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TETRA-ACETYLENIC METABOLITES FROM *MYCENA VIRIDIMARGINATA*

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Key Word Index—*Mycena viridimarginata*; Basidiomycetes; acetylenic compounds; tetraynamide; tetrayne lactone; chromophores of polyynamides.

Abstract—The structures of two new acetylenic compounds from *Mycena viridimarginata* were elucidated by spectroscopic methods and some chemical transformations. The major metabolite was 10-hydroxyundeca-2,4,6,8-tetraynamide and the minor one was 3,4,13-trihydroxytetradeca-5,7,9,11-tetraynoic acid γ-lactone. Another compound, methyl-3,4,13-trihydroxytetradeca-5,7,9,11-tetraynoate, was found to be an artifact from the γ-lactone produced on the Sephadex LH-20 column. Furthermore, model compounds were prepared to establish the chromophoric system of the major metabolite.

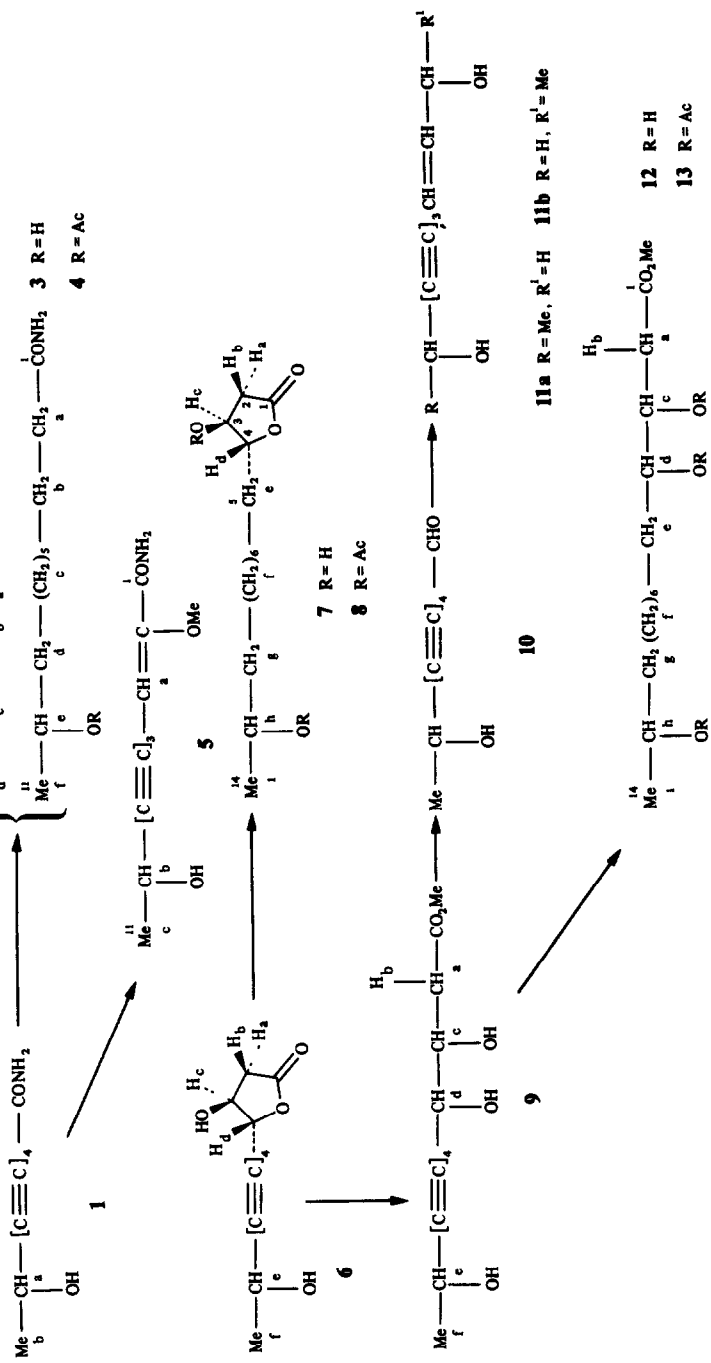
INTRODUCTION

Many acetylenic compounds are produced by plants and higher fungi (Basidiomycetes) [1, 2]. In addition, the ethyl acetate extract from the culture fluid of the Basidiomycete *Mycena viridimarginata* showed the presence of acetylenic compounds [3]. As these compounds were extremely unstable, model compounds were synthesized to establish the nature of the unknown chromophore. The results and the structure elucidation of the new compounds and their reaction products are described in this paper.

RESULTS AND DISCUSSION

From the culture fluid of *M. viridimarginata* a mixture of tetra-acetylenic compounds was isolated which could not be separated by CC or by TLC. Using the Sephadex LH-20–methanol combination, during each chromatographic separation process one of the tetrayne compounds was, in part, transformed to another slightly more polar tetrayne derivative which could be separated by TLC. A compound with an unknown UV absorption spectrum was the major metabolite which easily crystallized, but rapidly decomposed. The IR spectrum showed bands for acetylene, hydroxyl and for a carbonyl group. The frequency of the carbonyl agreed with the presence of a conjugated amide. In the ^{1}H NMR spectrum, only two clear signals were present, a methyl doublet at δ1.51 which was coupled with a more complex signal at δ4.61, while two broad bands around δ5.8 could not be assigned initially. No ^{13}C NMR spectrum could be obtained as the compound rapidly polymerized and in the mass spectrum no molecular ion either, under CI conditions (chemical ionization), could be detected. Reduction with alanate or reaction with acetic anhydride without, as well as in, the presence of Steglich catalyst [4] did not lead to definite compounds and the UV spectra indicated that the chromophoric system was destroyed. Catalytic hydrogenation gave a more polar product. To simplify its detection during purification, a sample of the major metabolite was also treated with a hydrogen–tritium gas mixture over platinum. Two radioactive products were separated by TLC. The minor, less polar product was identical with undecanamide (**2**) (Scheme 1) as shown by TLC, ^{1}H NMR (Table 1), ^{13}C NMR and IR spectral data, and the molecular formula ($C_{11}H_{23}NO$). However, this component obviously was the result of a hydrogenolysis of an allylic hydroxyl, as the natural compound doubtless was an alcohol. The more polar compound was soluble in both ether water. The IR, ^{1}H NMR (Table 1) and ^{13}C NMR spectral data agreed with structure **3** (Scheme 1), which was further supported by the mass spectrum with *m/z* 202 $[M+1]^+$ and *m/z* 184 $[202-H_2O]^+$ (chemical ionization). Acetylation afforded a less polar compound which showed *m/z* 200 $[M-Ac]^+$ ($C_{11}H_{22}NO_2$) and, by chemical ionization, *m/z* 244 $[M+1]^+$ and *m/z* 184 $[244-HOAc]^+$. The ^{1}H NMR spectrum (Table 1) established structure **4** (Scheme 1). Irradiation at δ4.89 (H_e) collapsed the doublet at δ1.20 (H_f) to a singlet, irradiation at δ1.46 (H_d) the quartet triplet at δ4.89 (H_e) to a quartet and irradiation at δ1.64 (H_b) the triplet at 2.22 (H_a) to a singlet. These results on the hydrogenated product agree with structure **1** for the metabolite (Scheme 1), in agreement with UV maxima which were between those of a tetrayne and an enetetrayne [1]. It was, however, still necessary to establish this structure. Addition of methanol in the presence of sodium methoxide [1] afforded a more stable derivative of **1**. Its UV maxima were at slightly longer wavelengths than those of a dienetriyne chromophore [1]. The ^{1}H NMR spectrum (Table 1) agreed with structure **5** (Scheme 1). Irradiation of H_b (δ4.62) sharpened the signal of H_a at δ6.14, in agreement with a Δ^2-double bond and with a nucleophilic attack at C-2. The molecular formula ($C_{12}H_{11}NO_3$) again supported the proposed structure (**1**) of the metabolite.

The minor metabolite showed UV maxima which were shifted to longer wavelengths when compared with those of a tetrayne [1] indicating functional groups adjacent to the triple bonds [5]. Unfortunately, this compound could not be separated completely from **1**. In addition to the bonds of **1**, the IR spectrum showed a carbonyl band at 1790 cm^{-1}, which could be due to a saturated γ-lactone.



Scheme 1. The acetylenic metabolites and their derivatives.

Table 1. ^{1}H NMR spectral data of the acetylenic metabolites and their derivatives ($CDCl_3$; 400 MHz; TMS as int. standard)

	1	2	3*	4	5	6	7	8	9	12	13
H_a	4.61 *q*	2.22 *t*	2.22 *t*	2.22 *t*	6.14 *brs*	2.94 *dd*	2.83 *dd*	2.92 *dd*	} 2.68†	} 2.56†	} 2.62†
H_b	1.51 *d*	1.64 *tt*	1.64 *tt*	1.64 *tt*	4.62 *q*	2.55 *dd*	2.52 *dd*	2.55 *dd*			
H_c	—	1.3 *m*	1.3 *m*	1.3 *m*	1.49 *d*	4.66 *ddd*	4.28 *ddd*	5.08 *ddd*	4.18 *ddd*	3.95 *m*	5.34 *m*
H_d	—	0.86 *t*	1.43 *td*	1.46 *td*	—	5.06 *d*	4.34 *m*	4.48 *m*	4.53 *d*	3.63 *m*	5.34 *m*
H_e	—	—	3.79 *qt*	4.89 *qt*	—	4.61 *q*	1.4 *m*	1.59 *m*	4.61 *q*	1.4 *m*	1.4 *m*
H_f	—	—	1.19 *d*	1.20 *d*	—	1.49 *d*	1.3 *m*	1.3 *m*	1.49 *d*	1.3 *m*	1.3 *m*
H_g	—	—	—	—	—	—	1.4 *m*	1.4 *m*	—	1.4 *m*	1.46 *m*
H_h	—	—	—	—	—	—	3.79 *qt*	4.89 *qt*	—	3.79 *qt*	4.89 *qt*
H_i	—	—	—	—	—	—	1.19 *d*	1.20 *d*	—	1.19 *d*	1.20 *d*
NH_2	5.82 *brs* 5.77 *brs*	5.35 *brs* 5.28 *brs*	5.50 *brs*	5.66 *brs* 5.40 *brs*	6.46 *brs* 5.37 *brs*	—	—	—	—	—	—
OAc	—	—	—	2.05 *s*	—	—	—	2.09 *s* 2.03 *s*	—	—	2.03 *s* (×3)
OMe	—	—	—	—	4.24 *s*	—	—	—	3.74 *s*	3.73 *s*	3.70 *s*

*270 MHz.

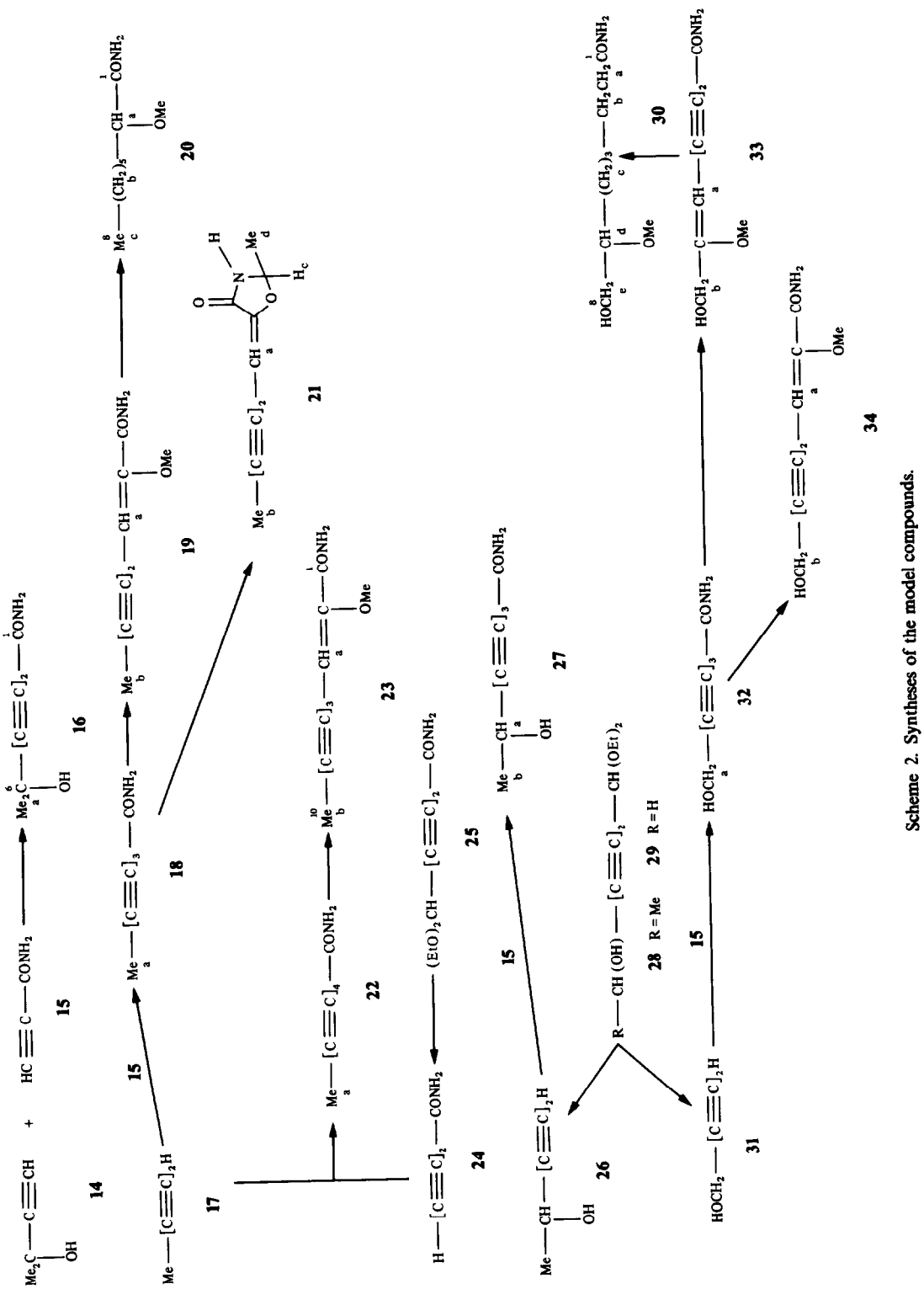
†Centre of an AB-multiplet.

J (Hz): Compound **1**: a, b = 6.8; compound **2**: a, b = 7.5; b, c ~ 7; c, d = 6.5; compounds **3** and **4**: a, b = 7.5; b, c ~ 7; d, e ~ 6; e, f = 6.5; compound **5**: b, c = 6.8; compound **6**: a, b = 18; a, c = 6.2; b, c = 2.2; c, d = 1.8; e, f = 6.8; compound **7**: a, b = 18; a, c = 6.8; b, c = 3.6; c, d ~ 2.5; g, h ~ 6; h, j = 6; compound **8**: a, b = 18; a, c = 7; b, c = 2; c, d ~ 1.5; g, h ~ 6; h, i = 6.5; compound **9**: a–c ~ virtual couplings 8 + 4.8; c, d = 4; e, f = 6.8; compounds **12** and **13**: a–c ~ virtual couplings 9 + 4.5; g, h ~ 6; compound **12**: h, i = 6; compound **13**: h, i = 6.5.

In the ^{1}H NMR spectrum (Table 1), spin decoupling allowed the assignment of all signals. Irradiation of the double doublet signal at δ2.94 (H_a) collapsed the double doublet at δ2.55 (H_b) to a doublet and changed the complex signal at δ4.66 (H_c), while irradiation at δ4.66 (H_c) collapsed the double doublets (H_a and H_b) to doublets and the doublet at δ5.06 (H_d) to a singlet. These results showed that, most likely, the lactone **6** (Scheme 1) was present. The stereochemistry at C-2–C-4 was deduced from the observed couplings, which agreed with those from inspection of a model. The structure of the hydrogenated product, deduced from the ^{1}H NMR spectrum (Table 1), was **7** (Scheme 1), but no molecular ion could be observed in the mass spectrum by chemical ionization. Acetylation afforded **8**, which could be separated from **4** by TLC. Again a small sample was prepared with tritium labelled acetic anhydride for easier detection. The IR spectrum showed carbonyl absorption at 1770 cm^{-1} and, in the ^{1}H NMR spectrum (Table 1), the signals for H_c and H_h were shifted downfield compared with those of **7**. The results of double resonance experiments were in agreement with structure **8** (Scheme 1). Chemical ionization showed *m/z* 343 for $[M+1]^+$ while electron impact only gave *m/z* 299 ($C_{16}H_{27}O_5$) formed by loss of acetyl. These data confirmed the carbon skeleton of **8** and, therefore, structure **6** was also established for the minor metabolite.

The UV absorption maxima of a further, slightly more polar tetrayne were shifted *ca* 1–2 nm to lower wavelengths. The IR spectrum showed a carbonyl band at 1730 cm^{-1} and, in the ^{1}H NMR spectrum (Table 1), a three proton signal at δ3.74 indicated the occurrence of a methyl ester. Spin decoupling allowed the assignments of all signals. Saturation of the broad signal at 4.18 (H_c) caused a change of the signals around δ2.68 (H_a and H_b) and the doublet at δ4.53 (H_d) collapsed to a singlet. These data led to the proposal that the methyl ester **9** (Scheme 1) was present. As, however, no molecular ion could be observed in the mass spectrum by chemical ionization, **9** was degraded by chemical reactions. Periodate oxidation gave a less polar product which showed absorption maxima of a tetraynon [5], which most likely was the aldehyde, **10**. As no molecular ion could be detected in the mass spectrum, this compound was reduced by sodium boranate leading to an alcohol (**11a** or **11b**) with an enetriyne chromophor indicating that, in addition to the carbonyl group, a triple bond was reduced. Chemical ionization showed a small $[M+1]^+$ peak (*m/z* 175), while the most prominent peak was *m/z* 157 for $[175-H_2O]^+$. The hydrogenation products of **6** contained a small amount of **12** (Scheme 1), as **6** was not completely free from **9**. The ^{1}H NMR spectral data of **12** and of its acetate **13** (Table 1) further supported the proposed structure, **9**, for the more polar tetrayne. In the spectrum of **12**, probably due to a hydrogen bridge between the 3-hydroxy and the ester groups, H_c and H_d showed separated signals, while in the spectrum of **13** only one signal was observed. However, the methyl ester, **9**, was not present in the original extract. Obviously, it was formed during separation on Sephadex LH-20 by addition of methanol from **6**. This clearly could be observed by following the UV spectra as **9** was eluted first, followed by **1** and, finally, by **6**. During each Sephadex separation process *ca* 5–10% of **6** was transformed to **9**.

As **1** had a so far unknown type of chromophore, the synthetic conformation of the chromophore and its derivative was desirable. The syntheses of the model compounds and their transformations are given in Scheme 2 and their ^{1}H NMR spectral data in Table 2. Glaser coupling [6, 7] of **14** and **17** with propiolamide (**15**) [8] gave the diynamide **16** and the triynamide **18**, respectively. The synthesis of a tetraynamide was achieved by first elongating **15** with 3,3-diethoxypropyne by Glaser coupling to the acetal, **25**, which by hydrolysis and elimination of carbon monoxide led to **24**, which was



Scheme 2. Syntheses of the model compounds.

Table 2. ^{1}H NMR spectral data of the model compounds and their derivatives ($CDCl_3$; 400 MHz; TMS as int. standard)

	16*†	18*	19	20	21	22	23	27	30†	32†	33	34
H_a	1.47 *s*	2.02 *s*	6.11 *brs*	3.63 *dd*	5.42 *brs*	2.03 *s*	6.12 *s*	4.64 *q*	2.16 *t*	4.36 *d*	4.98 *brs*	6.13 *brs*
H_b	—	—	2.00 *d*	1.3 *m*	2.00 *d*	—	2.01 *s*	1.51 *d*	1.58 *tt*	—	4.04 *brs*	4.40 *d*
H_c	—	—	—	0.88 *t*	5.68 *q*	—	—	—	1.3 *m*	—	—	—
H_d	—	—	—	—	1.56 *d*	—	—	—	3.15 *ddt*	—	—	—
H_e	—	—	—	—	—	—	—	—	3.48‡	—	—	—
NH_2	7.50 *brs* 7.03 *brs*	5.70 *brs*	6.47 *brs* 5.35 *brs*	6.46 *brs* 5.48 *brs*	—	5.90 *brs* 5.86 *brs*	6.48 *brs* 5.50 *brs*	5.76 *brs* 5.61 *brs*	6.74 *brs* 6.04 *brs*	7.67 *brs* 7.17 *brs*	5.68 *brs* 5.58 *brs*	6.48 *brs* 5.59 *brs*
OMe	—	—	4.22 *s*	3.42 *s*	—	—	4.24 *s*	—	3.32 *s*	—	4.13 *s*	4.22 *s*
OH	—	—	—	—	—	—	—	—	—	4.70 *t*	—	—

*270 MHz.

†In $(CD_3)_2CO$.

‡Centre of an AB-multiplet.

J (Hz): Compound **19**: a, b = 1.2; compound **20**: a, b ~ 7 + 4; b, c = 7; compound **21**: a, b = 1.2; c, d = 5.5; compound **27**: a, b = 6.8; compound **30**: a, b = 7.5; b, c ~ 7; c, d ~ 6; compound **32**: a and OH ~ 6.4; compound **34**: a, b = 1.

Table 3. UV spectral data of the polyynamides **16**, **18** and **22** (in Et_2O)

	16		18		22	
	λ_{max} (nm)	ε	λ_{max} (nm)	ε	λ_{max} (nm)	ε
low-intensity bands	270	2500	321	1900	367	1150
	255	3900	300	3000	341	1890
	241	3600	283	2440	318	1600
	230	3300	268	1620	299	930
					281 (sh)	700
high-intensity bands			219	117 500	246	125 600
			209	107 800	235	108 000
					225 (sh)	60 000

coupled with **17** to yield **22**. The UV maxima of the amides obtained (**16**, **18** and **22**) are presented in Table 3. The wavelengths of the absorption maxima of the amides and their intensities obviously were between those of a polyyne and the corresponding polyenyne chromophore. Plotting the λ^2 of a certain band in the spectra of the amides, e.g. that with longest wavelength, against the number of conjugated triple bonds gave a straight line. Further calculations showed that the contribution of an amide group to UV λ_{max} was, for the low-intensity bands about two-thirds and, for the high-intensity bands, about one-third of the contribution of a double bond. The data of the diacetylenic and triacetylenic amides, measured in ether, were slightly different from those published by Jones and coworkers [9–11], which were measured in ethanol. The UV spectral data of **22** were in agreement with those for the metabolite, **1**.

For further proof of the substituents of structure **1**, the model compound **27** was synthesized starting with **28**, formed by cross coupling of but-1-yn-3-ol and 3,3-diethoxypropyne. The ^{1}H NMR spectral data of **27** were identical with those of **1** and also the R_f values (TLC) of **1** and **27** were nearly the same thus confirming the presence of the (ω-1)-hydroxy group in the metabolite. Furthermore, the amides **16**, **18** and **22** were reacted with methanol–sodium methoxide under the same conditions as used in the reaction with **1**. While no reaction occurred with **16**, the addition product of **22** showed a UV spectrum similar, but with less fine structure, to that of a dienetriyne. It was of a similar shape to that of **5**, apart from the maxima being at shorter wavelengths (2–3 nm), due to the effect of the 10-hydroxy group which was not present in **22**. The structure of the addition product of **22** followed from the changed IR band (1680–1705 cm^{-1}) and the ^{1}H NMR spectral data: The broadened olefinic proton H_a at δ6.12 was sharpened on irradiation of the signal at δ2.01 (H_b) indicating a small coupling of H_a with the methyl group. With these results only structure **23** with a methoxy group at C-2 was compatible.

Reaction of **18** with methanol–sodium methoxide gave two products. One of them showed a UV spectrum with poor fine structure and maxima at 308, 292 and 278 (sh) nm and the more polar one a similar spectrum with maxima at 316, 297 and 286 (sh) nm. The IR spectrum of the former showed a carbonyl band again shifted to 1703 cm^{-1} as in **5** and **23** and the ^{1}H NMR spectrum displayed a doublet for H_b, which collapsed to a singlet on irradiation at δ6.11 (H_a) and decoupling of H_b sharpened the signal of the H_a. Accordingly, the structure most likely was **19**. The ^{1}H NMR spectrum of the hydrogenated product (**20**) established this assumption as the signal of H_a was a clear downfield double doublet. The more polar

product showed IR bands at 1730 as well as at 3440 and 1360 cm^{-1} indicating a secondary amide. The ^1H NMR spectrum showed two carbon–methyl signals, a narrowly split doublet at δ2.0 and a doublet at δ1.56 with a vicinal coupling (J = 5.5 Hz). Irradiation at δ5.42 (H_g) collapsed the signal at δ2.0 to a singlet and changed the signal at δ5.68 (H_c), whereas saturation of the latter signal collapsed the doublet at δ1.56 (H_d) to a singlet. Together with the molecular formula ($C_{10}H_9NO_2$), structure **21** was most likely. Obviously, **21** was formed by reaction of **18** with traces of acetaldehyde or its acetal present in commercial diethyl ether. Accordingly, **21** was easily produced by addition of acetaldehyde to a methanolic solution of **18** containing sodium methoxide. While **5**, **19** and **23** obviously were formed by nucleophilic attack of methoxide at C-2, reaction of agrocybin (**32**) was reported to lead to a different product [10]. We, therefore, have prepared agrocybin (**32**). Reaction with methanol–sodium methoxide afforded, in addition to the starting material, two addition products which turned out to be **33** and **34**. The spectral data of the more polar compound, **34**, again clearly showed that it was formed by nucleophilic attack at C-2, while the data of the main product, **33**, were different. The ^1H NMR spectrum of **33** displayed upfield shifted signals. The methoxy signal was at δ4.13 and the signal of the olefinic proton (H_a) was a broad singlet at δ4.98. The IR spectrum showed a carbonyl band at 1680 cm^{-1}. Hydrogenation led to **30** and its spectral data supported the proposed structure. No cyclization product, however, could be detected although one has been reported [10] from reaction of **32** with sodium hydroxide in ethanol and investigation of the UV spectrum of the reaction mixture obtained. The spectral data of the model compounds as well as the data of the reaction products again supported the proposed structure of the metabolite **1**. The biosyntheses of **1** and **6** are not clear yet.

EXPERIMENTAL

Metabolites. Fermentation of *M. viridimarginata* Karst and extraction of the culture fluid with EtOAc has already been described in our previous paper [3]. From this extract the solvent was evaporated. Separation of the residue by CC (silica gel, Et_2O) afforded a mixture of two acetylenic compounds (*ca* 30 mg per fermentation in a 20 l. apparatus [3]). Prep. TLC (silica gel, Et_2O) gave a band, of which the lower part gave **1** in a reasonably pure state. The upper part contained a mixture of **1** and **6**. This mixture was subjected to CC (Sephadex LH-20, MeOH). Compound **6** was eluted closely behind **1**. During the separation, however, **6** was partially transformed to **9**, which was eluted from this column faster than **6** and **1**. Therefore, **9**, was present as an impurity in the fraction containing **1**. The mixture of **1** and **9** was further separated by prep. TLC (Et_2O). The less polar fraction afforded **1** and the other **9**.

10-*Hydroxyundeca*-2,4,6,8-*tetraynamide* (**1**). Light yellow crystals, which immediately became brown, UV $\lambda_{max}^{Et_2O}$ nm: 367, 341, 319, 299, 281 (sh), 247, 236, 227 (sh); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 (OH), 3520, 3400, 1680, 1585 ($CONH_2$), 2160, 2100 (C≡C); ^1H NMR: see Table 1. To **1** (2.4 mg) in dry MeOH a soln of NaOMe (concn see below) was added. When the UV spectrum was changed the reaction mixture was filtered through a column of silica gel (MeOH–Et_2O, 1:4) and the product, **5**, purified by prep. TLC (Et_2O); UV $\lambda_{max}^{Et_2O}$ nm: 350, 327, 307, 289, 267, 257, 218 (sh), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 (OH), 3540, 3420, 1700, 1570 ($CONH_2$), 2180 (C≡C), 1620 (C=C–O), ^1H NMR: see Table 1; EIMS (probe) 70 eV, m/z (rel. int.). 217.0739 $[M]^+$ (100) (calc for $C_{12}H_{11}NO_3$), 202 $[M-Me]^+$ (21), 174 $[202-CO]^+$ (58). Two samples of **1** (a: 0.5 mg, b: 7.5 mg) in MeOH–Et_2O (1:1) (a: 2 ml, b: 10 ml) were hydrogenated (PtO_2; a: H_2–$^3H_2$ mixture). The products of reaction a were separated by prep. TLC (MeOH–Et_2O, 1:20) using a TLC scanner for their detection. The labelled compounds were used as indicators during separation and further purification of the compounds from reaction b, yielding 2.9 mg **2** and 4.9 mg **3**.

Undecanamide (**2**). Colourless crystals, mp 98° [12], identical with authentic material; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 3420, 1685, 1595 ($CONH_2$); ^1H NMR: see Table 1; ^{13}C NMR (20 MHz, $CDCl_3$): δ36.0 (C-2), 25.6 (C-3), 29.4–29.6 (C-4–C-8), 32.0 (C-9), 22.8 (C-10), 14.1 (C-11), (C-1 not detected); EIMS (probe) 70 eV, m/z (rel. int.): 185.178 $[M]^+$ (1.5) (calc. for $C_{11}H_{23}NO$), 59 $[H_3CCONH_2]$ (100) (McLafferty).

10-*Hydroxyundecanamide* (**3**). Colourless crystals (Et_2O), mp 104°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620 (OH), 3540, 3450, 1690, 1595 ($CONH_2$), ^1H NMR: see Table 1; ^{13}C NMR (20 MHz, $CDCl_3$): δ175.7 (C-1), 36.0 (C-2), 25.6 (C-3), 29.3–29.6 (C-4–C-7), 25.7 (C-8), 39.4 (C-9), 68.1 (C-10), 23.5 (C-11); CIMS (isobutane, probe) 300 eV, m/z (rel int.): 202 $[M+1]^+$ (100), 184 $[202-H_2O]^+$ (81). Compound **3** (1 mg) was acetylated [100 μl Ac_2O, 30 μl dry pyridine, *ca* 1 mg 4-pyrrolidinopyridine (Steglich catalyst), 20°, 3 hr] and the product purified by prep. TLC (MeOH–Et_2O, 1:20) yielding **4** (75%).

10-*Acetoxyundecanamide* (**4**). Colourless crystals, mp *ca* 56°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 3535, 3420, 1680, 1595 ($CONH_2$), 1730, 1270 (OAc); CIMS (isobutane, probe) 300 eV, m/z (rel. int.): 244 $[M+1]^+$ (32), 184 $[244-HOAc]^+$ (100); EIMS (probe) 70 eV, m/z (rel. int.): 200.165 $[M-Ac]^+$ (4) (calc. for $C_{11}H_{22}NO_2$)

3,4,13-*Trihydroxytetradeca*-5,7,9,11-*tetraynoic acid* γ-*lactone* (**6**). UV $\lambda_{max}^{Et_2O}$ nm: 240, 229, 219; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3400 (OH), 2170, 2100 (C≡C), 1790 (γ-lactone); ^1H NMR: see Table 1. A mixture (2.5 mg) of **1** and **6** in Et_2O was hydrogenated (Pd–$BaSO_4$) and the products acetylated with Ac_2O (a small sample with $[^3H]Ac_2O$) in the presence of Steglich catalyst. Separation by prep. TLC (Et_2O, TLC scanner) afforded **2**, **4** and **8**; compound **8** was further purified by CC (Sephadex LH-20, MeOH, radioactivity used for detection).

3,13-*Acetoxy*-4-*hydroxytetradecanoic acid* γ-*lactone* (**8**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770 (γ-lactone), 1730, 1250 (OAc); ^1H NMR: see Table 1; CIMS (isobutane, probe) 300 eV, m/z (rel. int.): 343 $[M+1]^+$ (20), 283 $[343-HOAc]^+$ (100), 223 $[283-HOAc]^+$ (27); EIMS (probe) 70 eV, m/z (rel. int.): 299.1858 $[M-Ac]^+$ (2) (calc. for $C_{16}H_{27}O_5$), 282.1831 $[M-HOAc]^+$ (2.5) (calc for $C_{16}H_{26}O_4$)

Methyl-3,4,13-*trihydroxytetradeca*-5,7,9,11-*tetraynoate* (**9**). UV $\lambda_{max}^{Et_2O}$ nm: 239, 228, 217; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3450 (OH), 2230, 2170 (C≡C), 1730 (C=O); ^1H NMR: see Table 1; CIMS (isobutane, probe) 300 eV, m/z (rel. int.): 202 $[M+1-CH_2CO_2Me]^+$ (100). To **9** (*ca* 1 mg) in Et_2O (0.5 ml) an aq. soln of $NaIO_4$ was added slowly (over *ca* 2 hr). The product was purified by TLC (Et_2O–petrol, 1:1) yielding the aldehyde **10**; UV $\lambda_{max}^{Et_2O}$ nm: 380, 353, 329, 309, 291, 265, 253. Compound **10** in MeOH (0.5 ml) was reduced by $NaBH_4$ at 20°. Purification by TLC (Et_2O–petrol, 4:1) gave **11a** or **11b**; UV $\lambda_{max}^{Et_2O}$ nm: 329, 308, 289, 273, 243, 231, 212 [1]; CIMS (isobutane, probe) 300 eV, m/z (rel. int.): 175 $[M+1]^+$ (8), 157 $[175-H_2O]^+$ (90) Catalytic (PtO_2) hydrogenation of **9** (*ca* 1 mg) in MeOH–Et_2O (1:1) afforded **12**, which was acetylated (Steglich catalyst, $[^3H]Ac_2O$). The product was purified by TLC (Et_2O) and CC (Sephadex LH-20, MeOH), yielding **13**; ^1H NMR of **12** and **13**: see Table 1.

Model compounds. Hydrolysis of acetals **25**, **28**, **29** *and elimination of* CO. The acetals (*ca* 100 mg) were shaken with 2 ml 2 M HCl at 50° for 15 min. The mixtures were immediately cooled to 0° and, after addition of 1.7 ml MeOH and 1.1 ml 10 M

NaOH (aq.), shaken at 50° for 5 min. Extraction with Et_2O and evaporation of solvent afforded the crude products **24**, **26**, **31**, which were directly used for Glaser coupling.

Glaser coupling (for the synthesis of **16**, **18**, **22**, **25**, **27–29** *and* **32**). The two compounds with acetylenic end grouping (see Scheme 2) in the ratio 1:1 or, in the reactions with **15** and **24** with an excess of non-amide component, were dissolved in MeOH. These solns were added to an aq. soln containing Cu_2Cl_2 and NH_4Cl, adjusted to pH 5.0 (used amounts for the reaction: total acetylenic compounds–MeOH–H_2O–Cu_2Cl_2–NH_4Cl, 1:9:9:1:3, by wt). The reaction mixtures were shaken under O_2 for 3–5 hr. The resulting products were purified by prep. TLC (in Et_2O for the amides, in Et_2O–petrol, 1:1, for **28** and **29**) and CC (Sephadex LH-20–MeOH). Yields: 30–60%.

Addition of MeOH. To the polyynamides **1**, **18**, **22** and **32** (2–5 mg) in 1 ml dry MeOH 1 ml methanolic 0.1 M NaOMe was added at 20°. When the bands of amides in the UV spectrum had disappeared (after 5–20 min) the products were extracted with Et_2O and separated by prep. TLC (Et_2O) yielding **5**, **19**, **23**, **33** and **34**, respectively.

6-Hydroxy-6-methylhepta-2,4-diynamide (**16**). Colourless crystals (Et_2O–MeOH–petrol), mp 147°; UV λ_{max}: see Table 3; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 3510, 3400, 1675, 1580 ($CONH_2$), 2250, 2160 (C≡C); ^1H NMR: see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.). 151.0633 $[M]^+$ (10) (calc. for $C_8H_9NO_2$), 136 $[M-Me]^+$ (83), 108 $[M-O{=}C{=}NH]^+$ (100).

Octa-2,4,6-triynamide (**18**). Colourless crystals (Et_2O–petrol), mp 120–130° (decomp.); UV λ_{max}: see Table 3; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530, 3410, 1680, 1585 ($CONH_2$), 2210 (C≡C); ^1H NMR: see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 131.0371 $[M]^+$ (100) (calc. for C_8H_5NO), 115 $[M-NH_2]^+$ (60), 103 $[M-CO]^+$ (57), 87 $[M-CONH_2]^+$ (52). The addition of MeOH to **18** afforded two more polar compounds, which were separated by prep. TLC (Et_2O). The less polar one was **19**, the other **21**. Compound **19**: UV $\lambda_{max}^{Et_2O}$ nm: 308, 292, 278 (sh), 231, 221; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 3420, 1703, 1575 ($CONH_2$), 2240 (C≡C), 1625 (C=C–O); ^1H NMR: see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 163.0633 $[M]^+$ (100) (calc. for $C_9H_9NO_2$). Catalytic hydrogenation (Pd–$BaSO_4$, MeOH) of **19** afforded **20**. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530, 3410, 1690, 1570 ($CONH_2$), 1110 (C–O–C); ^1H NMR: see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 173 $[M]^+$ (2), 129.1279 $[M-CONH_2]^+$ (52) (calc. for $C_8H_{17}O$), 97 $[129-MeOH]^+$ (47), 55 $[97-C_3H_6]^+$ (100). Compound **18** (21.6 mg) was dissolved in dry MeOH (12 ml) and treated for *ca* 20 min with NaOMe (2.5 ml 0.1 M soln) in the presence of MeCHO (7.3 mg). Compound **21** was the main product of this reaction; UV $\lambda_{max}^{Et_2O}$ nm: 316, 297, 286 (sh), 233, 225 (sh); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1730, 1360 (NHCO), 2240 (C≡C), 1665 (C=C–O); ^1H NMR: see Table 2; CIMS (isobutane, probe) 300 eV, *m/z* (rel. int.): 176 $[M+1]^+$ (100).

Deca-2,4,6,8-tetraynamide (**22**). Light yellow crystals (Et_2O–petrol), which decomposed when heated; UV λ_{max}: see Table 3; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3530, 3410, 1680, 1590 ($CONH_2$), 2190, 2160 (C≡C); ^1H NMR: see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 155.0371 $[M]^+$ (46) (calc. for $C_{10}H_5NO$), 139.018 $[M-NH_2]^+$ (18) (calc. for $C_{10}H_3O$), 127.042 $[M-CO]^+$ (28) (calc. for C_9H_5N), 111.024 $[M-CONH_2]^+$ (18) (calc. for C_9H_3), 86.016 $[C_7H_2]^+$ (100). Addition of MeOH to **22** yielded **23**; UV $\lambda_{max}^{Et_2O}$ nm: 348, 325, 305, 288, 266, 256; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3540, 3420, 1705, 1580 ($CONH_2$), 2240 (C≡C), 1630 (C=C–O); ^1H NMR: see Table 2; EIMS (probe) 70 eV, *m/z* (rel. int.): 187.063 $[M]^+$ (62) (calc. for $C_{11}H_9O_2N$), 100 $[M-C_3H_5NO_2]^+$ (100).

8-Hydroxynona-2,4,6-triynamide (**27**). UV $\lambda_{max}^{Et_2O}$ nm: 323, 302, 284, 268, 222, 213; ^1H NMR: see Table 2.

8-Hydroxyocta-2,4,6-triynamide (*agrocybin*) (**32**). UV $\lambda_{max}^{Et_2O}$ nm: 322, 301, 283, 267, 221, 212; ^1H NMR: see Table 2; CIMS (isobutane, probe) 300 eV, *m/z* (rel. int.): 148 $[M+1]^+$ (100). Reaction with NaOMe–MeOH yielded **33** (less polar) and **34**, which were separated by prep. TLC (Et_2O). Compound **33**. UV $\lambda_{max}^{Et_2O}$ nm: 299, 283, 268, 254, 230; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 (OH), 3530, 3410, 1680, 1590 ($CONH_2$), 2220 (C≡C), 1635 (C=C–O); ^1H NMR: see Table 2; CIMS (isobutane, probe) 300 eV, *m/z* (rel. int.): 180 $[M+1]^+$ (100), 148 $[180-MeOH]^+$ (31). Hydrogenation of **33** (Pd–$BaSO_4$, MeOH) afforded **30**; IR $\nu_{Max}^{CHCl_3}$ cm^{-1}: 3610 (OH), 3520, 3400, 1680, 1590 ($CONH_2$), 1110 (C–O–C); ^1H NMR: see Table 2; CIMS (isobutane, probe) 300 eV, *m/z* (rel. int.): 190 $[M+1]^+$ (100), 158 $[190-MeOH]^+$ (70). Compound **34**. UV $\lambda_{max}^{Et_2O}$ nm: 309, 293, 280 (sh), 232, 223; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610 (OH), 3540, 3420, 1702, 1580 ($CONH_2$), 2220 (C≡C), 1610 (C=C–O); ^1H NMR: see Table 2; CIMS (isobutane, probe) 300 eV, *m/z* (rel. int.): 180 $[M+1]^+$ (100), 162 $[180-H_2O]^+$ (16).

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