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Short Communication

Ritigalin, a New Thiocarbonic Acid Imide from *Glycosmis* Species#

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Summary. From the lipophilic leaf extracts of *Glycosmis mauritiana* and *G. parviflora* (Rutaceae-Aurantioideae), the novel thiocarbonic acid derived imide ritigalin was isolated as major component by MPLC and TLC. The structure was elucidated by spectroscopic evidence (^{1}H, ^{13}C NMR; MS; IR; UV).

Keywords. Thiocarbonic acid imide; Thiocarbamic acid S-methylester; Methylthiopropenoic acid amides; Imides; Rutaceae-Aurantioideae; *Glycosmis mauritiana; Glycosmis parviflora.*

Ritigalin, ein neues Thiocarbonsäureimid aus *Glycosmis-Arten* (Kurze Mitt.)

Zusammenfassung. Aus den lipophilen Blatt-Extrakten von *Glycosmis mauritiana* und *G. parviflora* (Rutaceae-Aurantioideae) wurde mit MPLC und DC das neue Methylthiocarbonsäureimid Ritigalin als Hauptkomponente isoliert. Seine Struktur wurde mit spektroskopischen Methoden aufgeklärt (^{1}H-, ^{13}C-NMR; MS; IR; UV).

Introduction

In previous papers we reported that the accumulation of 3-(methylthio)-propenoic acid derived amides represents a typical biogenetic trend in the leaves of the genus *Glycosmis* (Rutaceae/Aurantioideae – Citrus family). So far, 16 derivatives have already been identified from four species of *Glycosmis* [1–4]. Biogenetically, all acid moieties may be derived from the amino acid cysteine by deamination and S-methylation [2]. Interest in these compounds rests in their novel structures as well as in their biological role. Especially the illukumbins (3-(methylthio)-propenoic acid styryl amides) isolated from lipophilic leaf extracts of *Glycosmis mauritiana* (Lam.) Tanaka exhibit a pronounced antifungal activity [3].

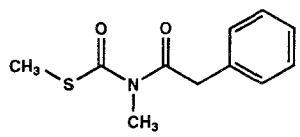
Herrn Professor Dr. *K. Schlögl* mit den besten Wünschen zum 70. Geburtstag gewidmet

Comparative HPLC analyses of samples of *G. mauritiana* collected from different localities in Sri Lanka showed a high degree of chemical variation between different geographical provenances and even between single individuals of the same population [3]. In the search for new bioactive compounds we concentrated our further screening to the northern provinces of Sri Lanka, where *G. mauritiana* represents the most common species in the dry, semi-evergreen forests [5]. As a consequence, we found a series of individuals whose amide pattern clearly deviates from all samples investigated so far. The leaf extracts were dominated by a single compound which was shown to be a novel sulphur-containing imide. With reference to the place of collection in the Ritigala Mts. it was designated as ritigalin (**1**). Later this compound was also isolated as the major component from the leaves of *G. parviflora* (Sims) Little (= *G. citrifolia* (Willd.) Lindley) grown from seeds in the Botanical Garden of the University of Vienna. The isolation and structure elucidation of this compound is reported in the present paper.

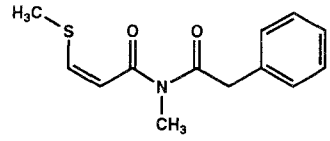
Results and Discussion

The methanolic extract of dried leaves of *G. mauritiana* was concentrated and fractionated with chloroform to give the lipophilic crude extract for qualitative HPLC and preparative MPLC analysis. The HPLC profile was characterized by a single dominating peak showing a simple UV-spectrum with a maximum at 231 nm (MeOH). Its IR spectrum with prominent bands at 1695 cm^{-1}, 1280 cm^{-1}, and 1082–1037 cm^{-1} (CCl_4) strongly resembles the previously isolated imide penimide B (**2**) [2]. Moreover, weak resonances at 3092 cm^{-1}, 3069 cm^{-1}, and 3035 cm^{-1} together with medium resonances at 709 cm^{-1} and 694 cm^{-1} point at a monosubstituted aromatic ring. This is also supported by the ^1H and ^{13}C NMR spectra showing relatively few signals. The proton spectrum is characterized by 5 aromatic protons for an aromatic ring, a singlet for 2H at 4.14 ppm, and two methyl signals at 3.31 and 2.35 ppm. The singlet character of the methylene group and the relatively low field position indicate an isolated $-CH_2-$ next to strongly anisotropic groups. NOE spectroscopy proved that one neighbouring moiety is the aromatic ring: irradiation at 4.14 ppm results in a strong NOE for the aromatic dublet of 2H at 7.24 ppm. The second downfield shifting neighbour of $-CH_2-$ is CO which follows from the ^{13}C NMR spectrum. The latter is characterized by two carbonyl resonances at 172.9 and 172.6 ppm, four aromatic C (one s and three d with two of them of about doubled intensity compared with the third one), a triplet at 44.7 ppm, and two quartets at 31.7 and 14.0 ppm. In combination with the two CO groups, the methyl group at 31.7 ppm, typical for N–Me, suggests an imide $CO-NCH_3-CO$ arrangement (compare $\delta = 31.9$ ppm for the corresponding imide N–Me of penimide B (**2**) [2, 4]). Regarding the previously isolated methylthio derivatives, the high field methyl group at 14.0 ppm in ^{13}C NMR and at 2.35 ppm in the ^1H NMR spectrum let expect a S–Me group. The presence of sulphur is also supported by the mass spectrum with $m/z = 223$ (i.e. 17%) as the heaviest ion. The sum of all protons and carbon atoms (+ 2 oxygen atoms for the two CO groups) derived from the NMR spectra amount to a mass of 191. Consequently, an additional mass of 32 is needed for the molecular ion of 223. The isotope pattern ($M^+ + 2$ due to ^{34}S) in the MS fits also in the molecular formula $C_{11}H_{13}NO_2S$. The 100% peak is represented by the

benzylic (tropolonium) unit $C_7H_7^+$ at $m/z = 91$. All this evidence (NMR and MS) allow only the structure of the methylthio-benzyl-imide ritigalin (**1**).



1 Ritigalin



2 Penimid B

With regard to the already known sulphur-containing amides and imides isolated from *Glycosmis species* (*e.g.* penimid B, **2**), the unusual thiocarbonic acid subunit of ritigalin (**1**) may also be derived from the amino acid cystein. In the present case, the sulphur-containing acid may be interpreted as the result of an additional chain shortening of a cysteine derived precursor from 3C to only 1C by β-oxidation.

Experimental

NMR: Bruker AM 400 WB (400 MHz) and WM 250 (250 MHz); IR: Perkin-Elmer 398; UV: Perkin-Elmer Lambda 5; MS: Varian MAT 311 A; HPLC: Hewlett Packard 1090 II LC with diode array detection, UV Signal 230 nm, column 290 × 4 mm (Spherisorb ODS, 5 µm), mobile phase MeOH (gradient 60–100%) in aqueous buffer (*o*-phosphoric acid 0.015 mol, tetrabutylammonium hydroxide 0.0015 mol, $pH = 3$), flow rate: 1 ml min^{-1}.

Plant material: Leaves of *G. mauritiana* were collected from different localities in Sri Lanka (august 1992): (i) Ritigala Mts. (near Habarana); (ii) near Anuradhapura. *G. parviflora* was grown from seeds in the Botanical Garden of the University of Vienna (RUT-69), received as *G. citrifolia* from South China Institute of Botany, Academia Sinica, Kwangchow. Voucher specimens are deposited at the Herbarium of the Institute of Botany, University of Vienna (WU).

88 g air-dried leaves were extracted with methanol at room temp. for 5 days. The resulting extract was concentrated and the aqueous solution extracted 3 times with chloroform yielding 1.2 g of raw material. 1 g was disolved in *n*-hexan and roughly separated by CC (Merck silica gel 60, 35–70 mesh) with *n*-hexan-Et_2O–MeOH mixtures of increasing polarity. The fractions eluted with 10%–25% Et_2O in *n*-hexan containing 200 mg of crude material were combined and further separated by prep. MPLC (400 × 38 mm home-made column packed with Merck LiChroprep Si 60, 25–40 µm, ca. 6000 theoretical plates, UV detection, 254 nm, ISCO UA-5) using 15% EtOAc in *n*-hexan as eluent. The resulting 20.3 mg of crude product were purified by TLC with 25% Et_2O in *n*-hexan to yield 7.4 mg of pure ritigalin (**1**) (corresponding to 0.011% of air-dried leaves).

Ritigalin, S-Methylthiocarbonic phenylacetic N-methylimide (**1**)

Colourless oil; UV (λ_{max}/nm): 226, 210 (Et_2O); 231 (MeOH); IR (CCl_4 ν/cm^{-1}): 3092w, 3069w, 3035w, 2936w, 1695s, 1662m, 1604w, 1495w, 1455m, 1421m, 1394w, 1343m, 1280s, 1227m, 1197w, 1082m, 1057s, 1037m, 961w, 709m, 694m, 663w, 587m; ^{1}H NMR ($CDCl_3$, δ/ppm, *TMS*): 7.33 (t, 2H, $J_{ortho} \sim 7.5$ Hz, aromat. 3-H + 5-H), 7.30 (t, 1H, $J_{ortho} \sim 7.5$ Hz, arom. 4-H), 7.24 (d, 2H, $J_{ortho} \sim 7.5$ Hz, aromat. 2-H + 6-H), 4.14 (s, 2H, Ar–CH_2–CO), 3.31 (s, 3H, N–CH_3), 2.35 (s, 3H, S–CH_3); NOE: irradiation at 4.14 showed a strong effect for the d at 7.24 ppm; ^{13}C NMR ($CDCl_3$, δ/ppm, *TMS*): 172.9 (s, C=O), 172.6 (s, C=O), 134.0 (s, aromat. C-1), 129.5 (d, aromat. C-2 + C-6), 128.6 (d, aromat. C-3 + C-5), 127.1 (d, aromat. C-4), 44.7 (t, benzyl. CH_2), 31.7 (q, N–CH_3), 14.0 (q, S–CH_3); assignments of the aromatic CH based on increment system and signal strengths; MS [70 eV, 60 °C, *m/z* (rel. int.)]: 223 (17, $M^+ = C_{11}H_{13}NO_2S^+$), 208 (4, $M^+ - CH_3$), 208 (2, $M^+ - SCH_3$), 118 (99), 91 (100, $C_7H_7^+$), 75 (17), 65 (22).

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