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Memnobotrins and Memnoconols: Novel Metabolites from *Memnoniella echinata*

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Four novel metabolites have been isolated from a rice culture of *Memnoniella echinata* (JS6308) by solvent extraction and radial silica chromatography. The structures were elucidated by spectroscopic techniques, and the absolute stereochemistry of memnobotrin A determined by X-ray crystallography.

As part of our research efforts to develop an HPLC method to characterize and quantify mycotoxins, we desired milligram quantities of the immunosuppressant phenylspirodrimane, stachybotrylactones and lactams.[1] These compounds are ubiquitous in cultures of *Stachybotrys atra*, although often at low levels. It was anticipated that *Memnoniella echinata*, which is closely related to *S. atra*, would produce these compounds based on our HPLC analysis and the literature.[2] Earlier, we reported that an isolate of *Memnoniella echinata* produced substantial amounts of dechlorogriseofulvins,[3] and we anticipated that a rice culture of this isolate would produce sufficient quantities of the phenyspirodrimane compounds. However, instead of producing the expected metabolites, four novel metabolites were recovered.

The memnobotrins A (**1**) and B (**2**), share similar structural features to the aforementioned phenylspirodrimanes,[1,4] hognoquercins,[5] stachybotrins,[6~8] staplabin,[9] the SMTP series,[10,11] stachyflin[12] and L-671,776.[13] The most closely related structure is that of kampanol A.[14] These previously reported compounds were isolated from *Stachybotrys* organisms, while *M. echinata* has been reported to produce the metabolite L-671,776[2] and three related compounds termed "Factors" A, B and C.[15]

Two other new metabolites were isolated and have been named memnoconol (**3**) and memnoconone (**4**). These compounds are closely related in structure to the diversely bioactive fungal metabolite mycophenolic acid (**5**)[16] as well as the cytotoxic hericenones,[17] originally isolated from a mushroom.[18]

This paper describes the fermentation, isolation and characterization of these four new compounds.

Results

Production and Isolation

The culture medium was prepared by autoclaving *Uncle Ben's Rice* (50 g) with water (50 ml) for 35 minutes (16 PSI, 130°C) in Erlenmeyer flasks (250-ml). The rice was allowed to sit for 24 hours, then re-autoclaved. Flasks (20) were inoculated with *Memnoniella echinata* (JS6308) from an agar slant. The cultures were incubated at ambient temperature for 5 weeks with shaking every 2~3 days.

The culture was extracted by coarsely grinding the heavily sporulated rice grains in a coffee-grinder for 1~3 seconds. The ground rice (980 g) was divided into three portions; each portion was slurried with MeOH (400 ml) and the mixture treated to ultrasound agitation and then allowed to sit for 12 hours (4°C). The mixture was filtered

This paper is dedicated to Prof. WOLF VON PHILIPSBORN on the occasion of his 70th birthday

Table 1. Physico-chemical properties of **1** and **2**.

	1	2
Appearance	Pale yellow glass	Cream powder
Molecular formula	$C_{25}H_{33}NO_5$	$C_{27}H_{37}NO_6$
Molecular weight	427	471
HR FAB-MS (*m/z*): calcd	428.2437 $[M+H]^+$	472.2699 $[M+H]^+$
found	428.2464	472.2690
UV λ_{max} nm (ε) (MeOH)	217 (26200), 257.5 (5600), 300 (2300)	218.5 (37200), 259 (10600), 302 (3300)
IR υ_{max} ($CHCl_3$) cm^{-1}	3596, 3455, 3316, 2931, 2854, 1716, 1692, 1612, 1458, 1368, 1136, 1069	3598, 3323 br, 2933, 2855, 1717, 1669, 1616, 1458, 1368, 1317, 1135, 1071
mp (°C)	decomposed 240-250	186-192
$[\alpha]_{25}$ (589 nm) (MeOH)	+9.6 (c=1.14)	+11.7 (c=1.25)
Rf[a]	0.50	0.42

[a] Silica gel 60 F_{254} plates (EM Science, P/N 5714-3, 0.25 mm); Solvent CH_2Cl_2-MeOH (93 : 7); Detection: UV or vanillin dip and heating.

and the rice re-extracted with MeOH : CHCl3 (1 : 1, 800 ml) and finally $CHCl_3$ (600 ml). The combined organic extract was concentrated to 400 ml by rotary evaporation. Water (500 ml) was added and the solution extracted three times with $CHCl_3$ (150 ml). The organic portion was evaporated to give the crude extract (25.5 g) as a brown tar.

The crude extract (25.0 g) was dissolved in CH_2Cl_2 and applied to a slurry packed polyethyleneimine[19] column (250 g, 200×60 mm, packed in CH_2Cl_2). The column was eluted with increasing proportions of MeOH in CH_2Cl_2 (0 to 10% in 1% increments, 500 ml each). Fractions (50 ml) were collected and combined based on TLC analysis to give seven fractions. Fraction 6 was comprised of dechlorogriseofulvins,[3] while 1 through 3 were of low mass and complex mixtures.

Fraction 4 (450 mg) was applied to a 2 mm chromatotron plate which was eluted with increasing proportions of MeOH in CH_2Cl_2 (1% (50 ml); 2%, (50 ml); 3.5%, (50 ml); 5%, (100 ml); 6.5%, (100 ml); 8.5%, (100 ml); 12%, (100 ml)). TLC analysis of the eluent revealed two major constituents and resulted in the isolation of memnobotrin A (**1**) (22.8 mg) and memnobotrin B (**2**) (25.0 mg). An earlier fraction from the plate (25.2 mg) required one further passage through a 2 mm chromatotron plate (gradient elution with 25~50% EtOAc : hexane (135 ml)) to yield pure memnoconone (**4**) (3.7 mg).

Fraction **5** (350 mg) was dissolved in the minimum CH_2Cl_2 : MeOH (1 : 1), diluted with a small volume of hexane, and the emulsion applied to a silica column (Whatman LPS-1, 15 g, 30×70 mm). Elution with EtOAc : hexane (25%, 40 ml; 50%, 100 ml; 80%, 100 ml) gave a total of 90.2 mg of memnoconol (**3**).

Physico-chemical Properties

The physico-chemical properties of memnobotrins A and B (**1** and **2**, respectively) are summarized in Tables 1 and 2; data for the metabolites **3** and **4** are summarized in Tables 3 and 4.

All compounds were readily soluble in MeOH and acetone, but memnobotrins A and B were only partially soluble in CH_2Cl_2 or $CHCl_3$. Similar solubility characteristics are observed with stachybotrylactones and lactams.[1]

Biological Activity

Compounds **1**~**3** were assayed in the 3-cell line panel currrently used by the National Cancer Institute.[20] Memnobotrin B (**2**) and memnoconol (**3**) exhibited appreciable cytotoxicity (*ca.* 80~90% cell death in all three cell lines, Table 5), while memnobotrin A (**1**) was significantly less cytotoxic. However, **1** exhibited selective cytotoxicity against the MCF7 (breast) cell line (Table 5).

Structure Elucidation

The molecular formula for memnobotrin A (**1**) was determined as $C_{25}H_{33}NO_5$ by HRFAB-MS. This formula requires 10 degrees of unsaturation, of which six are accommodated by an aromatic ring (^{13}C NMR δ 100.6, 123.6, 132.2, 151.3, 156.3, 114.7) plus two carbonyls (δ

Table 2. NMR data for **1** and **2** recorded in deuteroacetone.

	1[a]		**2**	
^{13}C	^{13}C / Dept	^{1}H / HMQC[b]	^{13}C / Dept	^{1}H / HMQC[b]
1	38.4 (t)	1.89 1H ddd 13.2, 3.4, 3.4 1.20 1H m	38.4 (t)	1.90 1H dt 13.3, 3.2 1.20 1H m
2	24.1 (t)	1.61 2H m	24.1 (t)	1.61 2H m
3	80.9 (d)	4.46 1H br dd 9.3, 6.3	80.9 (d)	4.46 1H dd 9.8, 6.7
4	38.3 (s)		38.3 (s)	
5	54.8 (d)	1.13 1H m	54.7 (d)	1.13 1H brd 9.8
6	18.5 (t)	1.55 1H m 1.70 1H m	18.5 (t)	1.63 2H m
7	40.8 (t)	1.70 m 2.16 brd 9.6	40.8 (t)	1.70 1H m 2.16 1H dd 2.0, 10.4
8	76.3 (s)		76.4 (s)	
9	49.0 (d)	1.57 1H m	49.0 (d)	1.57 1H m
10	38.8 (s)		38.7 (s)	
11	18.9 (t)	2.87 1H AB 18.8 2.71 1H ABX 18.8, 8.2	18.8 (t)	2.87 AB 18.9 2.71 ABX 18.9, 8.1
12	27.2 (q)	1.19 3H s	27.2 (q)	1.17 3H s
13	17.2 (q)	0.84 3H s	17.1 (q)	0.82 3H s
14	28.7 (q)	0.89 3H s	28.6 (q)	0.87 3H s
15	14.6 (q)	0.71 3H s	14.6 (q)	0.69 3H s
1'	114.7 (s)		114.4 (s)[a]	
2'	156.3 (s)		156.2(s)[a]	
3'	100.6 (d)	6.80 1H s	100.6 (d)[a]	6.81 1H s
4'	132.3 (s)		132.5 (s)	
5'	123.60 (s)[a]		121.6 (s)	
6'	151.3 (s)		151.0 (s)	
7'	171.8 (s)[a]		169.6 (s)	
8'	43.0 (t)[a]	4.21 2H br AB 16.9	49.2 (t)	4.36 2H AB 17.0
9'	-	-	46.0 (t)	3.65 2H t 5.4
10'	-	-	61.2 (t)	3.77 2H t 5.4
Ac	21.0 (q)	1.98 3H s	21.0 (q)	1.96 3H s
Ac	170.8 (s)		170.8 (s)	

[a] Signals appeared as doublets (Δ 0.1-0.2 ppm) at concentrations of 120 mM in deuteroacetone. At 10 mM this effect disappeared and all signals were observed as singlets.
[b] Numbers following multiplicities are coupling constants in Hz.

Table 3. Physico-chemical properties of **3** and **4**.

	3	**4**
Appearance	Light brown foam	Light brown film
Molecular formula	$C_{23}H_{32}O_6$	$C_{23}H_{30}O_5$
Molecular weight	404	386
HR FAB-MS (*m/z*): calcd	405.2277 $[M+H]^+$	387.2172 $[M+H]^+$
found	405.2296	387.2138
UV λ_{max} nm (ε) (MeOH)	219 (28700), 261.5 (11100), 290 (3000)	219 (38000), 261 (14600), 289 (2900)
IR υ_{max} ($CHCl_3$) cm^{-1}	3587, 3440, 3005, 2978, 2932, 1728, 1637, 1612, 1451, 1353, 1316, 1196, 1161	3587, 3435, 2972, 3934, 1761, 1639, 1612, 1462, 1384, 1355, 1316, 1124
mp (°C)	60-63	-
$[\alpha]_{25}$ (589 nm) (MeOH)	-14.0 (c=1.08)	achiral
Rf[a]	0.36	0.47

[a] Silica gel 60 F_{254} plates (EM Science, P/N 5714-3, 0.25 mm); Solvent EtOAc-hexane (1 : 1); Detection: UV or vanillin dip and heating.

Table 4. NMR data for **3** and **4** recorded in deuteroacetone.

	3		4	
^{13}C	^{13}C / Dept	^{1}H / HMQC[a]	^{13}C / Dept	^{1}H / HMQC[a]
1	172.9 (s)		173.0 (s)	
2	-		-	
3	70.6 (t)	5.15 2H s	70.7 (t)	5.22 2H s
3a	103.6 (s)		103.7 (s)	
4	163.8 (s)		163.8 (s)	
5	115.6 (s)		114.2 (s)	
6	155.7 (s)		155.7 (s)	
7	101.5 (d)	6.52 1H s	101.5 (d)	6.62 1H s
7a	147.4 (s)		147.5 (s)	
1'	22.1 (t)	3.30 2H d 6.9	22.1 (t)	3.36 2H brd 7.1
2'	123.0 (d)	5.20 1H brt 6.9	123.0 (d)	5.25 1H ddd 6.8,6.8,1.1
3'	135.4 (s)		135.4 (s)	
4'	40.3 (t)	1.91 2H brt 7.4	40.3 (t)	1.95 2H brt 7.5
5'	27.0 (t)	2.02 2H m	27.1 (t)	2.02 2H m
6'	124.6 (d)	5.07 brt 6.5	125.0 (d)	5.07 1H ddd 6.5,6.5,1.1
7'	135.7 (s)		134.9 (s)	
8'	37.4 (t)	1.87 1H m 2.15 1H brddd 4.4, 9.9, 13.7	34.1 (t)	2.10 2H brt 7.5
9'	30.6 (t)	1.26 1H dddd 4.4, 10.0, 10.1, 13.6 1.58 1H dddd 1.6, 6.8, 9.9, 13.6	39.5 (t)	2.49 2H m
10'	78.4 (d)	3.18 1H dd 10.1, 1.6	213.7 (s)	
11'	72.9 (s)		41.0 (d)	2.60 1H hept 7.0
12'	25.7 (q)	1.11 3H s	18.4 (q)	1.01 3H d 7.0
13'	24.9 (q)	1.11 3H s	18.4 (q)	1.01 3H d 7.0
14'	16.2 (q)	1.76 3H s	16.2 (q)	1.76 3H s
15'	16.1 (q)	1.55 3H s	16.1 (q)	1.55 3H s

[a] Numbers following multiplicities are coupling constants in Hz.

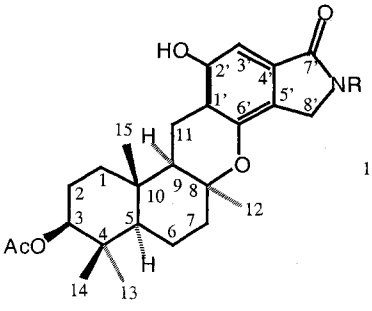
Table 5. Cytotoxicity/cell growth inhibition at 1×10^{-4} M for compounds **1**~**3**.[a]

Compound	Cell Line		
	NCI-H460 (Lung)	MCF7 (Breast)	SF-268 (CNS)
1	-12%	-64%	+4%
2	-80%	-92%	-87%
3	-81%	-80%	-89%

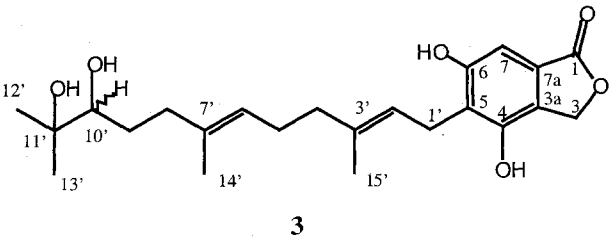
[a]Compounds which reduce the growth of cell lines to 32% or under (control = +100%) are considered active. Negative numbers indicate cell kill.[20]

170.8, 171.8); therefore, the molecule contains an additional four rings and must be pentacyclic. The carbonyl groups were observed in the IR spectrum as strong bands at 1728 (ester) and 1637 (amide) cm^{-1}, while a strong absorption at 1612 cm^{-1} was attributed to the aromatic ring. The ^{13}C NMR spectrum of **1** in $CDCl_3$ displayed 24 carbon resonances; however, the intensity of the quaternary signal resonating at 38.1 ppm indicated two quaternary signals were coincidental. This overlap was alleviated by recording NMR data in deuteroacetone, a solvent that **1** and **2** also displayed increased solubility. Proton NMR spectroscopy coupled with DEPT and HMQC experiments in deuteroacetone indicated there were five methyl, six methylene, four methine and ten quaternary groups (Table 2). The two remaining proton signals were observed as broad signals at δ 7.5 and 9.1.

A key ABX resonance in the ^{1}H NMR spectrum (δ 2.71, 2.87) that integrated for two protons was similar to an AB



1 R=H
2 R=CH_2CH_2OH



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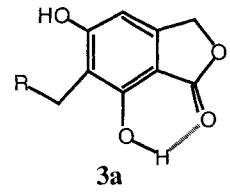
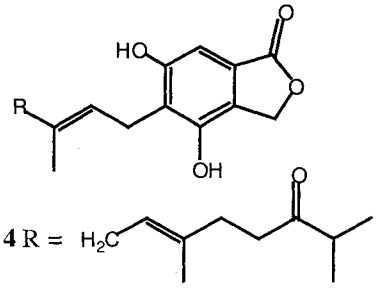
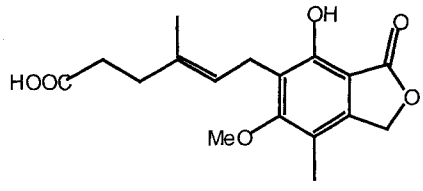


3a



4 R = H_2C



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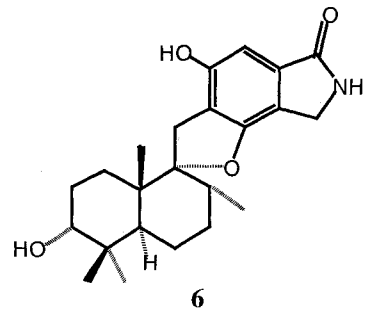

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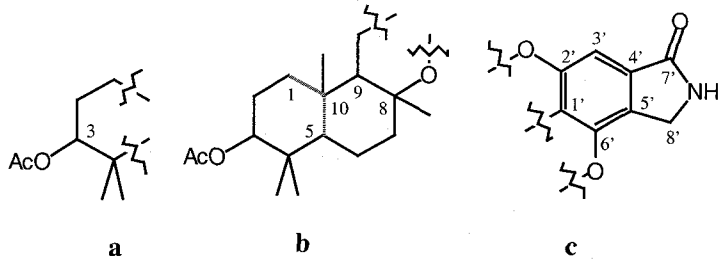
system observed for the spirodrimane lactones and lactams isolated previously in this laboratory.[1] Coupling this information with the similar UV spectrum (λ_{max} 219, 261.5, 290 nm) and many similarities in the ^{13}C NMR data, we strongly suspected a structure for **1** closely related to stachybotrylactam (**6**).

The presence of a geminal dimethyl system was clear from HMBC correlations. Further HMBC correlations connected this group to the down-field methine resonance at δ 4.46 (H-3), 80.9 (C-3), which in turn was extended by two methylene units on the basis of COSY data. The chemical shift of the methine indicated acetyl functionality; this was verified by the observation of a long-range through oxygen correlation from the acetyl methyl (δ 1.98) to the methine carbon δ 80.9 (C-3). This gives partial structure [**a**] (Fig. 1). Extension of [**a**] from the geminal methyl group to a methine was clear from HMBC data. This can be extended based on COSY correlations to include a further two methylene unit. The terminal C-7 methylene unit (^1H δ 1.70, 2.16; ^{13}C δ 40.8) displayed HMBC correlations to the quaternary oxygenated carbon δ 76.3 (C-8), the C-9 methine (^1H δ 1.57; ^{13}C δ 49.0) and a methyl group resonating at δ 1.19; ^{13}C δ 27.2. With the incorporation of the ABX methylene group based on a COSY correlation from δ 1.57 (H-9) to δ 2.71 (H-11), and including the remaining methyl (^1H δ 0.71; ^{13}C δ 14.6) and aliphatic quaternary carbon resonances δ 38.8 (C-10) based on HMBC correlations, we arrived at the partial structure [**b**]. At this point, HMBC data required careful scrutiny due to the close proximity of the methylene C-1 and quaternary C-4 and C-10 resonances (δ 38.4, 38.3 and 38.8, respectively). However, it was possible to deduce an unambiguous series of correlations which pointed to the drimane ring structure and closed the dotted connections of structure [**b**].

Partial structure [**c**] was determined by HMBC correlations. The lactam connection to the aromatic ring was defined by correlations from the amide proton (δ 7.5) to the lactam ring carbons: C-8′, C-5′, C-4′ and C-7′ (δ 43.0, 123.6, 132.3, 171.8, respectively). The aromatic proton displayed strong 3-bond correlations to C-1′ (δ 114.7) and C-5′ (δ 123.6), but importantly, only to C-7′ (δ 171.8) and not to C-8′ (43.0). Additional information which defined the orientation of the lactam unit, and also indicated the position of the oxygenated aromatic resonances, came from two and three bond HMBC correlations from the lactam methylene protons (δ 4.21) to the C-4′, C-5′ and C-6′ carbon resonances (δ 132.3, 123.6 and 151.3, respectively).

With all of the NMR resonances accounted for, excepting the phenolic proton (δ 9.1), the combination of [**b**] and [**c**] can give rise to two structures which satisfy the pentacyclic requirements. Connection of the C-11 methylene to C-1′ (δ 114.7) is clear from the distribution of HMBC correlations,

Fig. 1. Partial structures for memnobotrin A.



however, the pyran ring could be formed at either phenoxy position. The solution, structure **1**, was determined based on a long range HMBC correlation, across the pyran oxygen, from an H-7 proton (δ 1.70) to the aromatic resonance at δ 151.3 (C-6′). Further evidence came from a deuterium exchange experiment; upon deuteration of the alcohol proton, a 0.11 ppm upfield shift in the C-2′ carbon resonance was observed.[21] The final solution was in complete agreement with the spectral data reported for the closely related kampanol A which has the same structural features but contains a lactone rather than lactam ring.[13]

The structure, relative and absolute stereochemistry for **1** was unequivocally decided by X-ray crystallographic analysis (Fig. 2). The sesquiterpene portion is a *trans* fused labdane structure, *cis* fused to the furan system. NOESY data were in complete agreement with the stereochemical configuration determined by the X-ray solution. The absolute stereochemistry was obtained from the X-ray solution and allows **1** to be assigned (+)-3*S*-memnobotrin A. Invoking a common biogenetic mechanism would indicate **2** is also of (+)-3*S* absolute stereochemistry.

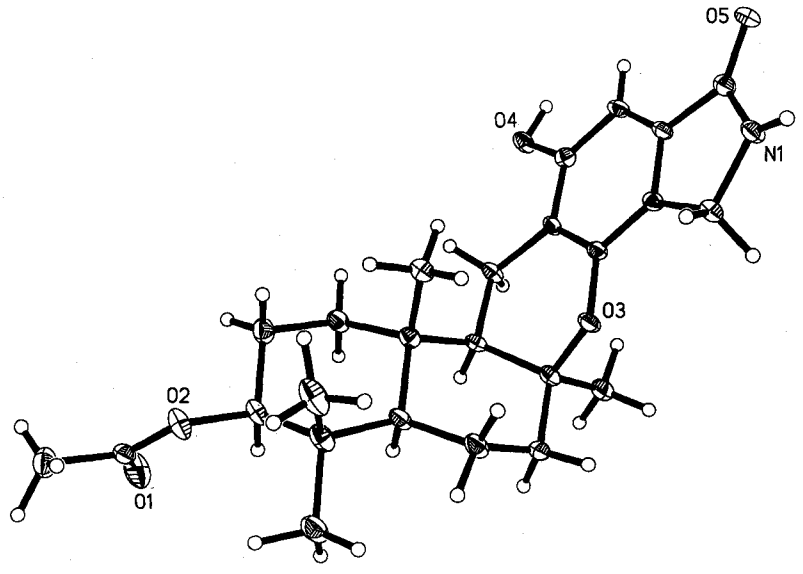
The molecular formula for memnobotrin B (**2**) was determined as $C_{27}H_{37}NO_5$ by HRFAB-MS. It is clear from ^{13}C NMR data that **1** and **2** are closely related in structure. As ^{13}C and DEPT experiments indicated that two additional methylenes are present in **2**, while no change in multiplicity occurred in the remaining carbon signals, it appears that the additional C_2H_4O must be attached to a hetero-atom. This was verified by the observation that no N-*H* proton was observed in the ^1H NMR spectrum, while the alcohol proton could still be detected resonating at δ 9.08. A self-consistent set of HMQC, COSY and HMBC experiments confirmed the structure as **2**, while a NOESY experiment indicated that **1** and **2** shared the same relative stereochemistry.

The ^1H NMR spectra of metabolites **3** and **4** contained some similarities to the spectra for **1** and **2**; however, the more extensive methylene envelope suggested an uncyclized isoprenoid chain. Metabolite **3** has a molecular formula $C_{23}H_{32}O_6$. This was confirmed by a HRFAB-MS peak of *m/z* 405.2296 AMU $(M+H)^+$ (Table 3). Carbon NMR data for **3** indicated 23 distinct resonances, while HMQC and DEPT experiments satisfied the formula $C_{23}H_{28}$, indicating four exchangeable protons which were all observed in the proton spectrum recorded in $CDCl_3$ as broad singlets (δ 8.1, 7.8, 2.9, 2.8). IR data indicated hydroxyl (3588 and 3435 cm^{-1}) and carbonyl (1731 cm^{-1}) functionality. Homo- and hetero-nuclear correlation experiments indicated that **3** is comprised of a γ-lactone fused to a di-hydroxylated aromatic ring, which in turn is connected to a farnesyl chain. Carbons 10′ and 11′ are oxygenated as evidenced by their ^{13}C chemical shifts (δ 78.4 and 72.9 respectively). A 1,2-diol arrangement satisfied the MS and ^{13}C NMR[22] spectral data and coupled with the observation that **3** formed an 11′-hydroxy,10′,4,6-triacetate indicated the structure memnoconol (**3**).

Previous workers had encountered difficulties in defining the orientation of the lactone ring with respect to the aryl ring for compounds closely related in structure to the memnoconols.[17,18] HMBC and COSY correlations indicated that the aryl proton and the methylene group of the lactone are in a *peri* relationship (as in **3a**); however, the lack of an intramolecular hydrogen bound hydroxyl proton (see **3a**), and no NOE from H-7 (δ 6.52) to H-3 (δ 5.15) suggested otherwise. To decide the orientation, the triacetate of **3** was prepared. After confirmation of the structure as **7** with HMQC, HMBC and COSY experiments, the NOESY spectrum was readily interpreted, and an NOE was observed between the aryl acetyl methyl resonances (δ 2.34, 2.35) and the H-3 protons (δ 5.35).

Fig. 2. X-Ray crystal structure of memnobotrin A (**1**).

For clarity, hydrogens are shown only at the stereogenic centers and at the OH and NH groups.



Such a through-space correlation would not be expected for **3a** and so the combined evidence confers the structure **3** to this compound.

Metabolite **4** had spectral data very similar to those of **3** excepting the NMR resonances associated with the terminal portion of the farnesyl chain. Mass spectral data indicated **4** had an additional unit of unsaturation and one fewer oxygen in comparison to **3**, *i.e.* molecular formula $C_{23}H_{30}O_5$ (Table 3). NMR data indicated that the 1,2-diol was replaced with a ketone at C-10′ (δ 213.7), forming an isopropyl group at positions 11′, 12′, and 13′ (^{1}H δ 2.60 (1H, heptuplet, J=7 Hz); 1.01 (6H, d, J=7 Hz)). A full set of self-consistent 2D NMR experiments confirmed the structure as memnoconone (**4**).

Storage of memnoconol **3** at 4°C resulted in the partial conversion to a new compound. This compound was not detected in the *M. echinata* crude extract (HPLC analysis). The appearance of two new methyl resonances in the proton NMR spectrum (δ 1.33, 1.42) and the corresponding carbon signals (δ 106.6, 28.6, 27.7) indicated the presence of an acetonide, presumably formed by the protection of the 1,2-diol in the presence of trace amounts of acetone during manipulations and storage. A corresponding shift was observed in the C-10′ and C-11′ carbon NMR resonances when comparing **3** (δ 78.4, 72.9) to **8** (δ 80.3, 83.1). The ^{13}C NMR data agree very well with the literature reports for similar systems.[23] The acetonide was readily converted back into the parent diol by treatment with aqueous acid.

Discussion

The filamentous fungus *Memnoniella echinata*, and the closely related species *Stachybotrys atra* produce a myriad of polar compounds. These metabolites are related in structure based on their very similar UV/Vis spectra, and may be extracted into organic solvents and are readily detected by HPLC analysis.[24,25] Previous isolates appeared to be abundant in phenylspirodrimanes, similar to those produced by *S. atra*,[1] but it is clear that a plethora of other compounds await discovery, and that these spirocyclic compounds are not always the major metabolites.

The relative stereochemistry of the drimane ring juncture and the C-12 methyl of memnobotrins A and B differs in comparison to the metabolites previously reported from *S. atra*, yet share the same relative stereochemistry as the kampanols from *S. kampalensis*.[14] The stereochemistry of the spirocyclic phenyldrimane from *M. echinata* has not been reported. However, the relative stereochemistry for the acetate (C-3) is retained. This suggests a different cyclization is occurring for the terpenoid portion of these pyran-fused compounds. Of interest is the production of

stachyflin[12] where the pyran ring is fused to the C-9 and C-10 carbons of the drimane ring, rather than C-10, C-11 in the memnobotrins or C-10, C-10 in the spirocyclicdrimanes.[1]

Metabolites **3** and **4** are further examples[6~11,18] of potential precursors to the bioactive series of benzodrimanes such as K-76,[26] stachbotrydial[27] and the phenylspirodrimanes.[1]

Experimental

General

Melting points were determined on a Lab Devices Mel-Temp and are uncorrected. Optical rotations were measured on a Jasco DIP-370 polarimeter. IR and UV/Vis spectra were recorded on Nicolet Magna-560 FT-IR and Beckmann DU-7 spectrophotometers, respectively. HRMS spectra were determined with a VG 7070E mass spectrometer. NMR spectra were recorded on Bruker DRX-400 and DRX-500 machines with spectra referenced to the solvent resonance. Radial chromatography was performed on a Harrison research 7924T chromatotron.

X-ray Crystallography

Crystals of Memnobotrin A (**1**) suitable for X-ray analysis were obtained by recrystallisation from EtOAc.

Crystal data:

$[C_{25}H_{33}NO_5][C_4H_8O_2]_{1.5}$, MW=559.68, Monoclinic, a=22.8684(14), b=10.1944(7), c=14.5195(10) Å, β=118.525(5)°, V=2974.0(3) Å^3, Z=4, D_x=1.250 gcm^{-3}, (MoKα)=0.71073 Å, (MoKα)=0.089 mm^{-1}, F(000)=1208, T=153K(2), R(F)=8.36%, R(wF^2)=18.89% for all 5244 independent reflections.

Data collection and processing:

A clear, colorless, block with dimensions 0.38×0.28×0.18 mm was placed and optically centered on an Enraf-Nonius CAD-4 diffractometer that was controlled by a Digital Equipment Corporation MicroVAX II (MVII) computer and Enraf-Nonius VAX\VMS CAD4 Express control program.[28] The crystals' final cell parameters and crystal orientation matrix were determined from 25 reflections in the range $17.1<\theta<18.5°$; these constants were confirmed with axial photographs. Data were collected [MoKα] with $\omega:2\theta$ scans over the range $2.6<\theta<27.5°$ with a scan width of $(0.51+0.62\tan\theta)°$ and variable scan speed of 2.4~4.1° min^{-1} with each scan recorded in 96 steps with the outermost 16 steps on each end of the scan being used for background determination. Six standard reflections well dispersed in reciprocal space were monitored at 30-minute intervals of X-ray exposure. Minor variations in intensity were observed; data were not corrected. An absorption correction was applied based upon crystal faces with transmission factors ranging from 0.9646~0.9846. Two forms of data were collected, indices h−k±l; and −hk±l (Friedel), resulting in the measurement of 5718 reflections; 5244 unique [R(int)=0.0281].

Structure analysis and refinement:

Data were corrected for Lorentz and polarization factors and reduced to Fo^2 and (Fo^2) using the program XCAD4.[29] The SHELXTL[30] program package program XPREP was implemented, clearly indicating the monoclinic space group C2. The structure was determined by direct methods with the successful location of the nearly all carbon, nitrogen and oxygen atoms present. The hydrogen atoms on the two solvent molecules were calculated and refined using a riding model. The final structure was refined to convergence [$\Delta/\sigma\leq0.001$] with R(F)=8.36%, wR(F^2)=18.89% and GOF=1.091 for all 5244 unique reflections [R(F)=6.20%, wR(F^2)=17.12% for those 4221 data with $Fo>4\sigma(Fo)$]. A final difference-Fourier map was featureless with the largest peak, $|\Delta\rho|\leq0.41$ e Å^{-3}. The correct enantiomorph was also determined using the Flack(x) parameter with x=−1.8(16) confirming the enantiomorph chosen.

The function minimized during the full-matrix least-squares refinement was w(Fo^2-Fc^2) where w=1/[^{2}(Fo^2)+(0.1296*P)^2+0.0*P] and P=(max(Fo^2, 0)+2*Fc^2)/3. An empirical correction for extinction was attempted but found to be negative and not applied.

Acetylation of 3

Memnoconol **3** (10.2 mg) was dissolved in pyridine (0.2 ml) and acetic anhydride (0.2 ml), and stirred for 17 hours at room temperature. The reaction mixture was diluted with dilute HCl (5%, 2 ml) and extracted three times with ether (3×5 ml). The organic portion was washed successively with dilute HCl (5%, 2×5 ml), water (5 ml), bicarbonate (5%, 5 ml), water (5 ml) and dried over sodium sulphate. Concentration and purification by radial chromatography (1 mm plate, 1 : 1 EtOAc/Hexane) gave the pure triacetate **7** (6.9 mg).

Memnoconol triacetate (**7**): pale yellow oil; $[\alpha]_D$ −2.9° (c 0.51, $CHCl_3$); FAB-MS, m/z 531 $[M+H]^+$; HRFAB-MS, m/z found 531.2597, calcd. 531.2594 for $C_{29}H_{39}O_9$; UV_{max} (MeOH) nm (ε) 212 (16800), 282 (1200); IR_{max} ($CHCl_3$) cm^{-1}, 3632 (O–H), 3498 br (O–H), 2933 (C–H), 1769 (C=O), 1729 (C=O), 1631 (C=C), 1599 (C=C), 1454, 1371, 1113, 1043, 1019; ^{1}H NMR (500 MHz, d-acetone) δ 7.24 (1H, s, 7-H), 5.33 (2H, s, 3-H), 5.08 (1H, brt, J=6.9 Hz, 6′-H), 5.01 (1H, brt, J=6.9 Hz, 2′-H), 4.70 (1H

dd, J=1.8, 9.7 Hz, 10′-H), 3.55 (1H, br s, OH), 3.30 (2H, d, J=6.9 Hz, 1′-H), 2.34 (3H, s, 2-Ac), 2.33 (3H, s, 4-Ac), 2.04 (1H, m, 6′-H), 2.01 (3H, s, 10′-Ac), 1.94 (2H, m, 4′-H), 1.93 (1H, m, 5′-H), 1.92 (1H, m, 8′-H), 1.84 (1H, m, 9′-H), 1.80 (1H, m, 8′-H), 1.77 (3H, s, 15′-H), 1.46 (1H, m, 9′-H), 1.56 (3H, s, 14′-H); 1.09 (6H, s, 12′ & 13′-H); ^{13}C NMR (100 MHz, *d*-acetone) δ 171.0 (s, 10′-Ac), 169.1 (s, 4-Ac), 168.9 (s, 6-Ac), 168.2 (s, 1-C), 155.7 (s, 4-C), 148.4 (s, 6-C), 147.8 (s, 7a-C), 137.0 (s, 3′-C), 135.3 (s, 7′-C), 129.0 (s, 5-C), 124.9 (d, 6′-C), 121.3 (d, 2′-C), 116.3 (s, 3a-C), 115.6 (d, 7-C), 79.9 (d, 10′-C), 71.7 (s, 11′-C), 69.7 (t, 3-C), 40.2 (t, 4′-C), 37.0 (t, 8′-C), 28.6 (t, 9′-C), 27.1 (t, 5′-C), 26.5 (q, 12′-C), 25.6 (q, 13′-C), 23.9 (t, 1′-C), 21.0 (q, 10′-Ac), 20.9 (q, 4-Ac), 20.5 (q, 6-C), 16.4 (q, 15′-C), 16.0 (q, 14′-C).

Memnoconol Acetonide Derivative of **3**

Memnoconol **3** (19.7 mg) was stored at 4°C in the presence of a trace amount of acetone. After two months a new compound was detected by TLC analysis. Separation by radial chromatography (2 mm, EtOAc : Hexane, 1 : 4 up to 10 : 1) gave unchanged memnoconol **3** (4.6 mg) and memonoconol acetonide **8** (7.6 mg).

Memnoconol acetonide (**8**): pale yellow oil; $[\alpha]_D$ −1.1° (*c* 0.46, $CHCl_3$); EI-MS, *m/z* 429 $[M-CH_3]^+$, 386 $[M-C_3H_4]^+$, 316, 259, 233, 219, 217, 207, 179; HRFAB-MS, *m/z* found 405.2265, calcd. 405.2277 for $C_{23}H_{33}O_6$ $[M-C_3H_6+H]^+$; IR_{max} ($CHCl_3$) cm^{-1}, 3363 (O–H), 3438 br (O–H), 2985 (C–H), 2941 (C–H), 1730 (C=O), 1639 (C=C), 1612 (C=C), 1452, 1371, 1116, 1047, 1016; UV_{max} (MeOH) nm (ε) 213.5 (14800), 280 (1200); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.09 (3H, s, 13′-H), 1.23 (3H, s, 12′-H), 1.33 (3H, s, acetonide), 1.42 (3H, s, acetonide), 1.46 (1H, m, 9′-H), 1.56 (3H, s, 14′-H), 1.60 (1H, m, 9′-H), 1.78 (3H, s, 15′-H), 1.96 (1H, ddd, J=2.7, 6.5, 10.2 Hz, 8′-H), 2.07 (2H, m, 4′-H), 2.10 (2H, m, 5′-H), 2.15 (1H, m, 8′-H), 3.38 (1H, dd (ABX), J=7.2, 15.9 Hz, 1′-H), 3.44 (1H, dd (ABX), J=7.2, 15.9 Hz, 1′-H), 3.66 (1H, dd, J=3.6, 10.0 Hz, 10′-H), 5.08 (1H, br t, J=6.8 Hz, 6′-H), 5.19 (2H, s, 3-H), 5.24 (1H, br t, J=7.0 Hz, 2′-H), 6.42 (1H, s, 7-H), 6.46 (1H, s, 4-OH),7.87 (1H, s, 6-OH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0 (s, 1-C), 163.0 (3, 4-C), 155.1 (s, 6-C), 145.9 (s, 7a-C), 139.4 (s, 3′-C), 134.8 (s, 7′-C), 124.0 (d, 6′-C), 121.0 (d, 2′-C), 113.9 (s, 5-C), 106.6 (s, acetonide), 103.8 (s, 3a-C), 101.4 (d, 7-C), 83.1 (d, 10′-C), 80.3 (s, 11′-C), 70.3 (t, 3-C), 39.6 (t, 4′-C), 36.6 (t, 8′-C), 28.6 (q, acetonide), 27.7 (q, acetonide), 27.0 (t, 9′-C), 26.1 (t, 5′-C), 26.0 (q, 13′-C), 23.0 (q, 12′-C), 21.5 (t, 1′-C), 16.2 (q, 14′-C), 16.3 (q, 15′-C).

Deprotection of **8**

Memnoconol acetonide **8** (1.0 mg) was stirred at room temperature in THF (1.0 ml) and 5% HCl (1.0 ml). After two hours no deprotection was observed and TFA (0.1 ml) was added to the reaction mixture. The reaction was stirred overnight, diluted with water (4 ml) and extracted with CH_2Cl_2 (2 ml). The evaporated organic layer yielded memnoconol (**3**) (0.7 mg); TLC and NMR analysis identical to those for authentic material.

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