. The results revealed that the *Z/E* pair of the imides krabin (**4**) and isokrabin (**5**) was detected in the acetone extracts together with the known imides penimides A (**1**) and B (**2**)[11] and ritigalin (**6**).[5] Furthermore, a small amount of the undescribed *Z*-isomeric methoxypenimide B (**3**) was isolated (Figure 1). The present paper reports on the
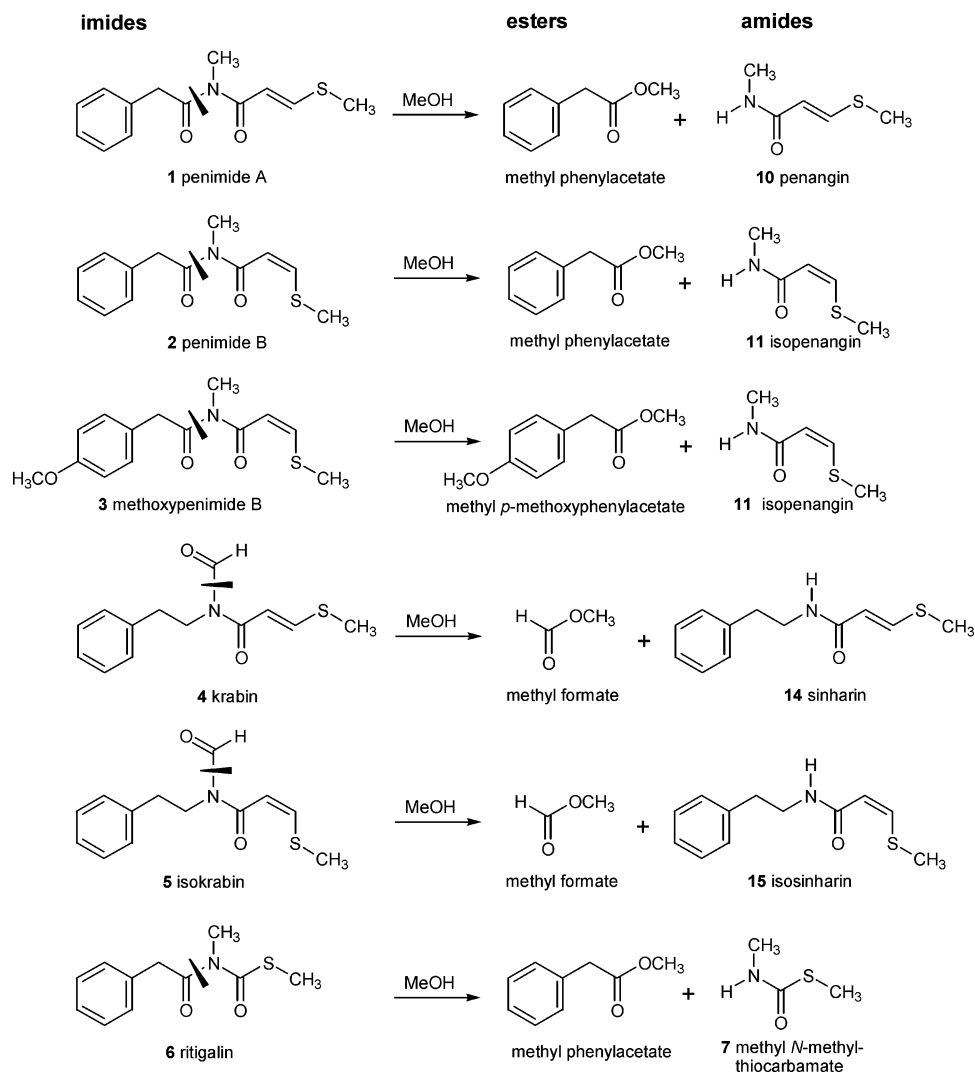
Figure 2. Methanolic cleavage of imides to corresponding methyl esters and amides.

isolation and structure elucidation of the new imides and describes the MeOH-induced cleavage of all imides (**1**−**6**) into their corresponding amides and methyl esters, as well as the associated changes in antifungal activities.

Results and Discussion

During our first field trips in Sri Lanka in 1991−1992, we collected *G. mauritiana*. In order to avoid formation of artifacts by drying the plant material and subsequent transport to Vienna, all samples were freshly chopped at the place of collection and immediately soaked and stored in MeOH. The lipophilic CHCl₃ fractions of the methanolic crude extracts of leaves, stem, and root bark were later investigated separately by HPLC-UV analyses. The results showed that amides and/or imides were accumulated mostly in the leaves. Parallel bioautographic tests on TLC plates sprayed with a spore suspension of the fungus *Cladosporium herbarum* (Persoon & Fries) Link exhibited clear inhibition spots for extracts containing methylillukumbins A (**8**) and B (**9**), ritigalin (**6**), sinharin (**14**), and methylsinharin (**16**) as active compounds, whereas those dominated by penangin (**10**) did not display notable antifungal activity. Penangin (**10**) was originally isolated and identified from a plant collected in Penang Island in Malaysia, erroneously named *G.* cf. *chlorosperma* (Bl.) Spreng. and later identified as *G.* cf. *puberula* Lindl.[14] The HPLC profile of this collection was characterized by a preponderance of penangin (**10**) along with small amounts of two isomeric imides, penimides A (**1**) and B (**2**).[11]

For use as authentic markers in chromatographic comparisons and for bioassays all isolated pure compounds were stored at −20 °C in MeOH solution. However, after three days at room temperature, penimide A (**1**) could not be recovered from the MeOH solution in HPLC-UV profiles. Instead, penangin (**10**) was detected as the main peak together with an additional small peak identified as methyl phenylacetate. These findings suggested methanolysis of penimide A (**1**), as shown in Figure 2, and explained the frequent presence of the inactive penangin (**10**) as the major component in many crude extracts stored in MeOH solution. Parallel experiments with penimide A (**1**) using EtOH, propanol, 2-propanol, or BuOH as solvents also resulted in the formation of penangin (**10**) and the corresponding alkyl phenylacetates. Penangin (**10**) was shown to be accompanied by small amounts of its *Z*-isomer isopenangin (**11**), suggesting the presence of the corresponding imide penimide B (**2**) (Figure 2).[11]

A new type of imide was found to dominate in the acetone extract of the leaves of *G. mauritiana* collected in south Thailand. Referring to the place of collection near the city of Krabi, this imide was designated as krabin (**4**). Its structure is similar to those of methylsinharin (**16**)[15] and could formally be described as "*N*-formylsinharin". The *N*-(2-phenylethyl)- and the (*E*)-*N*-3-(methylthio)prop-2-en-1-onyl moieties of krabin (**4**) were easily determined by comparison of their NMR data with those of methylsinharin (**16**).[15] The presence of the *N*-formyl group was determined by mass spectrometry and by ¹H and ¹³C NMR chemical shifts. Additionally,

the $^3J_{H-C}$ long-range couplings between the formyl proton and the N-bound ^{13}C atoms in the two other moieties indicated the presence of the new imide structure. Methanolysis of krabin (**4**) led to methyl formate and sinharin (**14**), as determined by HPLC (Figure 2). Krabin (**4**) was shown to be accompanied by small amounts of its Z-isomer, named isokrabin (**5**). The difference between the two structures was determined by $^3J_{H-H}$ coupling constants of the double bound. The E-isomer krabin (**4**) was identified by the large coupling constant of 14.5 Hz, while isokrabin (**5**) showed the intermediate coupling constant of 9.7 Hz, typical for 3-thioprop-2-(Z)-enone moieties.[15,16]

A small amount of an unknown *p*-methoxy derivative of penimide B (**2**), named methoxypenimide B (**3**), was isolated from the acetone extract of *G.* cf. *puberula* Lindley, collected in Khao Soi Dao Wildlife Sanctuary in southeast Thailand. Its structure was identified from ^{1}H NMR spectra, indicating a methoxy group being in the *para* position of the phenyl ring. A Z-configuration of the double bond similar to penimide B (**2**) was indicated by a $^3J_{H-H}$ coupling constant of 9.5 Hz. The UV spectrum and retention time in the HPLC-UV analysis also indicated the presence of the corresponding E-isomer methoxypenimide A in small amounts. However, due to limited plant material, no detailed spectroscopic analysis could be carried out.

Methanolysis of penimides A (**1**) and B (**2**) and krabin (**4**) led to the cleavage of the saturated acyl moieties and resulted in their corresponding methyl esters. A similar cleavage has been reported for coniothyriomycin [*N*-(3-chloro-4-hydroxyphenylacetyl)fumarate] in MeOH.[17] This acyclic imide was isolated from the fungus *Coniothyrium* and showed reasonably good stability in MeOH and MeOH/H_2O mixtures at neutral pH. However, addition of catalytic amounts HCl led to its complete methanolysis within a few days.[17] The lability of imides against hydrolysis in alkaline environment has been investigated in detail, for example, in the hydrolysis of substituted *N*-methylphthalimides[18] and maleimides.[19] Even poly[*N*-(4-sulfophenyl) dimethacrylamide], an imide that is stable under neutral and acidic conditions, is hydrolyzed at pH 12.3.[20] Additionally, transition metal ions further catalyze hydrolysis and methanolysis of amide bonds when the amide nitrogen is incorporated into their coordination. Such catalytic effects have been described in β-lactam hydrolysis[21] and were recently investigated with Cu^{2+} ions as a potential synthetic tool.[22] Mechanisms of the hydrolytic cleavage of C−N bonds in amides have been studied extensively[23,24] and are likely very similar to those of corresponding cleavages of the C−N bonds of imides.

The methanolysis of compounds **1**, **2**, and **4** in our crude and partly purified plant extracts is probably not caused just by the influence of the solvents. Small amounts of co-dissolved alkaloids or plant acids could slightly raise or lower pH values, respectively, which enhanced the methanolysis. The presence of transition metal ions in the plant extracts could not be excluded and might have further catalyzed the methanolysis of the imides, which have a reasonable potential to form complex structures with those ions.

In penimides A (**1**) and B (**2**) and krabin (**4**), the α,β-unsaturated acyl moieties did not cleave and remained as part of the formed *N*-alkyl amides. Such chemoselectivity also occurs in the comparable acid-catalyzed methanolysis of coniothyriomycin.[17] The rate-determining step of C−N bond hydrolysis in amides is the deterioration of the conjugated system between these two atoms, caused by sp^2 to sp^3 hybridization changes of the nitrogen atom.[25] This makes the carbon atom of the remaining carbonyl group a better target for a nucleophilic attack. In imides **1**, **2**, and **4** the two carbonyl groups are distinguished by their conjugation. The carbonyl group of the conjugated system is held in the sp^2 hybridization by the formation of a push−pull system with the electron-donating sulfur atom. The unconjugated carbonyl group, however, is a good target for the nucleophilic attack. The reduced chemoselectivity in the methanolysis of coniothyriomycin[17] is caused by the presence

of an electron-withdrawing carboxyl group, which replaces the sulfur atom and leads to reasonably higher electrophilicity of the imide carbonyl group. Isokrabin (**5**) and methoxypenimide B (**3**), however, also contain an electron-donating thioether moiety and, hence, quite likely underwent the same methanolysis as the imides **1**, **2**, and **4** (Figure 2).

Different rates of methanolysis were observed in the imides **1**−**6** by HPLC−UV comparison, exhibiting a relatively quick transformation of penimide A (**1**) into penangin (**10**) within ∼40 h at room temperature, whereas ritigalin (**6**) and krabin (**4**) were still present in large amounts after ∼60 h. In the latter compound an elimination of methyl formate led to the formation of sinharin (**14**), the first sulfur-containing amide reported for *Glycosmis* (Figure 2).[16,26] The structures of the decomposition products penangin (**10**), isopenangin (**11**), and sinharin (**14**) have been reported previously. The corresponding formation of methyl *N*-methylthiocarbamate (**7**) from ritigalin (**6**) has not yet been described. This methanolysis is interesting, as it does not lead to release of the amide, CO_2, and CH_3SH, but to the *S*-methyl-*N*-methylthiocarbamate (**7**) and methyl phenylacetate (Figure 2). Therefore, the methanolysis of ritigalin (**6**) was monitored in methanol-d_4 over several days by *in situ* ^{1}H NMR.[27,28] In Figure 3, stack plots of two different spectrum regions are shown, with indicative signals of the substrate ritigalin (**6**) as well as of the resulting products methyl phenylacetate and methyl *N*-methylthiocarbamate (**7**). The time course indicates the pseudo-first-order kinetics of the methanolysis and of the concomitant formation of the reaction products. H/D exchange in the α-position to the carbonyl groups was detected in negligible amounts (<5% after 400 h) in ritigalin (**6**) as well as in methyl phenylacetate. The slightly diminished formation of methyl *N*-methylthiocarbamate (**7**), as compared to methyl phenylacetate, might be caused by further decomposition of **7** or indicated by quite long longitudinal relaxation times of the two methyl groups.

The stability and structure of **7** were studied in further detail directly from the reaction mixture in methanol-d_4. HMBC indicated the *N*- and *S*-bound methyl groups to have $^3J_{C-H}$ couplings to the carbonyl function. Mass spectra taken from GC-MS coupling showed two compounds with the molecular mass of $m/z = 153$ and $m/z = 105$ for the methyl phenylacetate with a CD_3 moiety in both the ester function and **7**, respectively. Fragments of the latter compound were $m/z = 75$ and $m/z = 58$, which indicated the loss of −NH−CH3 and of −S-CH$_3$, respectively. Hence, methyl *N*-methylthiocarbamate (**7**) was identified as the stable product formed by methanolysis of ritigalin (**6**).

A hypothetical biosynthetic route of sulfur-containing amides has been proposed.[9] It suggests that the sulfur-containing acid moieties are derived from the amino acid cysteine, whereas phenylalanine is the precursor of the amine moieties. An elimination of a C-2 unit by β-oxidation of the acid moiety leads to the formation of methylthiocarbonic acid amides. As shown in Figure 4, methylsinharin (**16**) possibly assumes a central position and is transformed either to methylillukumbin (**8**, **9**) by dehydration or to dehydroniranin (**12**, **13**)[9] by dehydration and β-oxidation. Oxidation of the double bond next to the aromatic ring in methylillukumbin (**8**, **9**) and dehydroniranin (**12**, **13**), or the *N*-methyl group of methylsinharin (**16**), probably results in the formation of the imides **1**, **4**, and **6** (Figure 4).

On the basis of EC$_{50}$ values of germ-tube inhibition tests against the fungus *Cladosporium herbarum* and the rice pathogen *Pyricularia grisea* (Cooke) Sacc., significant antifungal activities were observed. Penimide A (**1**) showed the highest activity, with an EC$_{50}$ value of 0.9 µg/mL against *C. herbarum* and 3 and 5 µg/mL respectively against two different strains of *P. grisea*.[13] Even though the values of some imides and amides could not be determined explicitly, because nonsigmoid dose−response relationships impaired estimation, comparative bioautographic tests on TLC plates against *C. herbarum*[12] indicated that all sulfur-containing imides
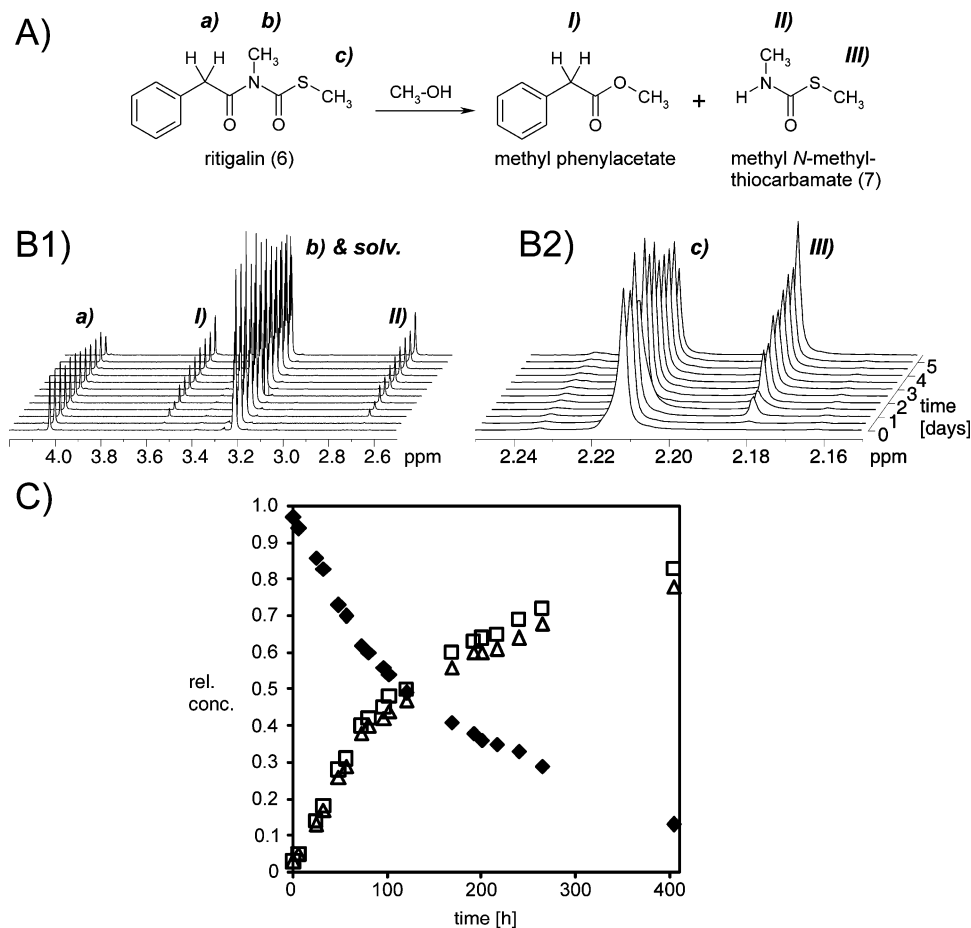
Figure 3. (A) Methanolysis of ritigalin (**6**) to methyl phenylacetate and methyl *N*-methylthiocarbamate (**7**) together with indication of protons showing indicative signals. (B) Corresponding stack plots of *in situ* ¹H NMR data monitored in methanol-d_4 for a time interval of 5 days. (C) Reaction progress over 400 h (ritigalin (**6**): solid diamonds; methyl phenylacetate: open squares; methyl *N*-methylthiocarbamate (**7**): open triangles).
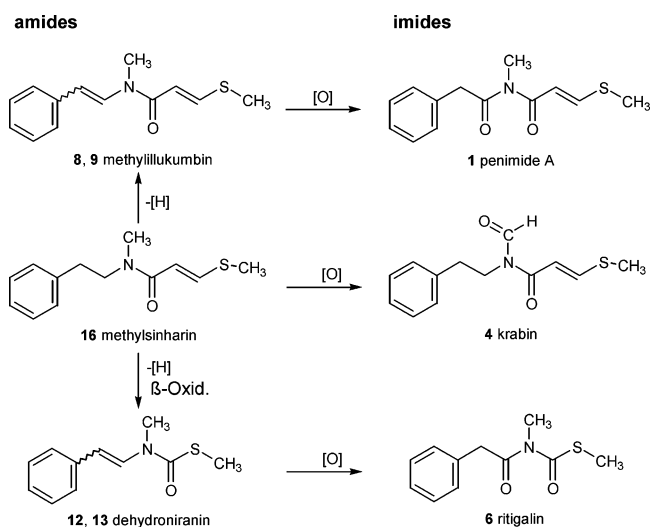
Figure 4. Supposed oxidation pathway from amides to imides.

have pronounced antifungal characteristics. Marked antifungal properties of acyclic imides were reported for fungi.[17,29] In the present investigation no significant differences in activity were observed between imides and their structurally related amides. However, the widely distributed transformation product penangin (**10**) clearly deviated with an EC$_{50}$ value of >200 μg/mL.[9,13] This lack of antifungal activity can probably be explained by the lack of an aromatic system. Since sinharin (**14**) was detected as the major

component together with methylsinharin (**16**) in some samples of *G. mauritiana*, collected in Sri Lanka, it cannot be excluded that sinharin (**14**) also represents an independent natural compound of its own, besides formation by decomposition of krabin (**4**).

On the basis of leaf extracts of two *Glycosmis* species, as compared by HPLC-UV analyses, it became evident that imides mostly co-occur with varying amounts of their corresponding sulfur-containing amides, a phenomenon supporting the biosynthetic hypothesis shown in Figure 4. Moreover, there may be special chemotaxonomic possibilities in view of the vicarious accumulation of two different types of sulfur-containing amides, characterized either by the above-mentioned amide/imide series or by methyl-sulfonylpropenoic acid amides with *para*-positioned prenyloxy or geranyloxy side chains of the aromatic ring.[1,2,30] In fact, HPLC-UV profiles showed a clear mutual exclusion of these two types of amides. However, due to the great difficulties of an infrageneric grouping of *Glycosmis*,[31,32] a clear correlation to a particular species was not always possible.[14] Further investigations will have to show to what extent these different accumulation trends of amide formation can be used as chemical markers and/or interpreted as an ecological function.

Experimental Section

source temperature 150 °C. EI-MS: MAT95. ESI-MS: MAT900. HRMS: MAT900. IR: Perkin-Elmer 16PC FT-IR. HPLC: Agilent 1100, UV diode array detection at 230 nm, column Hypersil BDS-C18 250 × 4.6 mm, 5 μm, mobile gradient either MeOH 20−100% or acetonitrile 20−60% in aqueous buffer (0.015 M H_3PO_4, 0.0015 M tetrabutylammonium hydroxide), flow rate 1 mL/min.

NMR Analyses. For NMR spectroscopy compound mixtures were dissolved in methanol-d_4 or $CDCl_3$ ($\sim$0.5−5.0 mg in 0.7 mL) and transferred into 5 mm high-precision NMR sample tubes. CHD_2OD or $CHCl_3$ was used as internal standard for ^{1}H (δ_H 3.31 or 7.24) and ^{13}C (δ_C 49.0 or 77.1) measurements. All spectra were measured at 300 ± 0.1 K at 400.13 MHz (^{1}H) or 100.61 MHz (^{13}C) and performed using the Bruker Topspin 2.1 software. 1D spectra were recorded by acquisition of 32k data points and after zero filling to 64k data points, and Fourier transformation spectra were performed with a range of 7200 Hz (^{1}H) and 20 000 Hz (^{13}C). To determine the 2D COSY, TOCSY, NOESY, HMQC, and HMBC spectra 128 experiments with 1024 data points each were recorded and Fourier transformed to 2D spectra with a range of 4000 Hz and 20 000 Hz for ^{1}H and ^{13}C, respectively. *In situ* ^{1}H NMR measurements were directly taken from the sample at regular intervals. The reaction was performed in the magnet and temporarily kept in a temperature-controlled water bath (300 ± 0.5 K).

Plant Material. Fresh leaves of *G. mauritiana* and *G.* cf. *puberula* were picked from plants cultivated in the Botanical Garden of the University of Vienna (HBV). These plants were originally collected from south Thailand and were kept in the greenhouse during winter. More specifically, *G. mauritiana* came from (i) Satun Province, Tarutao Island (HG 330, HG 331), and (ii) limestone in open deciduous forest near Krabi (HG 464). *G.* cf. *puberula* (HG 395, HG 397) came from Khao Soi Dao, southeast Thailand. Voucher specimens were identified by H.G. and deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

Extraction and Isolation. Dried leaves were ground and extracted with acetone at room temperature for 3 days, filtered, and concentrated, and the remaining residues were extracted with $CHCl_3$ from the aqueous solution. The $CHCl_3$ fractions were evaporated to dryness, and the lipophilic crude extracts were roughly separated by CC (Merck silica gel 60, 0.2−0.5 mm) with solvent mixtures of *n*-hexane, Et_2O, and MeOH. Further separation was achieved by preparative MPLC (400 × 40 mm column, Merck LiChroprep silica gel 60, 25−40 μm) with mixtures of EtOAc in *n*-hexane as mobile phase.

From 60 g of dried leaves of three collections of *G. mauritiana* (HG 330, HG 331, HG 464) the $CHCl_3$ fraction (1000 mg) was roughly separated by CC. The combined imide-containing fractions (208 mg), monitored by HPLC and TLC, were eluted with 50% Et_2O in *n*-hexane and 100% Et_2O and further separated by preparative MPLC with 10% EtOAc in *n*-hexane to afford 46 mg of krabin (**4**) and 9 mg of isokrabin (**5**). From 5 g of dried leaves of *G.* cf. *puberula* (HG 395, HG 397) the $CHCl_3$ fraction (220 mg) was separated in the same way, affording 2.5 mg of crystals of methoxypenimide B (**3**).

Methoxypenimide B (3): colorless crystals; mp 94−95 °C (Et_2O/*n*-hexane); UV (MeOH) λ_{max} 213sh, 238sh, 318 nm; IR (CCl_4) ν_{max} 3007w, 2927w, 2837w, 1701s, 1681s, 1621s, 1556s, 1521s, 1472w, 1432w, 1392w, 1292 m, 1257s, 1187m, 1108s, 1083m, 1058w cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 7.22 (1H, *d*, *J* = 9.9 Hz, CH-2), 7.14 (2H, *m*, CH-8), 6.83 (2H, *m*, CH-9), 6.59 (1H, *d*, *J* = 9.9 Hz, CH-3), 4.01 (2H, *m*, CH$_2$-6), 3.77 (3H, *s*, O-CH$_3$), 3.22 (3H, *s*, CH$_3$-11), 2.40 (3H, *s*, CH$_3$-1); FAB-MS *m/z* 279 [M + H]$^+$.

Krabin (4): colorless oil; UV (MeOH) λ_{max} 208sh, 237sh, 301 nm; IR (CCl_4) ν_{max} 3028w, 2928w, 2858w, 1718s, 1662s, 1568s, 1498w, 1454w, 1432w, 1342m, 1328s, 1256m, 1158s, 1140w, 972w, 942w, 890w, 700m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.27 (1H, *s*, CH-11), 7.97 (1H, *d*, *J* = 14.5 Hz, CH-2), 7.30 (2H, *m*, CH-8), 7.22 (2H, *m*, CH-9), 7.22 (1H, *m*, CH-10), 6.03 (1H, *d*, *J* = 14.5 Hz, CH-3), 3.94 (2H, *m*, CH$_2$-5), 2.86 (2H, *m*, CH$_2$-6), 2.37 (3H, *s*, CH$_3$-1); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 165.6 (C-4), 162.5 (C-11), 151.5 (C-2), 138.2 (C-7), 128.9 (C-8), 128.6 (C-9), 126.6 (C-10), 110.8 (C-3), 42.1 (C-5), 34.6 (C-6), 15.0 (C-1); EIMS *m/z* 249 [M]$^+$, 202, 130, 104, 101 (100), 91, 73; ESI-MS *m/z* 250 [M + H]$^+$; HRMS (ESI) calc ($C_{13}H_{16}O_2NS$) 250.0902, found 250.0904 ± 5 ppm.

Isokrabin (5): colorless oil; UV (MeOH) λ_{max} 208sh, 235sh, 314 nm; IR (CCl_4) ν_{max} 3028w, 2926m, 2856w, 1712s, 1654s, 1546s, 1498w,

1454w, 1432w, 1384w, 1342w, 1330m, 1302m, 1158s, 1082w, 1038w, 984w, 838w, 698m cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ_H) 9.24 (1H, *s*, CH-11), 7.35 (1H, *d*, *J* = 9.7 Hz, CH-2), 7.28 (2H, *m*, CH-8), 7.23 (2H, *m*, CH-9), 7.23 (1H, *m*, CH-10), 6.28 (1H, *d*, *J* = 9.7 Hz, CH-3), 3.93 (2H, *m*, CH$_2$-5), 2.86 (2H, *m*, CH$_2$-6), 2.45 (3H, *s*, CH$_3$-1); ^{13}C NMR (100 MHz, $CDCl_3$, δ_C) 166.6 (C-4), 161.3 (C-11), 156.8 (C-2), 137.8 (C-7), 128.9 (C-8), 128.6 (C-9), 126.6 (C-10), 109.7 (C-3), 41.5 (C-5), 37.7 (C-6), 19.9 (C-1); EIMS *m/z* 249 [M]$^+$, 202, 130, 104, 101 (100), 91, 83.

Methyl *N*-methylthiocarbamate (7). *In situ* from reaction: ^{1}H NMR (400 MHz, CD$_3$OD, δ_H) 2.65 (3H, *s*, CH-3), 2.18 (3H, *s*, CH-1); ^{13}C NMR (100 MHz, CD$_3$OD, δ_C) 170.1 (C-2), 27.4 (C-1), 13.1 (C-3); GC-MS, phenylacetic acid methyl ester/methyl *N*-methylthiocarbamate = 20:1 (15, rt =16 min); MS *m/z* 105 (20%, M$^+$, C_3H_7ONS), 75 (5%, M$^+$ − NHCH$_3$), 58 (100%, M$^+$ − SCH$_3$).

References and Notes

(1) Greger, H.; Hofer, O.; Zechner, G.; Hadacek, F.; Wurz, G. *Phytochemistry* **1994**, *37*, 1305–1310.
(2) Hofer, O.; Greger, H. In *Progress in the Chemistry of Organic Natural Products*; Herz, W.; Falk, H.; Kirby, G. W.; Moore, R. E., Eds.; Springer-Verlag: Wien, 2000; Vol. 80, pp 187−223.
(3) Hehenberger, T. MS Thesis, University of Vienna, Austria, 2007.
(4) Chansriniyom, C.; Ruangrungsi, N.; Lipipun, V.; Kumamoto, T.; Ishikawa, T. *Chem. Pharm. Bull.* **2009**, *57*, 1246–1250.
(5) Hofer, O.; Zechner, G.; Wurz, G.; Hadacek, F.; Greger, H. *Monatsh. Chem.* **1995**, *126*, 365–368.
(6) Greger, H.; Hofer, M.; Teichmann, K.; Schinnerl, J.; Pannell, C. M.; Vajrodaya, S.; Hofer, O. *Phytochemistry* **2008**, *69*, 928–938.
(7) Greger, H.; Zechner, G.; Hofer, O.; Hadacek, F.; Wurz, G. *Phytochemistry* **1993**, *34*, 175–179.
(8) Samuel, R.; Ehrendorfer, F.; Chase, M. W.; Greger, H. *Plant Biol.* **2001**, *3*, 77–87.
(9) Greger, H.; Zechner, G.; Hofer, O.; Vajrodaya, S. *J. Nat. Prod.* **1996**, *59*, 1163–1168.
(10) Shapiro, J. P.; Bowman, K. D.; Lapointe, S. L. *J. Agric. Food Chem.* **2000**, *48*, 4404–4409.
(11) Greger, H.; Hadacek, F.; Hofer, O.; Wurz, G.; Zechner, G. *Phytochemistry* **1993**, *32*, 933–936.
(12) Pacher, T. Ph.D. Thesis, University of Vienna, Austria, 2005.
(13) Engelmeier, D. Ph.D. Thesis, University of Vienna, Austria, 2002.
(14) Lukaseder, B.; Vajrodaya, S.; Hehenberger, T.; Seger, C.; Nagl, M.; Lutz-Kutschera, G.; Robien, W.; Greger, H.; Hofer, O. *Phytochemistry* **2009**, *70*, 1030–1037.
(15) Johnson, W. M.; Littler, S. W.; Strauss, C. R. *Aust. J. Chem.* **1994**, *47*, 761–756.
(16) Hinterberger, S.; Hofer, O.; Greger, H. *Tetrahedron* **1994**, *50*, 6279–6286.
(17) Krohn, K.; Franke, C.; Jones, P. G.; Aust, H.-J.; Draeger, S.; Schulz, B. *Liebigs Ann. Chem.* **1992**, 789–798.
(18) Niyaz Khan, M. *Int. J. Chem. Kinet.* **1987**, *19*, 142–153.
(19) Niyaz Khan, M. *J. Pharm. Sci.* **1984**, *73*, 1767–1771.
(20) Kozakiewics, J. J.; Huang, S.-Y.; Draney, D. R.; Villamizar, J. L. In *Chemical Reactions on Polymers*; Benham, J. L.; Kienstle, J. F.; Eds.; ACS Symposium Series 364; American Chemical Society: Washington DC, 1988; pp 291−299.
(21) Page, M. I. *Acc. Chem. Res.* **1984**, *17*, 144–151.
(22) Niclas, N.; Alsfasser, R. *J. Chem. Soc., Dalton Trans.* **2006**, 3188–3199.
(23) Lehn, J. L.; Wipff, G. *J. Am. Chem. Soc.* **1980**, *102*, 1347–1354.
(24) Brown, R. S.; Bennet, A. J.; Slebocka-Tilk, H. *Acc. Chem. Res.* **1992**, *25*, 481–488.
(25) Kresge, A. J.; Fitzgerald, P. H.; Chiang, Y. *J. Am. Chem. Soc.* **1974**, *96*, 4698–4699.
(26) Greger, H.; Hofer, O.; Kählig, H.; Wurz, G. *Tetrahedron* **1992**, *48*, 1209–1218.
(27) Brecker, L.; Ribbons, D. W. *Trends Biotechnol.* **2000**, *18*, 197–202.
(28) Brecker, L.; Mahut, M.; Schwarz, A.; Nidetzky, B. *Magn. Reson. Chem.* **2009**, *47*, 328–332.
(29) Maruyama, H. B.; Suhara, Y.; Suzuki-Watanabe, J.; Maeshima, Y.; Shimizu, N.; Ogura-Hamada, M.; Fujimoto, H.; Takano, K. *J. Antibiot.* **1975**, *28*, 636–647.
(30) Hofer, O.; Greger, H.; Lukaseder, B.; Vajrodaya, S.; Bacher, M. *Phytochemistry* **2000**, *54*, 207–213.
(31) Stone, B. C. *Proc. Acad. Nat. Sci. Phila.* **1985**, *137*, 1–27.
(32) Stone, B. C. *Garden Bull. Singapore* **1994**, *46*, 113–119.