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SYNTHESIS AND CORRECTED STRUCTURES OF SULPHUR-CONTAINING AMIDES FROM *GLYCOSMIS* SPECIES: SINHARINES, PENIMIDES, AND ILLUKUMBINS

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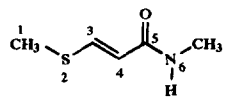
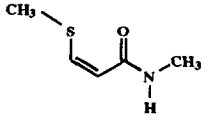
Abstract: The structures of sinharine and methylsinharine isolated from *Glycosmis cyanocarpa*, penimide A and penimide B from *G. chlorosperma*, and methylillukumbin A, illukumbin B and methylillukumbin B from *G. mauritiana* were revised on the basis of synthesis and additional spectroscopic evidence. The methylthio group and the aromatic moiety have to change their positions in the molecules. Thus the compounds represent amides of 3-(methylthio)-propenoic acid and not cinnamides as described previously.

In connection with our screening project on bioactive natural compounds of Sri Lankan and Malaysian Rutaceae, we have recently reported a series of new sulphur-containing amides from the methanolic leaf extracts of different *Glycosmis* species. Their structures were published in three preceding papers[1-3]. The fungitoxic derivatives sinharine (**3**) and methylsinharine (**4**) were originally isolated from *Glycosmis cyanocarpa* (Bl.) Spreng. collected in the Sinharaja rain forest of Sri Lanka[1], whereas the simple methylamides penangin (**1**) and isopenangin (**2**) were found in the Malayan *G. chlorosperma* (Bl.) Spreng. together with the two imides penimide A (**5**), and penimide B (**6**)[2]. In *G. mauritiana* (Lam.) Tanaka of the more humid and higher elevated mountain forests of Sri Lanka we detected three further related derivatives with high antifungal activity. They were designated as illukumbin B (**7**), methylillukumbin B (**8**) and methylillukumbin A (**9**)[3]. However, as already indicated in a 'note added in proof' in Ref.[3], our subsequent synthetic studies and NOE measurements have shown that the structures **3-9** have to be revised.

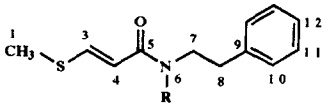
As shown in the formula diagram, the previously suggested structures of compounds **3-9** have to be corrected to their isomeric forms with phenyl and methylthio groups exchanged. However, this change also alters the chemical character of the compounds from originally published cinnamides to amides of 3-(methylthio)-propenoic acid. Moreover, a wrong biosynthetic sequence has been deduced from the suggested structures linking cinnamic acid to decarboxylated and methylated cysteine as amine component. Based on the new corrected structures, the acid moiety most likely results from deaminated and methylated cysteine, whereas the amine part may be derived from decarboxylated phenylalanine (compare the corrected structure **3** for sinharine with the corresponding synthetic product **10**).



1 penangin

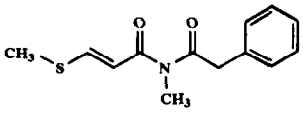


2 isopenangin

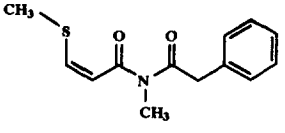


3 R = H sinharine

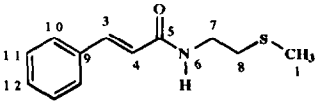
4 R = CH_3 methylsinharine



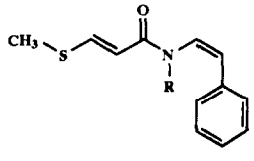
5 penimide A



6 penimide B

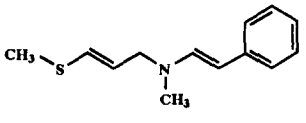


10 (synthetic)

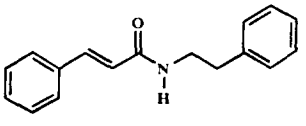


7 R = H illukumbin B

8 R = CH_3 methylillukumbin B



9 methylillukumbin A



11 (from *Clausena indica*)

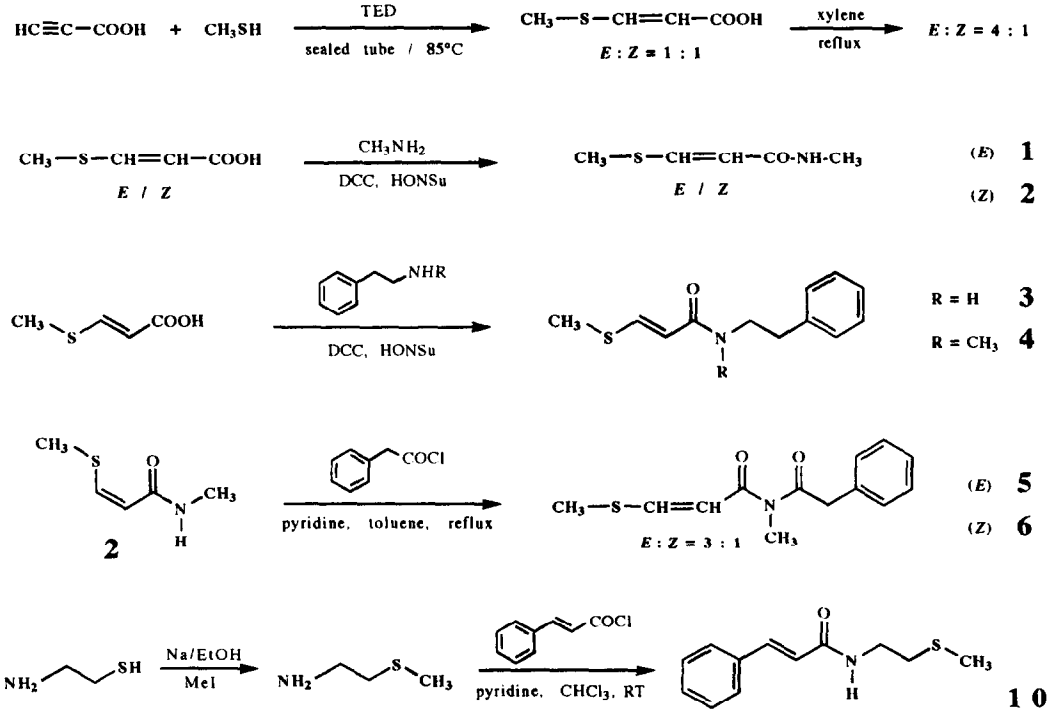
Cinnamides are common in several Rutaceae genera[4], e.g. *Aegle*[5], *Zanthoxylum*[6] or *Clausena*[7] which is closely related to *Glycosmis*. Consequently the presence of cinnamides in *Glycosmis* species seemed to fit well into the general biogenetic trends, even more as we had already isolated the known cinnamoyl-phenethylamide (**11**)[7a] together with other cinnamic acid derived amides from *Clausena indica*[8].

The synthesis of cinnamoyl-2-methylthioethylamide (**10**) revealed the wrong structure originally assigned to sinharine[1]. Further syntheses of 3-(methylthio)-propenoylphenethylamide = sinharine (**3**), methylsinharine (**4**), and penimide A (**5**) and B (**6**), together with NOE-difference measurements for sinharine (**3**) and methylillukumbin B (**8**) unambiguously showed that methylthiopropenoic acid was the acid component of all natural amides isolated so far from *Glycosmis* species[1-3]. Direct comparison of the NMR spectra of compounds **1-11** (Table 1) shows that there exist also some characteristic – although relatively small – differences in chemical shifts and coupling constants for the olefinic protons of methylthiopropenoyl and phenylpropenoyl derivatives.

Derivatives of 3-methylthiopropenoic acid seem to be rather scarce as natural constituents. So far this acid was reported only for liverworth[9] forming an aromatic ester, and for the legume *Entada phaseoloides* (l.) Merr. in three closely related amides named entadamide A, B and C[10].

Syntheses

3-(Methylthio)-propenoic acid was synthesized by an addition reaction of methylthiol to the triple bond of propiolic acid[10c]. (*E*) and (*Z*) Isomers were formed at about equal ratio, after reflux in xylene the thermodynamic equilibrium lies at an (*E*) content of 80%.



Pure (*E*) acid can be obtained via crystallization. Penangin (**1**), isopenangin (**2**), sinharine (**3**), and methylsinharine (**4**) can be obtained readily by reaction of (*E*) or (*Z*) acid (plus N-hydroxysuccinimide and dicyclohexylcarbodiimide) with the proper amine in 65-70% yield. The imides penimide A (**5**) and penimide B (**6**) were synthesized from synthetic isopenangin (**2**) and 2-phenylacetylchloride at the temperature of boiling toluene. At that temperature isomerization at the double bond takes place and a mixture of (*E*) and (*Z*) imide is formed which can be separated chromatographically (ratio of **5**:**6** = 3:1). The synthetic cinnamic acid amide **10** was obtained from S-methylated cysteamine and cinnamic acid chloride.

NMR Spectra

^{1}H NMR and ^{13}C NMR spectra are listed in Tables 1 and 2. It is interesting to note that the influence of the methylthio group and the phenyl group on the chemical shifts of neighbouring atoms (^{1}H as well as ^{13}C) is very similar, which led previously to wrong conclusions. For better comparison of the relevant positions 3 and 4, and 7 and 8 for compounds **3-11**, the numbering of cinnamides **10** and **11** is chosen in a way to obtain equal labels for analogous positions in both series, methylthiopropenamides and cinnamides

Table 1. ^{1}H NMR data of methylthiopropenoic acid and cinnamic acid amides ($CDCl_3$, δ/ppm, TMS, Bruker AM 400 WB)

No.	1	3	4	6	7	8	10	11	12
Methylthiopropenamides:									
1	2.32 s	7.62 d	5.61 d	2.88 d	5.31 br.q	—		—	
2	2.35 s	6.78 d	5.74 d	2.87 d	5.40 br.q	—		—	
3	2.30 s	7.62 d	5.54 d	5.34 br.t	3.59 ps.q	2.85 t	7.20 d	7.32 dd	7.23 dd
4[a]	2.23 br.s	7.58 d	5.78 d	2.98 s	3.58 t	2.87 t			
	2.35 br.s	7.71 d	6.07 d	2.96 s	3.63 t	2.87 t	7.15-7.30 m (5H)		
5	2.36 s	7.87 d	6.39 d	3.24 s	—	4.05 s	7.25 d	7.33 dd	7.26 dd
6	2.35 s	7.18 d	6.55 d	3.18 s	—	4.04 s	7.18 d	7.25 dd	7.19 dd
7	2.35 s	7.78 d	5.59 d	7.47 br.d	7.07 dd	5.78 d	7.30 br.d	7.40 dd	7.26 tt
8	2.25 br.s	7.67 br.d	6.14 d	3.03 s	6.40 br.d	6.17 br.d	7.29 br.d	7.32 ddd	7.24 tt
9	2.41 s	7.84 br.d	6.24 d	3.29 s	7.31 d	6.01 d	7.34 br.d	7.31 ddd	7.19 tt
Cinnamides:									
10	2.11 s	7.63 d	6.44 d	6.35 br.t	3.59 ps.q	2.70 t	7.48 d	7.33 m	7.31 m
11	—	7.62 d	6.31 d	5.59 br.t	3.67 ps.q	2.90 t	7.48 d[b]	7.31–7.37 m[b]	

Coupling constants: **1**: $J_{3,4}$ = 14.6 Hz, $J_{NH,NMe}$ = 4.9 Hz; **2**: $J_{3,4}$ = 9.9 Hz, $J_{NH,NMe}$ = 4.9 Hz; **3** : $J_{3,4}$ = 14.6 Hz, $J_{NH,7}$ = 7 Hz, $J_{7,8}$ = 7 Hz; **4**: $J_{3,4}$ = 14.5 Hz, $J_{7,8}$ = 7.5 Hz; **5**: $J_{3,4}$ = 14.4 Hz, $J_{ortho\text{-}Ar}$ = ca. 7.5 Hz; **6**: $J_{3,4}$ = 9.9 Hz; **7**: $J_{3,4}$ = 14.5 Hz, $J_{NH,7}$ = 10.5 Hz, $J_{7,8}$ = 9.6 Hz, $J_{10,11}$ = $J_{11,12}$ = 7.5 Hz, $J_{10,12}$ = 1.1 Hz; **8**: $J_{3,4}$ = 14.5 Hz, $J_{7,8}$ = 8.1 Hz, $J_{10,11}$ = $J_{11,12}$ = 7.5 Hz, $J_{10,12}$ = 1.2 Hz; **9**: $J_{3,4}$ = 14.3 Hz, $J_{7,8}$ = 14.5 Hz, $J_{10,11}$ = $J_{11,12}$ = 7.5 Hz, $J_{10,12}$ = 1.2 Hz; **10**: $J_{3,4}$ = 15.6 Hz, $J_{NH,7}$ = $J_{7,8}$ = 6.5 Hz; **11**: $J_{3,4}$ = 15.6 Hz, $J_{NH,7}$ = $J_{7,8}$ = 6.7 Hz; **3** - **11**: $J_{ortho\text{-}Ar}$ = ca. 7.5 Hz.

[a] Upper line: *s-cis*, lower line: *s-trans* conformer; [b] Aromatic protons of the cinnamic acid moiety, further aromatic resonances of the phenethylamine part at 7.31–7.37 (2H, m) and at 7.21–7.27 ppm (3H, m), comp. Ref.[7a]

Table 2. ^{13}C NMR data of methylthiopropenoic acid and cinnamic acid amides ($CDCl_3$, δ/ppm, TMS, Bruker WM 250)

No.	1	3	4	5	6-Me	7	8	9	10,11,12 (arom. CH)*
Methylthiopropenamides:									
1	14.6	142.6	115.7	165.2	26.3	—	—	—	—
3[a]	14.6	142.9	115.7	164.4	—	40.6	35.7	138.9	128.8, 128.6, 126.5
4[b]	14.4	143.8	112.1	165.0	34.0	51.5	33.7	138.1	
	14.6	144.6	112.3	164.6	35.9	50.2	35.1	139.1	126.0, 126.6, 128-129
6	20.0	155.2	114.6	168.8*	31.9	174.1*	44.5	134.6	127.1, 128.7, 129.6
8	14.7	145.1	113.3	176.2	34.4	128.9*	124.6*	138.6	128.6, 128.6, 128.0
Cinnamides:									
10	14.9	141.1	120.5	165.9	—	37.8	33.8	134.7	127.7, 128.7, 129.6
11[c]	—	140.8	120.5	165.6	—	40.7	35.6	134.6	128.6, 128.6, 129.4

* Interchangeable.

[a] In $CDCl_3$, note: in Ref.[1] in deuteromethanol; [b] Data from synthetic material (due to the small amount available the data in Ref.[1] were not complete and included some misinterpretations), upper line: *s-cis*, lower line: *s-trans* conformer; [c] Taken from Ref.[7a] (the aromatic cinnamic acid CH are listed).

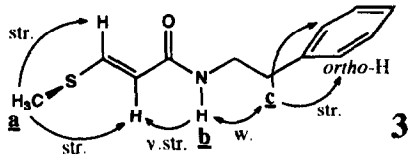
(comp. formula scheme and Tables 1 and 2). Especially the significant low field olefinic proton of position 3 is at very similar chemical shift values for both amide series. The deviations for corresponding resonances in the ^{13}C NMR spectra are also surprisingly small: e.g. 1.8 ppm for carbon atom no. 3, 4.8 ppm for no. 4, 2.8 ppm for no. 7, and 1.9 ppm for carbon no. 8 in direct comparison of sinharine (**3**) with the synthetic cinnamide **10**.

The ^1H coupling constants of the olefinic protons are also very similar. In methylthiopropenamides **1-9** $J(3,4)$ is uniformely 14.5 ± 0.1 Hz, in cinnamides $J(3,4)$ = 15.6 Hz. The difference of about 1 Hz is not very much but already significant, since coupling constants are not very sensitive to different substituent patterns. However, this small but significant difference was less clear previously.

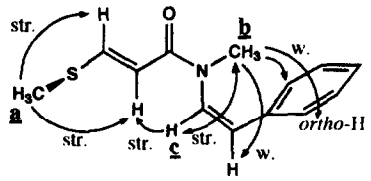
The partial misinterpretation of the lanthanide induced shifts (LIS) in Ref.[1] is also the result of the similar topologies of the sinharines **3/4** and the previously assumed structure of type **10**: the part including positions 3-8 is completely identical (compare formula scheme), positions 1 (S-Me) and the aromatic positions 9-12 are (although different) in a similar arrangement relative to the coordinating amide oxygen. This allowed a reasonable good fit for the wrong structures of *s-cis* and *s-trans* **4**. The assignment of the NMR resonances to the *s-cis* and *s-trans* conformers and the results concerning the torsion about the bond C4–C5 are still valid and correct, the calculated values for the complete LIS-simulation and details about torsions in the flexible phenethylamine part (with three successive single bonds) needs further careful calculations. However, preliminary calculations with variation of all torsional

angles within reasonable limits (not too far from staggered conformations) have already lowered the obtainable R-factor for both rotamers below 6%, which compares favourably to the original 'wrong' fits of about 9%. A detailed conformational analysis of this series of sulphur-containing amides is in progress.

The NOE results for compounds **3** and **8** are also clearly in favour of the methylthio-propenamide structures. Irradiation **a** (S-methyl, see scheme) shows very strong effects for both *trans*-olefinic protons in **3** and **8**. This indicates that the torsional angle C1–S2–C3–C4 is close to 90°, which agrees well with LIS-results and force field calculations. Irradiations **b** and **c** within the amine part of the amides **3** and **8** (comp. NOE scheme) give a complete picture of constitution and configuration, including some further informations concerning possible conformations of the molecules.



3



8

EXPERIMENTAL

(E)-3-(Methylthio)-propenoic Acid [10c]

7.5 g (0.107 mol) 2-Propynoic acid (propiolic acid), 0.13 g (1.16 mmol) triethylene-diamine, and 10.25 g (0.214 mol) methanethiol were mixed in an acetone/CO_2 cooled tube which was sealed with a teflon-tightened screwable stopper. The tube was placed in an autoclave and an external pressure of 60 atm was applied. The autoclave was heated to 85°C for 12 h. After cooling, the tube was opened. The orange-coloured reaction mixture solidified immediately. An NMR spectrum of the crude reaction product showed an (*E*):(*Z*) mixture of ca. 1:1. After 24 h reflux of the crude product in xylene the (*E*):(*Z*) ratio was 80:20, further heating did not cause any change (total yield 10.1g, 80%). However, almost pure (*E*) isomer [≤ 5 % (*Z*)] precipitated from the xylene solution. This material could be further recrystallized from ethyl acetate yielding 1.68g pure (*E*)-3-(methylthio)-propenoic acid. ^{1}H NMR ($CDCl_3$, δ/ppm): ca. 10.5 (v.br. s, COOH), 7.89 (d, 1H, *J*= 14.8 Hz, S-CH=), 5.66 (d, 1H, *J*= 14.8 Hz, CO-CH=), 2.36 (s, 1H, S-Me); ^{13}C NMR ($CDCl_3$, δ/ppm): 170.4 (s, COOH), 150.3 (d, S-CH=), 112.3 (d, CO-CH=), 14.4 (q, S-Me). [(*Z*) isomer: ^{1}H NMR ($CDCl_3$, δ/ppm): ca. 10.7 (v.br. s, COOH), 7.20 (d, 1H, *J*= 10.2 Hz, S-CH=), 5.85 (d, 1H, *J*= 10.2 Hz, CO-CH=), 2.40 (s, 1H, S-Me); ^{13}C NMR ($CDCl_3$, δ/ppm): 171.8 (s, COOH), 155.0 (d, S-CH=), 112.2 (d,CO-CH=), 12.9 (q, S-Me)].

General Procedure for the Synthesis of Amides **1-4**

0.88 g (7.5 mmol) 3-(Methylthio)-2-propenoic acid (an (*E*)/(*Z*) mixture for **1/2** and pure (*E*) product for **1**, **3**, and **4**) and 0.98 g (8.5 mmol) N-hydroxysuccinimide (HONSu) were

dissolved in 5 ml dimethylformamide (DMF) and cooled to 5°C. 1.5 g (7.3 mmol) dicyclohexylcarbodiimide (DCC) was also dissolved in DMF at 5°C and added dropwise to the former solution maintaining the mixture at 5°C. A colourless precipitate formed slowly. The mixture was stirred at 5°C overnight. Then a cooled solution of 12.5 mmol of the proper amine in 5 ml DMF was added dropwise and the stirred mixture kept again at 5°C overnight. Afterwards the colourless precipitate of dicyclohexylurea was filtered off, the filtrate was evaporated in vacuo to dryness and chromatographed on silica gel with ethyl acetate as eluent (column l = 40 cm, i.d. = 2 cm).

Penangin, (E)-3-(methylthio)-propenoic acid methylamide (**1**): From (*E*)-acid and methylamine (gas bubbled through DMF, weight control). Yield 0.63g (64 %).

Isopenangin, (Z)-3-(methylthio)-propenoic acid methylamide (**2**): The acid used was an (*E*):(*Z*) mixture of 1:1; the resulting amides **1** and **2** could be separated chromatographically (silica gel, column l = 30 cm, i.d.= 3 cm, ethylacetate). Yield 0.32 g of **1** and 0.31 g of **2** (together 64 %).

Sinharine, (E)-3-(methylthio)-propenoic acid phenethylamide (**3**): From (*E*)-acid and 2-phenylethylamine. Yield 1.04 g (65 %).

Methylsinharine, (E)-3-(methylthio)-propenoic acid N-methyl-phenethylamide (**4**): From (*E*) acid and N-methyl-2-phenylethylamine (synthesized from 2-phenylethylamine via LAH reduction of its formamide[11]). Yield after additional MPLC (l = 45 cm, i.d.= 4 cm, silica gel LiChroprep. Si60 Merck, particle size 25-40 μm, ethylacetate) 1.25 g (71 %).

Synthesis of Penimide A (**5**) *and Penimide B* (**6**)

(E)- and (Z)-3-(Methylthio)-propenoyl-phenylacetyl-N-methylimide

140 mg (1.186 mmol) Synthetic amide **2** [(*Z*) configuration] and 184 mg pyridine (1.32 mmol) were dissolved in 10 ml dry toluene. A second solution of 314 mg (2.04 mmol) 2-phenylacetylchloride (prepared from 2-phenylacetic acid and thionyl chloride) in 10 ml toluene was added dropwise and the resulting mixture was refluxed for 2 h. After evaporation in vacuo to dryness and chromatography (silica gel, column l = 25 cm, i.d. = 3 cm, chloroform) the material obtained was a mixture of penimide A [**5**, (*E*) configuration]: penimide B [**6**, (*Z*) configuration] with a ratio of 3 : 1. A second chromatography with petrol ether : ethylacetate = 8 : 2 as eluent allowed the separation into pure **5** and **6**. Yield: 201 mg **5** and 67 mg **6** (together 90 %).

The NMR spectra of the synthetic compounds **1-6** were identical with the spectra of the natural products listed in Tables 1 and 2.

Cinnamic Acid 3-(Methylthio)-ethylamide (**10**)

300 mg Cysteamine was dissolved in 5 ml absolute EtOH and 250 g sodium wire was added. After dissolution of the metal 700 mg (4.9 mmol) methyl iodide was added dropwise and the reaction mixture was stirred for 24 h. NaI formed was filtered off, and the filtrate was evaporated in vacuo to dryness (the colour changed from yellow to dark red). The residue was dissolved in NaOH (colourless solution) and extracted with chloroform. The organic phase was dried over $MgSO_4$ and evaporated to dryness giving 173 mg (51%) of S-methylcysteamine.

80 mg (0.9 mmol) S-Methylcysteamine was suspended in chloroform and 80 mg (1 mmol) pyridine was added. This solution was added slowly to a stirred solution of freshly prepared cinnamoyl chloride (cinnamic acid / thionyl chloride) in chloroform. After 1 h the solvent was evaporated and the crude product was chromatographed (silica gel, column l = 30 cm, i.d. = 2 cm, petrol ether : ethylacetate 2 : 8). Yield 85 mg (43%); m.p. 188-189°C.

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