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Investigation of the New Zealand Basidiomycete *Favolaschia calocera*: Revision of the Structures of 9-Methoxystrobilurins K and L, Strobilurin D, and Hydroxystrobilurin D

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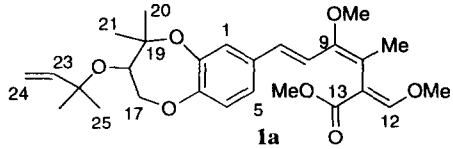
Abstract: 9-Methoxystrobilurin K has been obtained from *Favolaschia calocera*. The previously proposed epoxyprenyl structure for 9-methoxystrobilurin K **2c** and dioxan structure for 9-methoxystrobilurin L **3** have both been revised to the prenyldioxepin structure **1a**. The epoxyprenyl structures of strobilurin D **2a** and hydroxystrobilurin D **2c** should also be revised. © 1997 Elsevier Science Ltd.

Favolaschia calocera, a bright orange fungus found in the North Island of New Zealand, was investigated as part of a program for the identification of new biologically active compounds from basidiomycetes. This fungus was targeted for study when an extract of the fruit bodies showed significant cytotoxicity against the P388 murine cell line. Previous investigations of an Ethiopian *Favolaschia sp.* and *F. pustulosa*, in culture, had identified a series of strobilurins, an oudemansin and four new 9-methoxystrobilurins.[1,2] Strobilurins are a class of compounds which show significant antifungal activity, and some also exhibit cytostatic properties. Both these effects are attributed to inhibition of cellular respiration by the (*E*)-methoxyacrylate moiety.[3] The significance of this class of compounds is evident with some twenty pharmaceutical companies having published over 200 patents in this area along with the development of the commercial fungicides containing kresoxime methyl or azoxystobin, variants of the strobilurin skeleton.[4]

F. calocera fruit bodies were collected from rotting wood in a mixed forest at Frankton, North Island, New Zealand. A dichloromethane extract of the fruit bodies was evaporated to dryness and subjected to a series of bioassay-guided purification steps including normal phase (DIOL) and reverse phase (ODS) column chromatography. This gave **1a**[5] as the only cytotoxic component in the fungus.

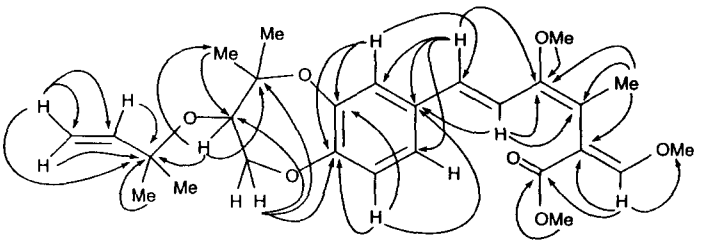


1a

High resolution mass spectrometry established the molecular formula of **1a** as $C_{27}H_{36}O_7$, corresponding to ten double bond equivalents. An absorption at 1709 cm^{-1} (FTIR) indicated the presence of a carbonyl group, while the ^1H NMR spectrum revealed three methoxyl signals (δ_H 3.64, 3.70 and 3.80 ppm), five methyl groups (between δ_H 1.22 and 1.88), a cluster of aromatic, or olefinic protons between δ_H 5.87 and 7.38, and a vinyl CH_2 (δ_H 5.14, 5.16). All one-bond C-H connectivities were obtained from correlations in the HMQC spectrum. The 9-methoxystrobilurin triene system (C7 - C13) was readily assigned with a series of

HMBC and NOE correlations. The starting point was a series of HMBC correlations from H12 as indicated (Fig. 1). The C9-C10 and the C11-C12 alkenes were each assigned as *E* as a consequence of an NOE interaction between Me14 and MeO27, and based on the chemical shift of H12 compared with data reported for other strobilurins.

Figure 1
HMBC correlations for **1a**



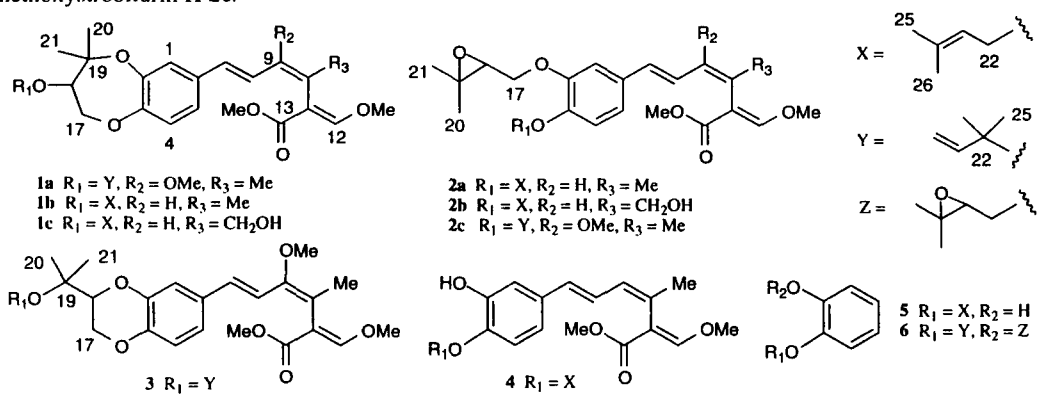
HMBC correlations from both H7 and H8 (δ_H 6.57 and 6.36 respectively) to a carbon at δ_C 132.7 allowed the connection of the triene system to a 1,2,4-tri-substituted aromatic ring. Further HMBC correlations from the H7 olefinic proton (δ_H 6.57) allowed the assignment of the carbons C1, C5 (δ_C 122.4 and 121.6) of the aromatic ring. From the HMQC spectrum the overlapping aromatic protons at δ_H 6.93 were both identified as *ortho*, and the proton at δ_H 6.81 as *meta* to the triene substituent. The two remaining aromatic carbons (δ_C 150.6 and 146.5) were oxygenated and linked to the remainder of the molecule *via* ether bonds (lack of an IR O-H stretch, and molecular formula requirements (2 remaining dbe)).

The remainder of the molecule comprised two oxygen-linked isoprene units which were established by COSY and HMBC correlations (Fig. 1). The vinyl protons (H23, H24a,b) were correlated to each other in the COSY spectrum, while in the HMBC spectrum they were correlated to the quaternary oxygenated carbon C22 (δ_C 76.1). An HMBC correlation to C22 from the Me25 and Me26 protons (δ_H 1.31) completed the assignment of the first isoprene unit. Connection of the two isoprene units was established by a crucial HMBC correlation between this same carbon, C22 (δ_C 76.1), and H18 (δ_H 3.68, δ_C 75.2). Connectivity through the second isoprene unit was determined by a COSY correlation between H18 and the H17 diastereotopic protons (δ_H 3.97, 4.20), which in turn were correlated in the HMBC spectrum to a quaternary carbon at δ_C 81.6 (C19) that in turn showed correlations from the Me20 and Me21 protons (δ_H 1.22, 1.41). Attachment of the isoprene units to the remainder of the molecule was established with an HMBC correlation from the H17 diastereotopic protons (δ_H 3.97, 4.20) to an oxygenated aromatic carbon C3 (δ_C 150.6) and an NOE between H1, the *ortho* aromatic proton (δ_H 6.93), and the Me20 and Me21 protons (δ_H 1.22 or 1.41; irradiation of the methyls). This NOE supported the final ether connection between the C19 quaternary carbon (δ_C 81.6) and the C2 aromatic carbon (δ_C 146.5; HMBC correlation to H1) leading to a 1,5-dioxepin ring, and the structure for 9-methoxystrobilurin K as **1a**. The 1,5-dioxepin structure is supported by an NOE between the methine proton H18 (δ_H 3.68) and the Me21 protons (δ_H 1.41) as well as the Me25 and Me26 protons (δ_H 1.31). These NOEs had been noted previously.[1]

Subsequent examination of the literature revealed the ^1H and ^{13}C NMR chemical shift data obtained for **1a** ($CDCl_3$ and CD_3OD) were indistinguishable from those published previously for 9-methoxystrobilurin K **2c** ($CDCl_3$)[1] and 9-methoxystrobilurin L **3** (CD_3OD).[2] The **only** significant difference from the reported NMR data[1] was our observation of an HMBC correlation from H18 (δ_H 3.68) to C22 (δ_C 76.1), which linked the 3-methylbut-1-ene unit by an ether bond to C18 of a dioxepin ring, a system not present in either **2c**[1] or **3**.[2] This same correlation was observed in several HMBC experiments (obtained in $CDCl_3$) using different optimisations for the long-range coupling constants (J_{nxh} 2, 4, and 8.3 Hz), but was most obvious when J_{nxh}

was small. When the HMBC spectra of **1a** were subsequently obtained in CD_3OD, correlations from H18 were less obvious due to the t_1 noise ridge of the overlapping methoxyl signal for 16Me.

To support the NMR based assignment of **1a** two key model compounds was synthesised.[6] Monoalkylation of 2-hydroxyphenol with 1-bromo-3-methylbut-2-ene gave **5**, which displayed ^1H and ^{13}C NMR data consistent with that published for the relevant fragment of strobilurin F **4**.[7] This result provided validation for this system as an appropriate model for comparison with the corresponding fragment in the natural products. Alkylation of **5** with 3-chloro-3-methylbutyne, followed by partial reduction of the alkyne and regioselective epoxidation gave **6**,[8] corresponding to the isoprenoid fragment published for 9-methoxystrobilurin K **2c**.



The ^1H and ^{13}C NMR chemical shift data[9] for **6** were inconsistent with those published for the natural product (**2c**).[1] In particular, the chemical shifts of the epoxide carbons were δ_C 13.7 and 23.4 ppm respectively upfield compared to those of the natural product (C18 and C19 in **2c**). The NOEs observed for **6** were also inconsistent with those reported.[1] Molecular modeling[10] of **6** showed that in the preferred conformations of the isoprenyl groups, they were sufficiently remote from each other that no NOE interaction could be expected, and was in fact not observed, for the epoxide methine proton (δ_H 3.17) and the methyl protons (δ_H 1.44 ppm) of the 1,1-dimethyl-2-propenyloxy group in **6**. This interaction had been reported for the natural product (**2c**), and can now be accounted for in the revised structure **1a**. The differences in the NOEs observed in the model compound, along with the unacceptably large chemical shift differences for supposed epoxide carbons, and our own observation of an HMBC correlation (H18/C22) linking the two isoprenyl units lead to the conclusion that 9-methoxystrobilurin K could **not** have the epoxyprenyl structure (**2c**) previously assigned,[1] and should be reassigned as **1a.**

The ^1H and ^{13}C NMR data (CD_3OD) published for 9-methoxystrobilurin L **3**[2] were also indistinguishable from those obtained for 9-methoxystrobilurin K **1a** (in CD_3OD). However, the dioxan structure proposed for **3** does not contain the ether linkage between C18 and C22, and the observed HMBC correlation between H18 and C22 would not be possible. It was possibly because this HMBC correlation was not observed that the dioxan structure was originally proposed.[2] As noted above, this correlation would have been difficult to observe in CD_3OD solution. Furthermore, when the ^1H and ^{13}C NMR data (CD_3OD) for a dioxan model compound with a dimethylcarbinol side chain[7] were compared with those assigned to 9-methoxystrobilurin L **3** significant differences were observed in chemical shifts for C17 and C18 ($\Delta\delta$ 6.3 and 2.7, respectively). On the basis of these data we suggest that the structure of 9-methoxystrobilurin L **3** should also be reassigned as 9-methoxystrobilurin K **1a.**

Strobilurin D **2a**[11] and hydroxystrobilurin D **2b**[12] had also been assigned as containing the epoxyprenyl structure. As noted already for 9-methoxystrobilurin K **2c**, the chemical shifts assigned as epoxide carbons to C18 and C19 in **2a** and **2b** (δ_C 83.2/81.6; 81.9/80.7 respectively) are inappropriate, but are totally in keeping with C18/19 in a 1,5-dioxepin structure such as **1a** (δ_C 75.2/81.6) after allowance is made for the upfield shift effect by C25 and C26 on C18 in **1a**. This suggests that strobilurin D and hydroxystrobilurin D may also have to be reassigned as 1,5-dioxepin structures (eg **1b** (previously reported as strobilurin G)[7] and **1c**).[13] To confirm these and other structures assigned, or postulated in the strobilurin series, model compounds covering all possible combinations in the epoxyprenyl and 1,5 dioxepin series are being prepared.

Biological testing of 9-methoxystrobilurin K **1a** showed it to be significantly cytotoxic (IC_{50} 0.5 ng/mL) against the P388 cell line and antifungal against *Candida albicans* (10 mm), *Trichophyton mentagrophytes* (5 mm), and *Cladosporium resinae* (5 mm), at a concentration of 10 µg/disk, in a zone of inhibition assay. No antibacterial activity was observed, which is consistent with other strobilurins.[3]

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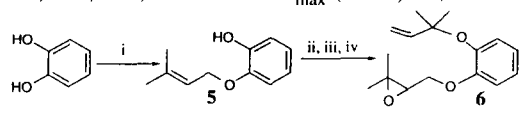
5. ^{1}H NMR ($CDCl_3$) δ 7.38 (1H, s, H12), 6.93 (2H, m, H1, H5), 6.81 (1H, d, *J* = 8.8 Hz, H4), 6.57 (1H, d, *J* = 15.8 Hz, H7), 6.36 (1H, d, *J* = 15.8 Hz, H8), 5.87 (1H, dd, *J* = 10.8, 17.5 Hz, H23), 5.16 (1H, d, *J* = 17.6 Hz, H24b), 5.14 (1H, d, *J* = 10.7 Hz, H24a), 4.20 (2H, dd, *J* = 3.3, 12.3 Hz, H17b), 3.97 (2H, dd, *J* = 7.7, 12.4 Hz, H17a), 3.80 (3H, s, H15), 3.70 (3H, s, H16), 3.68 (1H, m, H18), 3.64 (3H, s, H27), 1.88 (3H, s, H14), 1.41 (3H, s, H21), 1.31 (6H, s, H25,H26), 1.22 (3H, s, H20) ^{13}C NMR ($CDCl_3$) δ 169.0 (C13), 159.7 (C12), 152.4 (C9), 150.6 (C3), 146.5 (C2), 143.6 (C23), 132.7 (C6), 126.8 (C7), 122.4 (C1), 121.6 (C5), 120.9 (C8), 120.1 (C4), 118.0 (C10), 114.5 (C24), 110.4 (C11), 81.6 (C19), 76.1 (C22), 75.2 (C18), 71.6 (C17), 61.6 (C15), 59.4 (C27), 51.3 (C16), 28.1 (C21), 26.1 (C25,C26), 21.6 (C20), 18.0 (C14) IR (KBr) 2932, 2360, 1708, 1503, 1266, 1124, 1067 cm^{-1} UV λ_{max} (MeOH) 310, 232.

6. 

i 1-bromo-3-methylbut-2-ene, K_2CO_3, DMF
ii K_2CO_3, KI, 18-crown-6, 3-chloro-3-methylbutyne, acetone
iii H_2, 5%Pd/$BaSO_4$, quinoline, EtOAc
iv *m*-CPBA, CH_2Cl_2

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8. Each compound in the series **5** through to **6** was fully characterised by MS and NMR.
9. ^{1}H & ^{13}C NMR data for **1a-b**, **2a-c**, **3** and the synthetic model compound **6**.

H	**1a** $CDCl_3$ (CD_3OD)	**1b**[7] $CDCl_3$	**2a**[11] CD_3OD	**2b**[12] $CDCl_3$	**2c**[1] $CDCl_3$	**3**[2] CD_3OD	**6** $CDCl_3$
17a	3.97 (3.99)	3.95	4.00	3.98	3.98	4.00	4.09
17b	4.20 (4.20)	4.23	4.25	4.24	4.22	4.20	4.12
18	3.68 (3.70)	3.50	3.53	3.51	3.68	3.70	3.17
20	1.22 (1.22)	1.21	1.26	1.23	1.43	1.21	1.33
21	1.41 (1.38)	1.47	1.43	1.47	1.23	1.39	1.37
22a		4.15	4.12	4.06			
22b		4.06	4.22	4.16			
23	5.87 (5.93)	5.34	5.40	5.35	5.88	5.90	6.16
24a	5.14 (5.17)				5.15	5.19	5.07
24b	5.16 (5.23)				5.18	5.22	5.14
25	1.31 (1.32)	1.76	1.75	1.69	1.32	1.32	1.44
26	1.31 (1.32)	1.69	1.81	1.76	1.32	1.32	1.44

C	**1a** $CDCl_3$ (CD_3OD)	**1b**[7] $CDCl_3$	**2a**[11] CD_3OD	**2b**[12] $CDCl_3$	**2c**[1] $CDCl_3$	**3**[2] CD_3OD	**6** $CDCl_3$
17	71.6 (73.0)	68.7	69.4	67.3	71.8	72.8	68.1
18	75.2 (77.2)	81.9	83.2	81.9	75.5	77.7	61.5
19	81.6 (82.9)	80.6	81.6	80.7	81.7	82.9	58.2
20	21.6 (22.7)	20.8	22.0	20.8	21.8	22.6	18.9
21	28.1 (28.3)	27.7	27.6	27.7	28.1	28.2	24.6
22	76.1 (77.6)	67.3	67.9	66.7	76.1	77.4	80.9
23	143.6 (145.4)	120.9	122.1	124.7	143.6	145.3	144.1
24	114.5 (115.0)	137.5	135.2	133.0	114.4	114.9	115.2
25	26.1 (27.2)	25.8	18.1	18.0	26.3	26.4	26.5
26	26.1 (26.9)	16.1	25.9	25.7	26.7	26.8	26.6

10. Molecular modeling used PC Spartan 1.1 (Wavefunction Inc., Irvine, CA) at the AM1 level.
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