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ISOLATION AND SYNTHESIS OF RUFULAMIDE, AN OLIGOPEPTIDE ANALOGUE FROM *METZGERIA RUFULA**

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Key Word Index—*Metzgeria rufula*; Metzgeriaceae; Hepaticae; oligopeptide analogue; rufulamide; chemical synthesis.

Abstract—An oligopeptide analogue consisting of L-glutamic, malonic and 2 molecules of anthranilic acid combined via amide bonds was isolated from the liverwort *Metzgeria rufula*. Its structure was elucidated by spectroscopic methods and by chemical synthesis. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Metzgeria species are thalloid liverworts the taxonomy of which is often very difficult [1, 2]. As they are lacking oil bodies there are no reports of terpenoid compounds (apart from sterols e.g.) which are typical for many other liverworts [3–5]. On the other hand chemical investigations regarding phenolic metabolites revealed that *Metzgeria* species are rich sources of flavonoids, mainly flavone-C-glycosides [6–9]. In the course of our studies on *Metzgeria rufula* Spruce [10] from Ecuador we could also detect a range of flavonoids which will be reported elsewhere. This paper deals with the isolation, structure elucidation and independent chemical synthesis of a new glutamic acid derivative named rufulamide.

RESULTS AND DISCUSSION

Chromatographic separation of the 80% aqueous methanol extract by different methods yielded rufulamide (**1**) besides two further N-containing compounds. The FAB mass spectrum of **1** exhibited $[M-H]^-$ at *m/z* 470. A fragment peak appeared at *m/z* 426 indicating the cleavage of a carboxylic group. The ^1H NMR spectrum of **1** showed signals integrating for 18 protons (Table 1). On the basis of the ^1H–^1H COSY we could detect three sequences. The eight protons from δ 7.12 to 8.46 belonged to two 1,2-disubstituted phenyl rings with their typical coupling pattern. Another sequence consisted of two methylenes, a methine proton and another proton (δ 8.57) which disappeared after addition of D_2O to the sample. All these features

Table 1. ^1H and ^{13}C NMR data of **1** (400 MHz, DMSO-d_6)

H		C	
		1	172.8
2	4.30 *m*	2	51.5
3a	2.11 *m*	3	26.5
3b	1.95 *m*		
4a/b	2.50* *m*	4	33.5
		5	170.2
NH-2	8.57 *d* (7.6)		
		6	166.2
7a	3.45 *d* (15.0)	7	45.3
7b	3.37 *d* (15.0)		
		8	165.5
		1′	116.8
		2′	140.6
3′	8.45 *d* (8.3)	3′	120.0
4′	7.55 *dt* (1.4/7.4)	4′	133.7a
5′	7.12 *dt* (1.0/7.6)	5′	122.5
6′	7.95 *dd* (1.6/7.9)	6′	130.9
		7′	169.3
NH-2′	11.14 *s*		
		1″	117.1
		2″	140.2
3″	8.46 *d* (8.3)	3″	120.2
4″	7.55 *dt* (1.4/7.4)	4″	133.8a
5″	7.14 *dt* (1.1/7.5)	5″	122.7
6″	7.95 *dd* (1.6/7.9)	6″	130.9
		7″	168.9
NH-2″	11.31 *s*		

* Partially overlapped by the DMSO-signal.
a: Assignment exchangeable.

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were in agreement with the respective data of a glutamyl moiety [11]. The third sequence only consisted of an isolated methylene group which was split into two doublets at δ 3.45 and 3.37 (J = 15.0 Hz each). Its chemical shift was typical for a position between two carbonyls. In addition the ^{1}H NMR spectrum still delivered signals for two chelated protons at δ 11.31 and 11.14 (singlet each) which also disappeared at the presence of D_2O. The analysis of the ^{13}C NMR and DEPT spectra of **1** afforded 10 quaternary, eight tertiary and three secondary carbons. The HMQC spectrum additionally showed that the signal at δ 130.9 comprised two tertiary carbons. Six of the quaternary carbons represented carbonyl groups (δ 165–171).

From the NMR data discussed so far and from the FAB mass spectrum we supposed a molecular composition of $C_{22}H_{21}N_3O_9$ for **1**. Regarding the substructures we concluded that rufulamide is composed of glutamic, malonic and two molecules of anthranilic acid. Moreover three amide bonds had to be implicated in order to meet with the molecular formula of **1**. On account of the ^{13}C–^{1}H long-range couplings (Table 2) it was evident that the malonyl part is bound to glutamic acid via the amino group. Starting from the NH-proton at δ 8.57 (NH-2) couplings arose to the carbons C-1, C-2 and C-3 of glutamic acid and to a carbonyl of malonic acid at δ 166.2 (C-6). A connectivity also existed between H-2 and C-6, from which the sequence continued to the methylene protons of malonic acid (H-7a/b), and from these protons to the carbonyl at δ 165.5 (C-8). The connectivities could be pursued to the chelated proton at δ 11.31 which was assigned to the NH-proton of one anthranilic acid moiety. In the same way we proved that the second anthranilyl moiety was bound to the C-5 atom of glutamic acid (δ 170.2). Acid hydrolysis of **1** yielded L-glutamic acid, malonic acid and anthranilic acid as shown by HPLC and TLC.

Another amide (**2**) which proved to be much more unstable in solution than **3** consisted of only glutamic and anthranilic acid. The methine carbon of the glutamyl part of **2** resonated at δ 3.68 indicating a free amino group in comparison with **1**. Hence anthranilic acid had to be bound at C-1 or C-5 of glutamic acid. The exact site of attachment could not be elucidated by NMR analysis. Unfortunately a signal for the NH-proton of anthranilic acid was not visible, so heteronuclear long-range couplings did not exist between the two substructures of **2**. In addition we isolated and identified anthranilic acid (**3**) itself.

Table 2. Long-range ^{13}C, ^{1}H correlations detected in the HMBC spectrum of **1**

H	Correlated carbons
2	1, 3, 4, 6
3a/b	1, 2, 4, 5
4a/b	2, 3, 5
NH-2	1, 2, 3, 6
7a/b	6, 8
3′	1′, 2′, 5′, 7′
4′	2′, 6′
5′	1′, 3′
6′	2′, 4′, 7′
NH-2′	5, 1′, 3′
3″	1″, 2″, 5″, 7″
4″	2″, 6″
5″	1″, 3″
6″	2″, 4″, 7″
NH-2″	8, 1″, 3″

Secondary metabolites of bryophytes containing nitrogen are very rare. Prenylated indole derivatives were earlier reported from *Riccardia* species [12]. Sakai *et al.* [13] found some macrocyclic maytansinoids in two mosses. The so-called anthocerodiazonin and some amides of glutamic acid with a cinnamoyl derivative were isolated from a sterile culture of the hornwort *Anthoceros agrestis* [11]. Recently two harman-type alkaloids were detected in the aquatic moss *Fontinalis squamosa* [14].

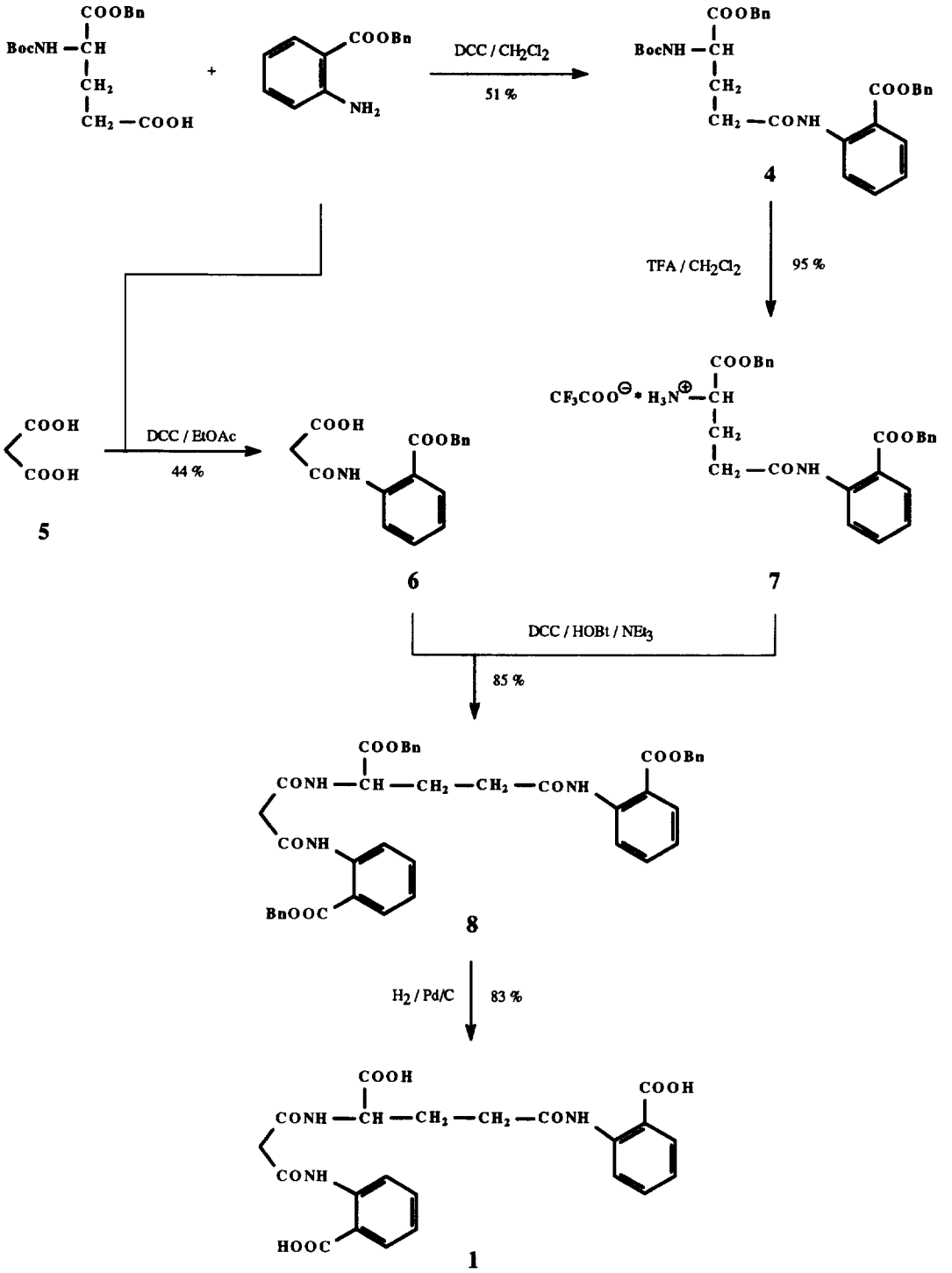
Synthesis of rufulamide (Scheme 1)

Rufulamide **1** was synthesized by a straightforward convergent strategy from the building blocks **6** and **7**, which were accessible by well-established methods of peptide synthesis (see Scheme 1). Firstly the *N*-tert-butoxycarbonyl protected monobenzylglutamate **2** was reacted with benzyl anthranilate **3** and DCC yielding the anthranilic amide **4**, in which the BOC group was removed by treatment with trifluoroacetic acid thus giving rise to the trifluoroacetate **7**. Secondly malonic acid **5** was transformed to the monoamide **6** with benzyl anthranilate **3** by a DCC-assisted coupling reaction. Finally the free carboxyl and amino functions of the building units **6** and **7** were combined using DCC and *N*-hydroxybenzotriazole as coupling reagents in the presence of triethylamine to achieve the formation of tribenzyl rufulamide **8**. Catalytic debenzylation of **8** led to rufulamide **1** being identical in all spectroscopic data with the natural product.

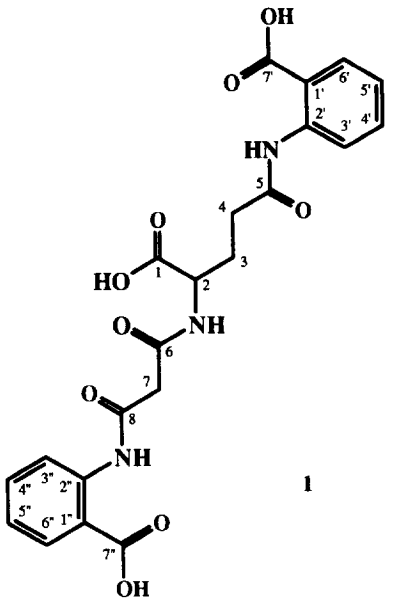
EXPERIMENTAL

Plant material. Metzgeria rufula was collected in September and October 1988 at the following localities in Ecuador (Pichincha region): in a secondary rain forest in Quebrada Alisal at the road from Quito to Santo Domingo, 2800 m (locality A), and in a rain forest at the road from Lloa to Rio Cristal, Hacienda Las Palmas, 2900 m (locality B). The plants were identified by Prof. S. R. Gradstein, Göttingen, F.R.G. Voucher specimens are deposited in the herbarium SAAR, no. 5076, Saarbrücken.

Extraction and isolation. 133 g of cleaned, air-dried, gametophytic plant material (33 g from locality A, 100 g from locality B) of *M. rufula* were extracted. The two samples could be combined for extraction as their patterns of secondary metabolites were identical



according to TLC- and HPLC-results. The ground plants were pre-extracted with $CHCl_3$ (×4). Afterwards they were extracted with 80% aq. MeOH (×4). The crude MeOH extract was purified from chlorophylls and lipophilic compounds by SPE on RP 18. Further fractionation of the extract was achieved by MPLC on RP 18 using a gradient between 20 and 80% aq. MeOH (in 2% HOAc) to yield 11 frs (from which we isolated 9 flavonoids). From fr. 11 (eluted between 50 and 80% aq. MeOH) we sepd compound **3** (fraction 11.1) from **1** and **2** (fraction 11.2) by renewed MPLC on RP 18 (50% aq. MeOH in 2% HOAc as solvent). Afterwards **1** was sepd from **2** (**2** eluted prior to **1**) by HPLC on RP 18 (55% aq. MeOH in 2% HOAc). Final purification of **1**–**3** was achieved by CC on Sephadex LH-20 with 70% aq. MeOH as eluent. The different chromatographic steps yielded 17.2 mg of **1**, 3.3 mg of **2** and 2.1 mg of **3**.



1

Chromatography. For TLC- and HPLC-procedures see ref. [15]. The compounds **1**–**3** were easily detected by their blue fluorescence in UV-light (mainly at 254 nm; compound **3** also exhibited a strong fluorescence at 365 nm) on cellulose layers and by their dark absorption (**1**–**2**) or faint blue fluorescence (**3**) on sheets of silica gel and RP 18 with fluorescence indicator.

Acid hydrolysis. Compound **1** (*ca* 5 mg) was hydrolysed with 1 M CF_3COOH (TFA) (1 hr, reflux). After removal of the TFA glutamic and malonic acid were sepd from anthranilic acid by SPE on RP 18. Malonic acid was analysed by HPLC on RP 18 (solvent: 1% H_3PO_4, detection at 210 nm). Chromatographic analysis of L-glutamic acid was achieved by TLC on a chiral plate (Macherey–Nagel; solvent: MeCN–MeOH–H_2O 4:1:1; cochromatography with commercial L- and D-glutamic acid; detection: ninhydrin; heating to 110).

Spectroscopic data. NMR: 400 MHz (1D), 500 MHz (2D; HETCOR: inverse technique). The carbon atoms of **2** (see below) were characterized by the DEPT pulse sequence in the following way: quat. = quaternary, tert. = tertiary, sec. = secondary carbon. The optical rotation value of **1** was determined at 20 at 578 nm in MeOH (Uvasol). FABMS, xenon, glycerol as matrix, 5–6 keV. CIMS, CH_4, 120 eV.

Compound **1**. NMR data: see Table 1. UV λ_{max}^{MeOH} nm, ($\log\varepsilon$). 302 (3.89) −260 *sh* (4.23) −252 (4.37) −226 *sh* (4.62) −221 (4.67). $[\alpha]_D$ +21.4 (*c* 1.7). FABMS *m/z* 470 $[M-H]^-$. Compound **2**. UV λ_{max}^{MeOH}, nm. 303 −251 −227. ^1H NMR (DMSO-d_6). δ 2.10 (2H, *m*, H-3a/3b), 2.50 (1H, *m*, H-4b), 2.62 (1H, *m*, H-4a), 3.68 (1H, *t*, J = 6.0 Hz, H-2), 7.02 (1H, *dt*, J = 1.0 and 7.5 Hz, H-5′), 7.40 (1H, *dt*, J = 1.5 and 7.8 Hz, H-4′), 7.96 (1H, *dd*, J = 1.7 and 7.8 Hz, H-6′), 8.45 (1H, *dd*, J = 1.0 and 8.4 Hz, H-3′). ^{13}C NMR (DMSO-d_6). δ 26.2 (sec., C-3), 33.3 (sec., C-4), 52.5 (tert., C-2), 119.0 (tert., C-3′), 120.7 (quat., C-1′), 121.9 (tert., C-5′), 131.1 (tert., C-6′), 131.9 (tert., C-4′),140.5 (quat. C-2′), 170.0 (quat. C-7′*), 170.0 (quat. C-5*), 171.0 (quat. C-1*). Numbering of atoms according to **1**. All assignments are based on ^1H–^1H COSY, HMQC and HMBC, *assignments exchangeable. FABMS *m/z* 265 $[M-H]^-$.

Compound **3**. UV (λ_{max}, nm, MeOH). 334–247. ^1H NMR (DMSO-d_6). δ 6.48 (1H, *dt*, J = 1.1 and 7.4 Hz), 6.71 (1H, *d*, J = 8.3 Hz), 7.20 (1H, *dt*, J = 1.6 and 7.7 Hz), 7.66 (1H, *dd*, J = 1.6 and 8.0 Hz). The ^1H NMR spectrum of an authentic sample of anthranilic acid was identical to that of **3**. Cochromatography of **3** with anthranilic acid also proved its identity.

Synthesis of rufulamide. (a) 2-(4-Benzyloxycarbonyl-4-tert-butoxycarbonylamino-butyrylamino) -benzylbenzoate **4**: *N*-BOC-1-benzylglutamate [16] (2.25 g, 6.6 mM) and benzylanthranilate [17] (1.68 g, 7.35 mM) were dissolved in dry CH_2Cl_2 (60 ml) and dicyclohexylcarbodiimide (DCC) (1.50 g, 7.35 mM) was added. The reaction mixt. was stirred for 1 hr at 0 and 2 days at 20 . The pptd dicyclohexylurea (DCU) was filtered off, washed with CH_2Cl_2 (30 ml) and the solvent was removed *in vacuo*. The residue was treated with Et_2O at 0 , filtered and the solvent was removed. The crude product thus obtained was purified by flash chromatography on silica gel (eluent: Et_2O–*n*-hexane 2:1). Yield: 1.86 g (51%) **4**, colourless crystals, mp 160 . IR (KBr) ν cm^{-1}: 3300 (NH), 1740, 1700, 1685, 1525, 1270, 1165. ^1H NMR (400 MHz, DMSO-d_6): δ 10.51 (1H, *bs*), 8.23 (1H, *d*, J = 8.3 Hz), 7.93 (1H, *dd*, J = 1.4 and 7.9 Hz), 7.59 (1H, *m*), 7.46 (1H, *d*, J = 8.3 Hz), 7.42–7.31 (10H, *m*), 7.17 (1H, *m*), 5.33 (2H, *s*), 5.15 (1H, *d*, J = 12.4 Hz), 5.10 (1H, *d*, J = 12.4 Hz), 4.08 (1H, *m*), 2.44 (2H, *m*), 2.05 (1H, *m*), 1.89 (1H, *m*), 1.36 (9H, *s*). ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.1, 170.7, 168.0, 155.4, 141.6, 135.5, 134.7, 130.9, 128.8, 128.6, 128.5, 128.4, 128.2, 122.5, 120.5, 115.0, 80.0, 67.2, 67.1, 53.3, 34.2, 28.3, 27.8. CIMS *m/z* (rel. int.): 546 $[M]^+$ (7), 490 (23), 294 (29), 227 (23), 91 (100), 65 (10), 57 (28), 41 (8).

(b) 2-(4-Amino-4-benzyloxycarbonyl-butyrylamino)-benzylbenzoate-trifluoroacetate **7**: The amide **4** (1.29 g, 2.35 mM) was dissolved in dry CH_2Cl_2 (25 ml) and a soln of TFA (8 ml) in CH_2Cl_2 (25 ml) was added slowly with stirring at 0 . The reaction mixt. was stirred at 0 for 4 hr, the solvent and the excess of TFA was removed *in vacuo*. The oily residue crystallized on treatment with Et_2O. Yield: 1.26 g (95%) **7**, colourless solid, mp 120 . IR (KBr) ν cm^{-1}: 3305, 3270, 3030 (NH_3^+), 1745, 1700, 1680, 1660, 1595, 1535. ^1H NMR (400 MHz, DMSO-d_6): δ 10.62 (1H, *bs*), 8.57 (3H, *bs*), 8.26 (1H, *d*, J = 8.4 Hz), 7.97 (1H, *dd*, J = 1.3 and 7.9 Hz), 7.61 (1H, *m*), 7.49–7.32 (10H, *m*), 7.20 (1H, *m*), 5.35 (2H, *s*), 5.27 (1H, *d*, J = 12.4 Hz), 5.20 (1H, *d*, J = 12.4 Hz), 4.20 (1H, *d*, J = 6.6 Hz), 2.67–2.55 (2H, *m*), 2.21–2.12 (2H, *m*). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.7, 169.1, 166.8, 139.6, 135.7, 135.0, 134.1, 130.5, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 123.3, 121.1,

117.4, 67.2, 66.5, 51.5, 31.9, 25.3. CIMS *m/z* (rel. int.): 446 [M–CF_3COOH]$^+$ (17), 440 (21), 439 (53), 411 (16), 322 (16), 321 (56), 319 (15), 318 (49), 311 (17), 310 (63), 229 (20), 228 (78), 227 (100), 220 (27), 91 (46), 84 (18).

(c) 2-(2-Carboxyacetyl)-benzylbenzoate **6**: To a soln of malonic acid (2 g, 19.2 mM) and benzylanthranilate (4.30 g, 19.2 mM) in dry EtOAc (100 ml) at 0° DCC (4.30 g, 21.0 mM) was added. The reaction mixt. was stirred at 0° for 12 hr. The pptd DCU was filtered off, washed with EtOAc (30 ml) and the solvent was removed *in vacuo*. The residue was dissolved in Et_2O (100 ml) and extracted ×3 with a satd soln of $NaHCO_3$ in H_2O (25 ml). The aq. extracts were combined and acidified with 6 M HCl. The ppt. was filtered off and dried *in vacuo*. Yield: 2.66 g (44%) **6**, yellowish solid, mp 109°. IR (KBr) ν cm^{-1}: 3000 (COOH), 1735, 1725, 1700, 1650, 1590, 1525, 1300. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.91 (1H, *bs*), 10.14 (1H, *bs*), 8.27 (1H, *d*, J = 8.4 Hz), 7.93 (1H, *dd*, J = 1.3 and 8.0 Hz), 7.59 (1H, *m*), 7.48–7.32 (5H, *m*), 7.19 (1H, *m*), 5.36 (2H, *s*), 3.41 (2H, *s*). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.0, 166.5, 165.2, 139.3, 135.7, 133.9, 130.5, 128.4, 128.1, 128.0, 123.4, 121.3, 117.9, 66.5, 44.6. CIMS *m/z* (rel. int.): 270 (23), 269 [M–CO_2]$^+$ (100), 227 (45), 163 (19), 162 (16), 120 (15), 106 (12), 91 (73), 65 (13).

(d) Tribenzyl rufulamide **8**: The trifluoroacetate **7** (0.80 g, 1.4 mM) and the monoamide **6** (0.46 g, 1.4 mM) were dissolved in dry CH_2Cl_2 (50 ml) and anhyd. $N(C_2H_5)_3$ (0.28 ml, 2.1 mM) was added. The reaction mixt. was stirred for 10 min at 0°. *N*-hydroxybenzotriazole (0.22 g, 1.65 mM) and DCC (0.36 g, 1.8 mM) were added and the reaction was stirred for 12 hr at 0°. The pptd DCU was filtered off, the solvent was removed in vacuo and the oily residue was purified by chromatography on silica gel (eluent: Et_2O). Yield: 0.90 g (85%) **8**, colourless crystals, mp 96°. IR (KBr) ν cm^{-1}: 3270 (NH), 1740, 1690, 1645, 1605, 1590, 1530, 1450, 1260. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.36 (1H, *bs*), 11.02 (1H, *bs*), 8.64 (2H, *m*), 8.02 (2H, *m*), 7.96 (1H, *d*, J = 7.5 Hz), 7.47 (2H, *m*), 7.43–7.25 (15H, *m*), 7.04 (1H, *m*), 6.99 (1H, *m*), 5.33 (2H, *s*), 5.30 (2H, *s*), 5.18 (2H, *s*), 4.79 (1H, *m*), 3.45 (2H, *s*), 2.52 (2H, *m*), 2.37 (1H, *m*), 2.23 (1H, *m*). ^{13}C NMR (400 MHz, $CDCl_3$): δ 171.3, 170.7, 167.9, 167.7, 166.1, 166.0, 141.5, 140.8, 135.5, 134.7, 134.6, 130.9, 130.9, 128.7, 128.6, 128.5, 128.3, 123.2, 122.5, 120.9, 120.5, 115.8, 115.0, 67.3, 67.2, 67.0, 52.2, 44.9, 34.1, 27.3. CIMS *m/z* (rel. int.): 725 (15), 546 (16), 452 (15), 321 (27), 319 (24), 318 (75), 317 (50), 228 (52), 227 (100), 226 (20), 210 (17), 91 (37).

(e) Rufulamide **1**: The tribenzyl ester **8** (0.51 g, 0.7 mM) was dissolved in dry EtOAc (150 ml), pd on charcoal (5%, 0.30 g) was added and the soln was hydrogenated at a H_2 pressure of 3.5 bar for 12 hr. The catalyst was filtered off, washed with EtOH (100 ml) and the solvents were removed *in vacuo*. Yield: 0.27 g (83%) **1**, colourless crystals, mp 194°. IR (KBr) ν cm^{-1}: 3260–3100 (COOH, NH), 1700, 1650, 1610, 1590, 1520. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.31 (1H, *bs*), 11.14 (1H, *bs*), 8.58 (1H, *d*, J = 7.6 Hz), 8.49 (1H, *d*, J = 8.4 Hz), 8.48 (1H, *d*, J = 8.4 Hz), 7.97 (2H, *dd*, J = 1.3 and 8.0 Hz), 7.57 (2H, *m*), 7.15 (2H, *m*), 4.34 (1H, *m*), 3.47 (1H, *d*, J = 15.0 Hz), 3.40 (1H, *d*, J = 15.0 Hz), 2.54 (2H, *m*), 2.15 (1H, *m*), 1.97 (1H, *m*). ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.8, 170.2, 169.4, 168.9, 166.3, 165.6, 140.7, 140.2, 133.8, 133.7, 130.9, 122.8, 122.5, 120.2, 120.0, 117.1, 116.7, 51.5, 45.3, 33.5, 26.5. FABMS *m/z* (rel. int.): 562 [M + Gly]$^+$ (6), 474 (5), 473 (27), 472 [M + 1]$^+$ (100), 341 (5), 335 (9).

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REFERENCES

1. Schuster, R. M., in *The Hepaticae and Anthocerotae of North America, Vol. V*. Field Museum of Natural History, Chicago, 1992.
2. Kuwahara, Y., *Review of Bryology and Lichenology*, 1978, **44**, 351.
3. Asakawa, Y., in *Progress in the Chemistry of Organic Natural Products*, Vol. 65, ed. W. Herz, E. W. Kirby, R. E. Moore, W. Steglich and Ch. Tamm. Springer, Wien, 1995, p. 1.
4. Huneck, S., in *New Manual of Bryology*, Vol. 1, ed. R. M. Schuster. The Hattori Botanical Laboratory, Nichinan, 1983, p. 1.
5. Zinsmeister, H. D., Becker, H. and Eicher, T., *Angewandte Chemie*, 1991, **103**, 134.
6. Theodor, R., Zinsmeister, H. D., Mues, R. and Markham, K. R., *Phytochemistry*, 1980, **19**, 1695.
7. Theodor, R., Zinsmeister, H. D., Mues, R. and Markham, K. R., *Phytochemistry*, 1981, **20**, 1851.
8. Theodor, R., Mues, R., Zinsmeister, H. D. and Markham, K. R., *Zeitschrift für Naturforschung*, 1983, **38c**, 165.
9. Mues, R., *Berichte der Deutschen Botanischen Gesellschaft*, 1982, **95**, 115.
10. Kuwahara, Y., *Bryophytorum Bibliotheca*, 1986, **28**, 99.
11. Trennheuser, F., Burkhardt, G. and Becker, H., *Phytochemistry*, 1994, **37**, 899.
12. Benesova, V., Samek, Z., Herout, V. and Sorm, F., *Collection of Czechoslovak Chemical Communications*, 1969, **34**, 1807.
13. Sakai, K., Ichikawa, T., Yamada, K., Yamashia, M., Tanimoto, M., Hikita, A., Ijuin, Y. and Kondo, K., *Journal of Natural Products*, 1988, **51**, 845.

14. Salm, R., Dissertation, Universität des Saarlandes, Saarbrücken, 1995.
15. Kraut, L., Scherer, B., Mues, R. and Sim-Sim, M., *Zeitschrift für Naturforschung*, 1995, **50c**, 345.
16. Nefkens, G. H. L. and Nivard, R. J. F., *Recueil des Travaux Chimiques des Pays-Bas*, 1964, **83**.
17. Venuti, M., *Synthesis*, 1982, 266.