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Polyenylpyrroles and Polyenylfurans from an Australian Isolate of the Soil Ascomycete *Gymnoascus reessii*

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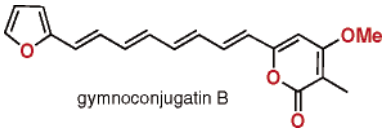
ABSTRACT



An Australian isolate of the soil ascomycete *Gymnoascus reessii* yielded a series of cytotoxic metabolites, including the known polyenylpyrroles rumbrin (1) and auxarconjugatin A (2), and the new rumbrin stereoisomer 12*E*-isorumbrin (3), as well as an unprecedented class of polyenylfurans exemplified by gymnoconjugatins A (4) and B (5). Structures were assigned with detailed spectroscopic analysis.

In recent years our research has focused on the isolation, identification, and evaluation of bioactive metabolites from Australian marine and terrestrial biodiversity. In this report we describe our investigations into the terrestrial soil ascomycete *Gymnoascus reessii* Baranetzki, collected near Sussex Inlet on the southern coast of New South Wales, Australia. The MeOH extract of a liquid culture of *G. reessii* displayed significant growth inhibitory activity against the bacterium *Bacillus subtilis*, the nematode *Haemonchus contortus*, the plant fungal pathogen *Septoria nodorum*, and a tumor cell line (murine NS-1). Quite apart from these biological properties, we also took note of the fact that the genus *Gymnoascus* had not featured prominently in the natural products literature, and were intrigued by preliminary HPLC-DAD analyses which revealed an extract rich in structurally diverse metabolites. In earlier studies on this isolate we determined the antibacterial principle to belong to the rare roquefortine class of isoprenylated diketopiperazines, and went on to describe a new example of this structure class, roquefortine E.[1] Likewise, we succeeded in attributing the antifungal activity in the *G. reessii* extract to a series of novel aromatic butenolides, designated gymnoascolides A−C.[2] This report represents the third installment in our investigation, and reveals the *G. reessii* cytotoxic agents as the polyenylpyrrole metabolites rumbrin (**1**) and auxarconjugatin A (**2**), and the new stereoisomer 12*E*-isorumbrin (**3**). This represents the first reported observation that such polyenylpyrroles display cytotoxic properties. Significantly, this study also discovered an unprecedented class of polyenylfurans, exemplified by gymnoconjugatins A (**4**) and B (**5**).

Concentration of a MeOH extract derived from a liquid fermentation of *G. reessii* yielded a cytotoxic red precipitate. HPLC-DAD analysis of the precipitate revealed a complex mixture comprising many components, all possessing similar and highly distinctive UV−vis spectra (see Figure 1).

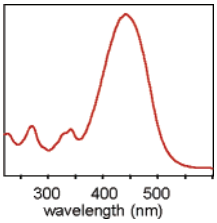


Figure 1. Representative UV−vis spectrum of polyene metabolites from *Gymnoascus reessii* strain MST-F9977.

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Table 1. NMR (600 MHz, d_6-DMSO) Data for Compounds **3**−**5**[a]

	12*E*-isorumbrin (**3**)		gymnoconjugatin A (**4**)			gymnoconjugatin B (**5**)
	δ_C	δ_H (m, *J*(Hz))	δ_C	δ_H (m, *J*(Hz))	gHMBC (H to C)[b]	δ_H (m, *J* (Hz))
1		11.47 (dd, 2.6, 2.9)				
2	120.4	6.90 (dd, 2.9, 3.0)	143.4	7.68 (br s)	C-3, C-4, C-5	7.68 (dd, 1.1, 1.1)
3	109.1	6.13 (dd, 2.6, 3.0)	112.3	6.53 (br s)[c]	C-2, C-4, C-5	6.53 (br s)[d]
4	111.8		109.9	6.54 (br s)[c]	C-2, C-3, C-5	6.54 (br s)[d]
5	126.0		152.7			
6	120.5	6.52 (d, 15.6)	121.1	6.59 (d, 13.9)	C-4, C-5, C-8	6.59 (d, 15.6)
7	124.8	6.78 (dd, 11.2, 15.6)	127.2	6.76 (dd, 10.8, 13.9)	C-5, C-9	6.76 (dd, 10.3, 15.6)
8	136.3	6.61 (dd, 11.2, 14.7)	135.2	6.61 (m)	C-10	6.59 (dd, 10.3, 14.7)[e]
9	131.7	6.47 (dd, 10.4, 14.7)	133.5	6.58 (m)		6.55 (dd, 10.3, 14.7)[e]
10	138.6	6.72 (dd, 10.4, 15.2)	138.3	6.75 (dd, 10.7, 14.4)	C-8, C-12	6.74 (dd, 10.3, 14.8)
11	127.9	6.77 (dd, 11.1, 15.2)	128.8	6.78 (dd, 12.3, 14.4)	C-9	6.51 (dd, 11.3, 14.8)
12	131.1	7.05 (d, 11.0)	130.8	7.05 (d, 12.3)	C-10, C-14, 13-Me	7.06 (dd, 11.3, 15.2)
13	125.8		126.5			6.36 (d, 15.2)
14	158.8		158.7			
15	93.7	6.57 (s)	94.0	6.59 (s)	C-13, C-14, C-16, C-17	6.67 (s)
16	166.0		166.0			
17	100.3		100.5			
18	163.4		163.4			
13-Me	12.4	2.05 (s)	12.4	2.05 (s)	C-12, C-13, C-14	
16-OMe	56.7	3.95 (s)	56.7	3.95 (s)	C-16	3.89 (s)
17-Me	8.7	1.81 (s)	8.7	1.81 (s)	C-16, C-17, C-18	1.81 (s)

[a] Assignments made with the assistance of gHSQC and gHMBC correlations and by comparison to known compounds. [b] Extensive overlap of ^{1}H NMR signals at ~6.76 and ~6.60 ppm means some uncertainty exists in gHMBC correlations from these protons. [c−e] Assignments may be interchanged.

Fractionation of a portion of this material with Sephadex LH-20 (MeOH), silica flash chromatography (CH_2Cl_2/MeOH/AcOH), and reversed-phase HPLC (MeOH/H_2O/TFA) yielded the known compounds rumbrin (**1**)[3] and auxarconjugatin A (**2**)[4] as relatively minor components, together with a new major isomer of **1** identified as 12*E*-isorumbrin (**3**).

High-resolution ESI(+)MS analysis of **3** suggested a molecular formula ($C_{20}H_{20}O_3ClN$) requiring 11 DBE. Examination of the ^{1}H NMR (d_6-DMSO) data for **3** revealed resonances closely related to those for auxarconjugatin A (**2**), with a major difference being replacement of an olefinic methine (δ_H 6.32) with an olefinic methyl resonance (δ_H 2.05). Detailed analysis of the NMR data for **3** revealed resonances consistent with the conjugated chloropyrrole, tetraene, and pyrone moieties characteristic of this structure class (Table 1). Large values for $J_{6,7}$ (15.6 Hz), $J_{8,9}$ (14.7 Hz), and $J_{10,11}$ (15.2 Hz) supported assignment of *E* stereochemistry about these three double bonds. However, significant differences in the ^{1}H NMR chemical shift for H-12, and the ^{13}C NMR chemical shift for the 13-Me, in rumbrin (**1**) (δ_H 6.47 and δ_C 21.1) compared to those of **3** (δ_H 7.05 and δ_C 12.4), suggested an *E* $\Delta^{12,13}$ stereochemistry for **3**. Thus **3** was attributed the trivial name 12*E*-isorumbrin, and the structure assigned as indicated.

Further HPLC-DAD-MS analysis of the crude red precipitate suggested a complex of related polyenyl stereoisomers. Such stereoisomers have been shown to exist in the auxarconjugatin series,[4] and as might be expected, acid treatment of the *G. reessii* red precipitate resulted in a simplified HPLC-DAD profile, as *Z* double bond isomers equilibrated to more stable *E* isomers. Consistent with this observation, attempts to isolate components incorporating *Z* double bonds by HPLC proved challenging, due to facile equilibration of the purified materials to *E* isomers. Having identified the major constituents in the *G. reessii* red

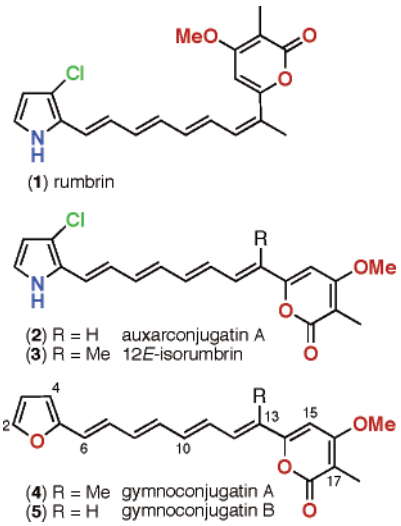

Figure 2. Polyenes isolated from *Gymnoascus reessii*.

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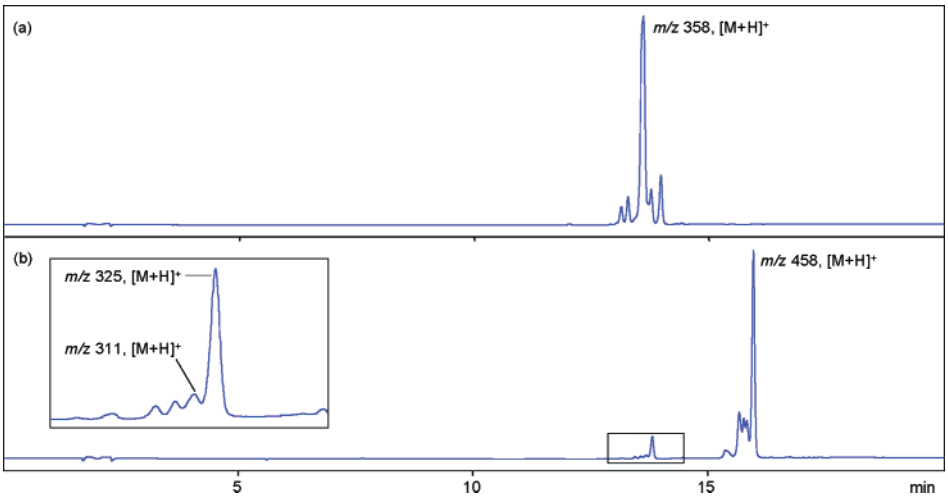

Figure 3. HPLC-DAD-MS (400 nm shown) analysis of (a) polyenylpyrroles and polyenylfurans from the *G. reessii* red precipitate (eluting over a narrow and overlapping range of retention times). (b) BOC-protected polyenylpyrroles eluting later than polyenylfurans. The expansion box reveals the complex of minor polyenylfurans. Major ESI(+)MS ions are displayed for selected peaks.

precipitate as polyenylpyrroles we turned our attention to a series of minor unhalogenated analogues. The first of these to be isolated by reverse-phase HPLC was gymnoconjugatin A (**4**). High-resolution ESI(+)MS analysis of **4** suggested a molecular formula ($C_{20}H_{20}O_4$) that, unlike the polyenylpyrroles described above, lacked both Cl and N atoms. Comparison of the ^{1}H and 2D NMR data for **4** (see Table 1) with those for 12*E*-isorumbrin (**3**) revealed resonances indicative of a 1,8,8-trisubstituted tetraene chain conjugated to a pyrone—i.e., a methoxy (δ_H 3.95), two olefinic methyls (δ_H 2.05 and 1.81), a pyrone methine (δ_H 6.59), and seven olefinic protons. The most noticeable ^{1}H NMR differences between the polyenylpyrroles and **4** were the replacement of the two pyrrole methine resonances (cf. **3**, δ_H 6.90 and 6.13) with three deshielded methine resonances (cf. **4**, δ_H 7.68, 6.54, and 6.53). Likewise, the most significant changes in the ^{13}C NMR spectra of **4** involved large downfield shifts for both C-2 (cf. from δ_C 120.5 in **3** to δ_C 143.4 in **4**) and C-5 (cf. from δ_C 126.0 in **3** to δ_C 152.7 in **4**). These observations suggested that gymnoconjugatin A (**4**) was the polyenylfuran pyrone shown (less stereochemistry). Assignment of an *E* stereochemistry about $\Delta^{6,7}$ and $\Delta^{10,11}$ in **4** was achieved by consideration of $J_{6,7}$ (13.9 Hz) and $J_{10,11}$ (14.4 Hz), while assignment of an *E* stereochemistry about $\Delta^{12,13}$ was achieved through consideration of the ^{1}H NMR chemical shift for H-12 in **4** (δ_H 7.05) relative to those of 12*E*-isorumbrin (**3**) (δ_H 7.05) and rumbrin (**1**) (δ_H 6.47). This latter assignment was further supported by (a) the ^{13}C NMR chemical shift for 13-Me in **4** (δ_C 12.8) compared to **3** (δ_C 12.4) and **1** (δ_C 21.1), and (b) a diagnostic 2D NMR NOESY correlation between the 13-Me and H-11 in gymnoconjugatin A (**4**). While $J_{8,9}$ could not be directly measured from the ^{1}H NMR spectrum of **4**, assignment of an *E* $\Delta^{8,9}$ stereochemistry could be inferred from analysis of the 2D NMR data and comparison of the assigned chemical shifts (see Table 1) with those for **1** and **3**.

Given the high relative concentration of polyenylpyrroles over polyenylfurans in the crude mixture, together with their similiar HPLC and NMR properties, it was speculated that additional very minor polyenylfuran co-metabolites might exist. To test this hypothesis, a sample of the red precipitate was first defatted by using a DMSO/hexane partition to yield material enriched in polyenes. This enriched material was then reacted with di-*tert*-butyl-dicarbonate[5] to convert polyenylpyrroles to their corresponding *N*-BOC protected derivatives. HPLC-DAD-MS analysis of this material (Figure 3) revealed a suite of new underivatized minor polyenylfurans. Reverse-phase SPE and HPLC of this material permitted isolation of additional quantities of gymnoconjugatin A (**4**), in addition to small amounts of a minor co-metabolite designated gymnoconjugatin B (**5**). HRESI(+)MS analysis of **5** indicated a molecular formula ($C_{19}H_{18}O_4$) differing from **4** by the loss of a single methylene unit. Although the yield of **5** (0.4 mg) precluded 2D NMR analysis, the ^{1}H NMR and UV−vis data indicated that **5** was a close analogue of gymnoconjugatin A (**4**). In particular, the absence of the 13-Me resonance in the ^{1}H NMR spectrum suggested that **5** was a demethyl homologue of **4**, while comparison of the UV−vis spectra and analysis of the tetraene coupling constants supported an all *E* stereochemistry. Thus gymnoconjugatin B (**5**) was tentatively assigned the structure shown.

Reports on the isolation of polyenylpyrroles are rare. Rumbrin (**1**) has been previously isolated from the fungus *Auxarthron umbrinum*,[3,6] while auxarconjugatin A (**2**) and its demethyl and dechloro derivatives, auxarconjugatins B

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and C, were first isolated from another *Auxarthron* species.[4] Limited biosynthetic studies on **1** have confirmed its polyketide origin, and it has also been reported to possess activity as a cytoprotective agent.[7] The only other closely related polyenylpyrroles on record are the keronopsins, brominated metabolites from the marine ciliate *Pseudokeronopsis rubra*,[8] and the wallemias,[9] which were isolated from the mitosporic ascomycete *Wallemia sebi*.[10] Polyenylfurans are even less common: to our knowledge, the gymnoconjugatins represent the first occurrence of polyketide polyenyl-2-furans. However, the known polyenylpyrone mycotoxin citreoviridin[11] certainly bears a strong resemblance to the gymnoconjugatins.

Rumbrin (**1**) has been reported to possess cytoprotective activity,[7] and has been patented as a calcium accumulation inhibitory agent and lipid peroxide production inhibitor.[12] Given these literature reports and the potent cytotoxic activity of the crude extract, the biological activities of the polyenes isolated in the current study were of great interest. Compounds **2**−**4** were tested for antimicrobial and cytotoxic activities: **1** and **5** were not tested due to the small amounts of material available. Minimal antimicrobial activity was revealed, but both auxarconjugatin A (**2**) and 12*E*-isorumbrin (**3**) possessed potent cytotoxic properties against an NS-1 cell line (LD_{99} 2.3 and 0.41 μg/mL), while gymnoconjugatin A (**4**) was significantly less active (LD_{99} 50 μg/mL).

Because cytotoxicity can be indicative of anticancer properties, 12*E*-isorumbrin (**3**) was sent for further assays against human cancer cell lines, the results of which are given in Table 2. These studies revealed potent and selective activity against ovarian cancer (JAM) compared to the control cell line (NFF). In most cases the crude mixture was less active than purified **3**; however, for a few cancer types, in particular lung (A549) and melanoma (MML96L) cancer cell lines, the crude red precipitate was of greater toxicity, possibly indicative of the presence of other cytotoxic agents or of synergistic effects. For example, given that 12*E*-isorumbrin (**3**) may very likely be an isomerized artifact of the less stable *Z*-isomer rumbrin (**1**), it is possible that the improved activity against A549 and MML96L displayed by the crude polyene mixture is due to the presence of **1**. Gymnoconjugatin A (**4**) was also tested, but showed significantly lower levels of activity against all cell lines.

Table 2. Anticancer Properties of 12*E*-Isorumbrin (**3**) and the Crude Polyene Mixture

		IC_{50} (ng/mL)	
cell line	cancer type	crude	**3**
A549	lung	4.94	14.3
C180−13S	ovarian	55.9	47.2
DU145	prostate	10.0	7.61
HT29	colon	>1000	>1000
JAM	ovarian	1.68	0.46
MML96L	melanoma	106.4	418.3
NFF	control line	96.3	65.2

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Note Added after ASAP Publication. The compound shown in the TOC and the Abstract was gymnoconjugatin B, not A, in the version published ASAP January 19, 2006; the corrected version was posted ASAP February 1, 2006.

Supporting Information Available: Full details of the collection and culturing of the *Gymnoascus reessii* strain MST-F9977, the isolation and spectroscopic characterization of compounds **1**−**5**, and chemical derivatization studies. This material is available free of charge via the Internet at http://pubs.acs.org.

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