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Short Communication

A Bis-formamidodiphenylbutadiene from the Fungus *Hamigera avellanea*

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(*E,E*)-1,4-Diphenyl-1,3-butadienes, carrying identical nitrogen substituents at the 2,3-positions, constitute a small, but curious group of fungal metabolites, encompassing the xanthocillins, e.g. **1–3**,[1,2] and the bisformamido-analogue (**4**).[2] We report an additional member of this group, isolated from the fungus *Hamigera avellanea* and identified as (*Z,Z*)-*N,N'*-[1-[(4-hydroxyphenyl) methylene]-2-[(4-methoxyphenyl)methylene]-1, 2-ethanediyl]bis-formamide (**5**).

The chromatographically homogeneous, colourless metabolite, possessing the composition $C_{19}H_{18}N_2O_4$ according to high-resolution mass spectrometry, unexpectedly exhibited at least 43 observable signals in its ^{13}C NMR spectrum, parallelled by an equally complex ^1H NMR picture, totally dominated by low-field signals within the δ 6.4–9.8 ppm range. Selective decoupling and $^1H,^1H$-COSY experiments precluded homonuclear couplings as responsible for most of the observed splittings, which were therefore to be ascribed to equilibrating species. The presence of two formamido groups, diagnosed by characteristic $^1J_{CH}$ couplings as large as 196 Hz along with ^1H–^1H couplings to exchangeable protons (NH) at δ 9.2–9.5 ppm, combined with high-extinction UV-absorption at 338 nm, characteristic of the (*E,E*)-1,4-diphenyl-1,3-butadiene system, established **5** as the structure of the *Hamigera* metabolite. Compatible m.p., UV, IR, MS, but no NMR, data were formerly reported for **5**, produced by acid hydrolysis of xanthocillin-X monomethyl ether (**2**).[3]

The dimethyl ether **6**, produced upon reaction of **5** with diazomethane, exhibited a far less complex ^{13}C NMR spectrum with 25 distinguishable lines. On heating, the stereomutation rate of the amide bonds increased, resulting in spectral coalescence at about 95 °C to the simple pattern expected from the molecular half of **6**. The observed ^{13}C NMR patterns of **5** and **6** in DMSO at room temperature pointed to the existence of the equilibrating individual rotamers **8–11** in the case of **5** ($Ar^1 \neq Ar^2$), and **8**(=**10**), **9** and **11** in the case of **6** ($Ar^1 = Ar^2$) (see Experimental). Totally resolved ^{13}C NMR spectra of the rotamer populations of **5** and **6** should exhibit 60 and 32 lines, respectively.

Upon acetylation of **5**, a crystalline triacetyl derivative was obtained that showed no signs of forming stable rotamers in solution. Spectroscopic characterization, including the determination of direct and long-range ^1H–^{13}C connectivities, established the structure of the acetyl derivative as **7**.

When tested against a variety of pathogenic fungi and bacteria, **5** exhibited only marginal activity.

Experimental

General. NMR spectra were acquired on a Bruker AC300P instrument equipped with a ^1H–^{13}C dual probe operating at 300.13 and 75.47 MHz for ^1H and ^{13}C, respectively. Chemical shifts (δ-values) were measured relative to solvent peaks at 2.50 and 7.27 ppm (^1H) and 39.5 and 77.0 ppm (^{13}C) for DMSO-d_6 and chloroform-*d*, respectively. High resolution fast atom bombardment (HR-FAB) mass spectrometry was performed on a AX505W instrument (JEOL). EI spectra were recorded on the same instrument at 70 eV ionization potential and are presented as *m/z* (% rel. int.). IR spectra were recorded (in KBr) on a Perkin-Elmer 1720 instrument and UV spectra on a Hitachi U-2000 instrument. Melting points are uncorrected.

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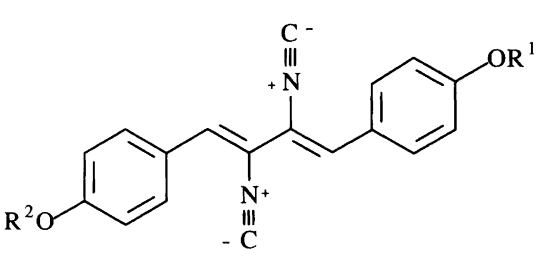
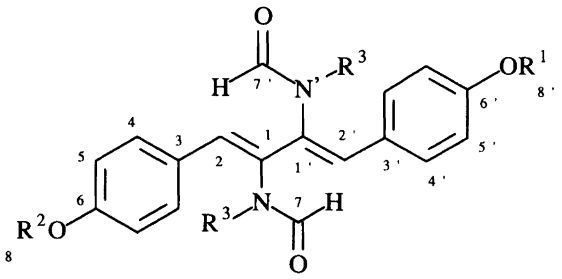
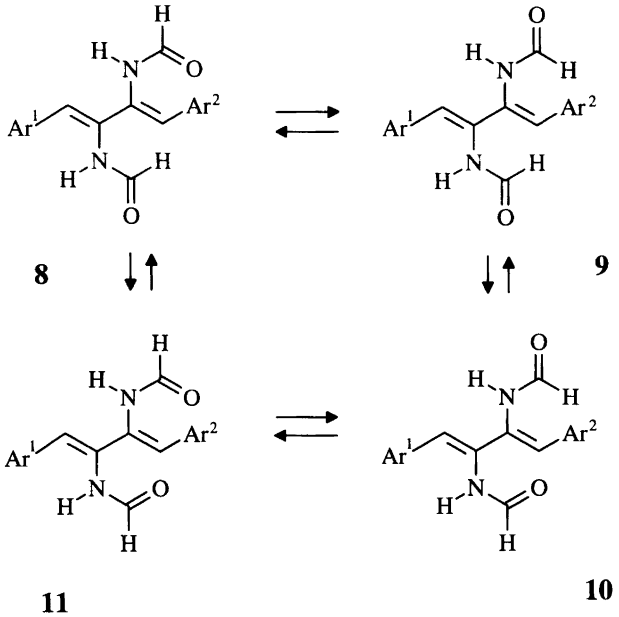




1 $R^1=R^2=H$ **4** $R^1=R^2=R^3=H$ **6** $R^1=R^2=CH_3$; $R^3=H$

2 $R^1=CH_3$; $R^2=H$ **5** $R^1=CH_3$; $R^2=R^3=H$ **7** $R^1=CH_3$; $R^2=R^3=COCH_3$

3 $R^1=R^2=CH_3$



8 **9**

11 **10**

Shake flask fermentation. The fungus *Hamigera avellanea* (CBS 501.94) was propagated on agar slants containing 12 mL of potato dextrose agar (PDA) for 16 days at 26 °C. The slants were washed with sterile, distilled water containing 0.1% of Tween 20 (10 mL/slant), and the resulting spore/mycelium suspension was used to inoculate the shake flasks (3 mL/flask). The fermentation was conducted in 500 ml Erlenmeyer flasks with two baffles, each containing 100 mL of a soy-based growth medium [soybean meal (40 g L^{-1}), potato flour (75 g L^{-1}), $Na_2HPO_4 \cdot 12H_2O$ (9 g L^{-1}), Ban 800 MG (0.075 g L^{-1}), and Pluronic (0.1 mL L^{-1})]. The medium was sterilized at 121 °C for 40 min. The pH after sterilization was 6.4. The flasks were shaken (200 rpm) for 7–12 days at 26 °C.

Extraction and isolation. The contents of 20 shake flasks were separated by vacuum filtration. The mycelium was extracted with EtOH (1 L) for 1 h under mechanical stirring and filtered off. The filter cake was extracted for another 2 h with EtOH (1 L) and again filtered off. After refrigeration (5 °C) of the extract, a brownish precipitate formed which was recovered by centrifugation. Repetitive re-precipitations from hot methanol resulted in a pale yellow, amorphous powder (100 mg). The powder, dissolved in DMSO, was subjected to preparative reversed-phase HPLC [Dupont ODS (10 μm) 250 × 20 mm, eluted with a linear gradient of 10–45% aqueous acetonitrile at a flow rate of 10 mL min^{-1}, UV detection at 210 and 340 nm]. The metabolite (**5**) precipitated from the water–acetonitrile mixture as a colourless solid (75 mg), m.p. 210–219 °C (dec.) (lit.[3] 216–219 °C). Anal. $C_{19}H_{18}N_2O_4$: C,H,N. ^1H NMR (DMSO-d_6, *cis*-to-*trans* ratio 1.2:3): δ 9.64 [s(br), OH], 9.54/9.51/9.43/9.40 [all s, NH(*cis*)], 9.37/9.33/9.28/9.24 [all d, *J* 11 Hz, NH(*trans*)], 8.20/8.19/8.18 [all s, CHO(*cis*)], 7.86/7.79 [both d, *J* 11 Hz, CHO(*trans*)], 7.5–7.3 (d's, H-4/H-4'), 6.9–7.0/6.7–6.8 (d's, H-5/H-5'), 6.53/6.52/6.51/6.49/6.46/6.45 (all s, H-2/H-2'), 3.77/3.76 (both s, CH_3-8'). ^{13}C NMR data in Table 1. IR: 3441 (br), 3240 (br), 1674, 1643, 1605, 1509, 1440, 1388, 1254, 1176, 1031 and 823 cm^{-1}. UV (MeOH) 338 nm (log ε 4.40). HR-FAB: $[M+H]^+$ 339.1319. Calcd. for $C_{19}H_{19}N_2O_4$ 339.1345. EI-MS: 338 (100), 320 (23), 309 (39), 294 (52), 293 (56), 292 (31), 290 (27), 281 (41), 266 (28), 265 (29), 264 (19), 250 (13), 231 (22), 217 (19), 203 (12), 189 (12), 186 (22), 185 (30), 169 (11), 158 (11), 146 (13), 121 (28), 107 (16) and 77 (10).

(Z,Z)-N,N'-[1,2-bis-[(4-methoxyphenyl)methylene]-1,2-ethanediyl]bis-formamide (**6**). To a solution of **5** (10 mg) in a mixture of DMSO (0.2 mL) and chloroform (1 mL) was added an excess of ethereal diazomethane. After 1 h at room temperature, ice was added, and the organic phase was dried and evaporated. The solid residue was purified by reversed phase HPLC (Dupont ODS (10 μm) 250 × 10 mm, elution with a linear gradient of 25 to 100% aqueous acetonitrile at a flow rate of 7.5 mL min^{-1}; UV

Table 1. ^{13}C NMR data for **5–7**. Spectra of **5** and **6** are recorded in DMSO-d_6, and **7** in chloroform-*d*.

	5	**6**	**7**
1	131,9, 129.9	130.2	127.9
1′	131.7, 129.3	130.7	130.7
2	121.7, 121.4, 121.0, 120.0	123.2, 122.1	126.9
2′	123.7, 122.9, 122.6, 122.4	121.8, 120.3	128.7
3	126.4, 126.1, 126.1, 125.8	131.8	130.5
3′	128.1, 127.8, 127.7, 127.5	131.6	125.0
4	~130.6[a]	130.6, 130.5	129.7
4′	~130.5[a]	130.4, 130.3	130.5
5	114.1, 114.0, 113.9, 113.8	114.1, 114.1	122.5
5′	115.5, 115.4, 115.3, 115.2	113.9, 113.8	114.8
6	157.2, 157.0, 157.0, 156.8	158.8, 158.6	151.4
6′	158.7, 158.5, 158.5, 158.3	158.6, 158.3	160.8
7	164.5, 164.5[b] (*trans*)	164.5[c] (*trans*)	162.3[d]
7′	160.2, 160.2[b] (*cis*)	160.3, 160.2[c] (*cis*)	162.3[d]
8	—	55.1	21.1/169.8
8′	55.1	55.1	55.4
NAc	—	—	22.8/173.3
		—	22.9/173.6

[a] Overlapping prevents precise assignment. [b] $^1J_{CH} = 196$ Hz. [c] $^1J_{CH} = 194$ Hz. [d] $^1J_{CH} = 215$ Hz.

detection at 210 and 340 nm) to yield the title compound (**6**) as a colourless solid (5 mg), m.p. 221–226 °C. ^1H NMR (DMSO-d_6, *cis*-to-*trans* ratio 1.3:1): δ 9.59/9.47 [both s, NH(*cis*)], 9.38/9.31 [both d, *J* 11 Hz, NH(*trans*)], 8.20/8.19 [both s, CHO(*cis*)], 7.88 [d(br), CHO(*trans*), 7.80 (d, *J* 11 Hz, CHO(*trans*)], 7.50–7.42 (d's, H-4/H-4′), 6.96–6.90 (d's, H-5/H-5′), 6.56/6.54/6.50 (s's, H-2/H-2′), 3.78 (s, CH_3-8/CH_3-8′). ^1H NMR (DMSO-d_6, 373 K): δ 9.00 [1H, NH(br)], 8.09 [1H, CHO(br)], 7.47 (2H, d, *J* 8.5 Hz, H-4/H-4′), 6.94 (d, *J* 8.5 Hz, H-5/H-5′), 6.58 (1H, s, H-2/H-2′), 3.80 (3H, s, CH_3-8/CH_3-8′). ^{13}C NMR data in Table 1. IR: 3440 (br), 3250, 1654, 1604, 1508, 1389, 1252, 1180 and 1033 cm^{-1}. UV (MeOH): 340 nm (log ε 4.57). EI-MS: 352 (100), 334 (47), 324 (29), 323 (34), 309 (16), 308 (28), 307 (63), 306 (65), 305 (52), 295 (35), 291 (26), 280 (30), 279 (25), 275 (24), 264 (16), 248 (11), 323 (36), 227 (11), 203 (18), 199 (22), 185 (15), 160 (11), 158 (12), 153 (13), 132 (15), 121 (70), and 77 (10).

(Z,Z)-N,N′-bis-formyl-N,N′-[1-[(4-acetoxyphenyl)-methylene]-2-[(4-methoxyphenyl)methylene]-1,2-ethanediyl]bis-acetamide (**7**). To an ice-cooled solution of **5** (20 mg) in pyridine (1 mL), acetic anhydride (0.5 mL) was added dropwise. After 11 h at room temperature, ice, followed by 1 M H_2SO_4, were added, and the mixture was extracted with dichloromethane (2 × 2 mL). After washing with water, sat. aqueous $NaHCO_3$, and water, drying and evaporation, the product was purified by HPLC as described above to give the title compound as a colourless solid (10 mg), that was recrystallized from MeOH, m.p. 185–188 °C. ^1H NMR (chloroform-*d*): δ 9.72 (1H, s, CHO), 9.70 (1H, s, CHO), 7.30 (2H, d, *J* 8.5 Hz, H-5), 7.25 (2H, d, *J* 8.5 Hz, H-5′), 7.11 (2H, *J* 8.5 Hz, H-4), 6.90 (2H, *J* 8.5 Hz, H-4′), 6.65 (2H, s, H-2/H-2′), 3.81 (3H, s, CH_3-8′), 2.32 (3H, s, OAc-8), 2.22/2.21 (both 3H s, N-Ac). ^{13}C NMR data in Table 1. IR: 1761, 1732, 1701, 1605, 1510, 1372, 1262, 1230, 1204, 1183, 1035 and 919 cm^{-1}. UV (MeOH): 340 nm (log ε 4.64). HR-EIMS: M^+ 464.1576. Calcd. for $C_{25}H_{24}N_2O_7$ 464.1583. EI-MS: 464 (100), 436 (24), 422 (31), 408 (22), 394 (18), 380 (10), 376 (13), 365 (32), 362 (22), 351 (38), 349 (18), 335 (23), 334 (21), 323 (37), 320 (27), 309 (25), 307 (29), 293 (30), 292 (22), 291 (25), 281 (29), 266 (13), 264 (14), 265 (28), 259 (20), 245 (22), 217 (21), 203 (13) and 121 (20).

References

1. Ando, K., Tamura, G. and Arima, K. *J. Antibiotics 21* (1968) 587.
2. Pfeifer, S., Bär, H. and Zarnack, J. *Pharmazie 27* (1972) 536.
3. Cable, K. M., Herbert, R. B., Knaggs, A. R. and Mann, J. *J. Chem. Soc., Perkin Trans. 1* (1991) 595.

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