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NOTE

Antibiotically active metabolites from *Talaromyces wortmannii*, an endophyte of *Aloe vera*

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Endophytes are microbes that colonize the internal tissues of plants without causing any immediate overt negative effects.[1] They are considered as a promising source of new natural drug leads that are of great potential for medicinal and agricultural applications.[2,3] For instance, many of the products currently used for human or animal therapy, in animal husbandry and in agriculture, are produced by microbial products, or are derived from them.[4] Furthermore, with the increasing incidence of drug resistance in human pathogenic bacteria, which are among the major causes of deaths worldwide,[5,6] there is an urgent need for new bioactive secondary metabolites.

As part of our ongoing research focused on the discovery of new bioactive natural products from endophytic fungi,[7,8] we studied the chemical constituents of the strain *Talaromyces wortmanii*, which was isolated from healthy tissues of *Aloe vera* collected in Alexandria, Egypt. Fungi of the genus *Talaromyces* have been found in solitary, as well as endophytic states in various climates worldwide from terrestrial to marine-influenced environment.[9] This genus has been investigated by many researchers as it produces a plethora of interesting compounds such as tetraene lactones,[9] diphenyl ether derivatives and anthraquinones,[10] which show various biological activities ranging from antibiotics to cytotoxins.[9–11] In the present study, we report the isolation and structure elucidation of a new atropisomer (**1**) a new wortmannin derivative (**2**) and two wortmannin derivatives (**3-4**) that were hitherto known only as synthetic products, as well as seven known metabolites (**5-11**). Furthermore, we evaluated the antibiotic activity of the isolated compounds against several pathogenic Gram positive and negative bacteria.

The endophytic fungus was isolated from healthy inner tissues of *Aloe vera* and then identified as *T. wortmanii* according to a molecular biological protocol by DNA amplification and sequencing of the ITS region. The fungal strain was cultured on rice medium for 4 weeks and then extracted with EtOAc to give 5 g of extract, which was fractionated by vacuum liquid chromatography with *n*-hexane, *n*-hexane/EtOAc, CH_2Cl_2 and CH_2Cl_2/MeOH gradient elution. Obtained fractions were separated by Sephadex LH-20 using MeOH as solvent system, and the compounds (**1**-**11**) (Scheme 1) were finally purified by semi-preparative HPLC.

Compound **1** was obtained as an orange amorphous powder. It showed UV absorption maxima identical to those of emodin.[12] ESIMS data of **1** showed a base peak at *m*/*z* 537.3 $[M\text{-}H]^-$ (−ve), which inferred that **1** has a MW of 538 g mol^{-1}. The molecular formula was established as $C_{30}H_{18}O_{10}$ based on the prominent signals detected at *m*/*z* 539.0971 $[M+H]^+$ and 561.0787 $[M+Na]^+$ (+ve) in the HRESIMS. In contrast, the ^{13}C NMR data of **1** (Table 1) showed signals corresponding to 15 carbons only, indicating that **1** is a symmetrical dimer consisting of two identical monomers. ^1H NMR showed proton signals of one aromatic methyl group resonating at δ_H 2.49 p.p.m., one aromatic singlet at δ_H 7.47 p.p.m. and two aromatic broad singlets resonating at δ_H 7.17 and 7.62 p.p.m. The ^{13}C NMR data (Table 1) exhibited 15 carbon peaks attributable to one methyl group, three methine carbons and 11 quaternary carbon atoms. Further analysis of the ^{13}C spectrum disclosed signals for two carbonyl groups resonating at δ_C 186.0 (C-9/9′) and 182.0 (C-10/10′), three oxygenated aromatic carbon atoms at δ_C 164.0 (C-1/1′), 156.0 (C-6/6′) and 163.5 (C-8/8′) and nine aromatic carbons at δ_C 124.9 (C-2/2′), 150.0 (C-3/3′), 121.5 (C-4/4′), 134.2 (C-4a/4a′), 109.0 (C-5/5′), 114.1 (C-7/7′), 110.1 (C-8/8a′), 115.0 (C-9a/9a′) and δ_C 137.0 (C-10a/10a′). Comparison of NMR data and UV spectra of **1** with those of emodin, as well as consideration of the MW, suggested that **1** is a symmetrical dimer of two emodin units. The linkage between the emodin building blocks was confirmed by 2D NMR analysis, including ^1H–^1H COSY and J^2/J^3 HMBC correlations (Supplementary Figure S1). ^1H–^1H COSY spectrum showed that the aromatic methyl signal correlated to the *meta*-coupled protons H-2 and H-4. The absence of the second set of *meta*-coupled protons and the presence of one aromatic singlet in **1** compared with emodin,

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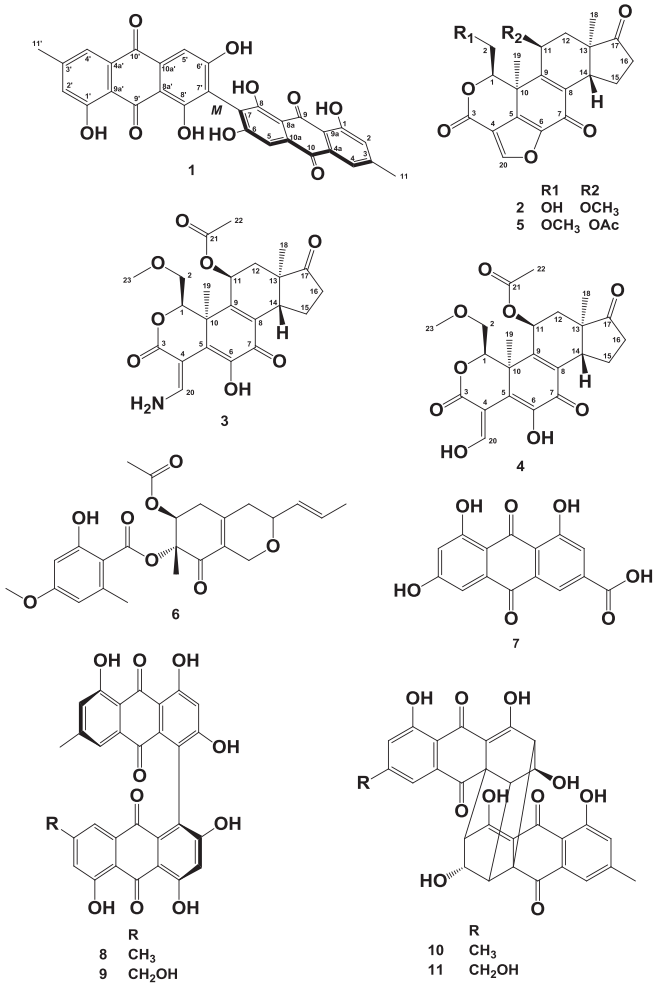

Scheme 1 Structures of isolated compounds.

confirmed the previous assumption and indicated either a 5,5′- or a 7,7′-linkage of the two emodin moieties. Inspection of the HMBC spectrum showed that CH_3-11 correlated to C-2, C-3 and C-4, and the aromatic singlet proton observed at δ_H 7.47 p.p.m. together with H-4/4′ showed strong J^3 correlations to the carbonyl carbons C-10/10′ (Supplementary Figure S1). Thus, the protons resonating at δ_H 7.47 p.p.m. were assigned to H-5/5′ and a 7,7′-linkage was established. Furthermore, comparison with NMR data of structurally related anthranoids, isolated in our previous studies,[7,8,12] revealed that in such systems the two *meta*-coupled aromatic protons H-5/5′ and H-7/7′ resonate at considerably different chemical shifts with H-5/5′ consistently appearing more downfield than H-7/7′ (ca. $7.0 < \delta_H < 7.2$). In addition, a 5,5′-linkage between the two emodin moieties forms the known skyrin,[13] which was likewise isolated from *T. wortmanii* in this study. Accordingly, the 7,7′-linkage of the emodin building blocks to form the new atropisomer **1** was unambiguously confirmed. Compound **1** is an atropisomer resulting from hindered rotation of two symmetrical moieties (emodin) around the biaryl axis, thus the axial chirality was determined by a CD experiment. The CD spectrum exhibited a positive Cotton effect at shorter wavelength and a negative one at longer wavelength, which is typical for the *M*-conformer (also known as 'A-type' or a*R*). This was also in agreement with the $[\alpha]_D$ value of -156. Accordingly, **1** is a new natural product consisting of two emodin moieties, for which the name biemodin is proposed.

Table 1 NMR spectroscopic data of 1 and 2 at 600 (^{1}H) and 150 (^{13}C) MHz ($CDCl_3$)

	1			*2*	
Nr.	δ_C[a]	δ_H(*J* in Hz)	*Nr.*	δ_C[a]	δ_H(*J* in Hz)
1/1′	164.0		1	84.9	4.65 (1H; t; 4.0)
2/2′	124.9	7.17 (1H; br s)	2	74.3	2.96 (1H; dd; 4.0, 10.5)
					3.07 (1H; dd; 4.0; 10.6)
3/3′	150.0		3	160.7	
4/4′	121.5	7.62 (1H; br s)	4	116.8	
4a/4a′	134.2		5	136.6	
5/5′	109.0	7.47 (1H; s)	6	148.2	
6/6′	156.0		7	174.2	
7/7′	114.1		8	139.0	
8/8′	163.5		9	149.1	
8a/8a′	110.1		10	45.5	
9/9′	186.0		11	53.7	5.11 (1H; br. d, 7.1)
9a/9a′	115.0		12	35.4	1.44 (1H; br.d; 11.0)
					2.53 (1H; dd; 7.0; 11.0)
10/10′	182.0		13	49.5	
10a/10a′	137.0		14	45.5	2.98 (1H; m)
11/11′	21.9	2.49	15	24.6	2.02 (2H; m)
OH/OH′		12.09	16	38.3	2.25 (1H; m)
		12.58			2.61 (1H; m)
			17	—	
			18	18.8	1.01 (3H; s)
			19		1.76 (3H; s)
			20		8.23 (1H; s)
			21	171.3	
			22	—	
			23	59.6	2.98 (3H; s)
			OH		3.47 (1H; br.s)

[a]Derived from HMBC spectrum.

Compound **2** was obtained as a light brown amorphous powder with UV absorption maxima resembling those of wortmannin (**5**), likewise isolated during this study. The HRESIMS exhibited a prominent peak at *m/z* 387.1436 $[M+H]^+$ (+ve), with a 42 mass unit decrease compared with the MW of **5**, which established the molecular formula $C_{21}H_{22}O_7$ for **2**. Comparison of the ^{1}H NMR spectra of **2** and **5** showed a close relationship between both compounds, except for the presence of an additional hydroxyl group and the absence of the *O*-acetyl group in **2**. Further analysis of 2D NMR data of **2** and **5** confirmed that both share similar skeletons and substituents, except for the replacement of the acetyl function in **5** by an aliphatic methoxy group and the presence of a free hydroxyl group at C-2 in **2** (Supplementary Figure S2). In analogy to **5**, three spin systems, including $CH(1)CH_2(2)OH$, $CH_2(12)CH(11)$ and $CH_2(16)CH_2(15)CH(14)$ were detected in the ^{1}H–^{1}H COSY spectrum of **2**. In addition, the HMBC spectrum confirmed the assignments of these spin systems. In contrast to **5**, the signal of the aliphatic methoxy group of **2** exhibited a strong J^3 HMBC correlation to a carbon resonating at δ_C 53.7 p.p.m. which was attributed to C-11. Hence, CH_2-2 is connected to the additional free hydroxyl group, whereas the methoxy group is attached to C-11. Thus, the planar structure of **2** was accomplished. The relative configuration of **2** was deduced from analysis of the coupling constants and comparison to those of **5**. Furthermore, comparison of the $[\alpha]_D$ values of **2** (+18.8) and **5** (+44) and biogenetic considerations suggest that both compounds share the same absolute configuration. Accordingly, **2** was identified as a new wortmannin derivative for which the name wortmannin B is proposed.

Compound **3-11** were identified on the basis of their ^{1}H and ^{13}C NMR, mass spectrometric data, $[\alpha]_D$ values and comparison with published data as amino adduct 3a (**3**),[14] wortmannin-diol (VIII) (**4**),[15] wortmannin (**5**),[15] wortmin (**6**),[16] emodic acid (**7**),[17] skyrin (**8**),[13]

oxyskyrin (**9**),[18] rugulosin A (**10**) and B (**11**).[19] We report here the complete NMR data (^{1}H, ^{13}C, DEPT, COSY, HMBC, HMQC and ROESY, Supplementary Figures S11 to S24) for compounds **3** and **4** for the first time.

All compounds, except **7** and **9** due to their small amounts, were tested for their antibiotic activity against pathogenic Gram negative bacteria, including *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter* sp. and *Enterococcus cloacae* and Gram positive bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, *Streptococcus pneumonia*, and *Enterococcus faecalis* (Supplementary Table S3). Among the tested compounds, **8** and **10** exhibited considerable antibiotic activity against Gram positive pathogenic bacteria with MIC values ranging between 4 and 16 μg ml^{-1}. The new biemodin (**1**) also showed strong activity against Gram positive bacteria, especially against MRSA, but was less active compared to compounds **8** and **10**.

Biemodin (**1**): orange amorphous powder. $[\alpha]_D^{20}$-156 (*c* 0.02, $CHCl_3$). UV: λ_{max} (PDA) = 223, 255, 291 and 450 nm. CD ($CHCl_3$, $c = 3.71\ 10^{-3}$ M): λ_{max} ($\Delta\varepsilon$) = 263 (0.624), 291 (–1.80), 316 (–1.59), 374 (–1.08), 470 (–2.94), 507 (–2.38). ESIMS (−ve): *m/z* = 537.3 [M-H]$^-$. HRESIMS: calcd. for $C_{30}H_{19}O_{10}$ [M + H]$^+$ 539.0978; found 539.0971 [M + H]$^+$ and for $C_{30}H_{18}O_{10}Na$ [M + Na]$^+$ 561.0798; found 561.0787. ^{1}H and ^{13}C NMR data, see Table 1.

Wortmannin B (**2**): light brown amorphous powder. $[\alpha]_D^{20}$ + 18.8 (*c* 0.02, MeOH). UV: λ_{max} (PDA) = 210, 259 and 300 nm. ESIMS (+ve): *m/z* = 386.8 [M + H]$^+$ and 794.9 [2M + Na]$^+$; (−ve): *m/z* 385.0 [M-H]$^-$ and 770.8 [2M-H]$^-$. HRESIMS: calcd. for $C_{21}H_{23}O_7$ [M + H]$^+$ 387.1444; found 387.1436. ^{1}H NMR data, see Table 1.

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1 Bacon, C. W. & White, J. F. *Microbial Endophytes* (Marcel Dekker, Inc., New York, 2000).
2 Aly, A. H., Debbab, A. & Proksch, P. Fungal endophytes: unique plant inhabitants with great promises. *Appl. Microbiol. Biotechnol.* **90,** 1829–1845 (2011).
3 Debbab, A., Aly, A. & Proksch, P. Endophytes and associated marine derived fungi-ecological and chemical perspectives. *Fungal Divers.* **57,** 45–83 (2012).
4 Donadio, S. *et al.* Microbial technologies for the discovery of novel bioactive metabolites. *J. Biotechnol.* **99,** 187–198 (2002).
5 Knight, G. M. *et al.* Shift in dominant hospital-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) clones over time. *J. Antimicrob. Chemother.* **67,** 2514–2522 (2012).
6 MacDougall, C., Powell, J. P., Johnson, C. K., Edmond, M. B. & Polk, R. E. Hospital and community fluoroquinolone use and resistance in *Staphylococcus aureus* and *Escherichia coli* in 17 US hospitals. *Clin. Infect. Dis.* **41,** 435–440 (2005).
7 Debbab, A. *et al.* New anthracene derivatives—structure elucidation and antimicrobial activity. *Eur. J. Org. Chem.* 1351–1359 (2012).
8 Aly, A. H. *et al.* NF kappa B inhibitors and antitrypanosomal metabolites from endophytic fungus *Penicillium* sp. isolated from *Limonium tubiflorum*. *Bioorg. Med. Chem.* **19,** 414–421 (2011).
9 Yuesheng, D. *et al.* Cathepsin B inhibitory tetraene lactones from the fungus *Talaromyces wortmannii*. *Helv. Chim. Acta.* **92,** 567–574 (2009).
10 Liu, F. *et al.* The bioactive metabolites of the mangrove endophytic fungus *Talaromyces* sp. ZH-154 isolated from *Kandelia candel* (L.) Druce. *Planta Med.* **76,** 185–189 (2010).
11 Wakelin, S., Warren, R., Harvey, P. & Ryder, M. Phosphate solubilization by *Penicillium* spp. closely associated with wheat roots. *Biol. Fert. Soils* **40,** 36–43 (2004).
12 Debbab, A. & Aly, A. H. *et al.* Bioactive metabolites from the endophytic fungus *Stemphylium globuliferum* isolated from *Mentha pulegium*. *J. Nat. Prod.* **72,** 626–631 (2009).
13 Vargas, F., Rivas, C., Zoltan, T., López, V., Ortega, J., Izzo, C., Pineda, M., Medina, J., Medina, E. & Rosales, L. Antioxidant and scavenging activity of skyrin on free radical and some reactive oxygen species. *Av. Quím.* **3,** 7–14 (2008).
14 Norman, B. H., Shih, C., Toth, J. E., Ray, J. E., Dodge, J. A., Johnson, D. W., Rutherford, P. G., Schultz, R. M., Worzalla, J. F. & Vlahos, C. J. Studies on the mechanism of phosphatidylinositol 3-kinase Inhibition by wortmannin and related analogs. *J. Med. Chem.* **39,** 1106–1111 (1996).
15 MacMillan, J., Vanstone, A. E. & Yeboah, S. K. Fungal products. Part III. Structure of wortmannin and some hydrolysis products. *J. Chem. Soc. Perkin Trans.* **1,** 2898–2903 (1972).
16 Merlini, L., Mondelli, R., Nasini, G. & Hesse, M. Structure of wortmin, a new metabolite from *Penicillium wortmanni*. *Helv. Chim. Acta.* **56,** 232–239 (1973).
17 Alvi, K. A., Nair, B., Gallo, C. & Baker, D. Screening of microbial extracts for tyrosine kinase inhibitors. *J. Antibiot.* **50,** 264–266 (1997).
18 Shibata, S., Takido, M., Ohta, A. & Kurosu, T. Metabolic products of fungi. XIII. The structure of oxyskyrin. *Pharm. Bull* **5,** 573–575 (1957).
19 Hiroyuki, Y., Nobuhiro, K., Satoshi, O. & Hiroshi, T. new rugulosins, anti-MRSA antibiotics, produced by *Penicillium radicum* FKI-3765-2. *Org. Lett.* **12,** 1572–1575 (2010).

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