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Hesseltin A, a Novel Antiviral Metabolite from *Penicillium hesseltinei*

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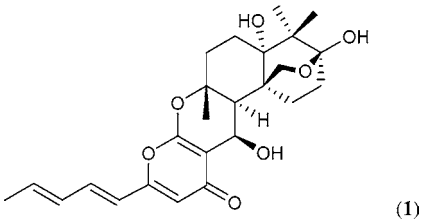
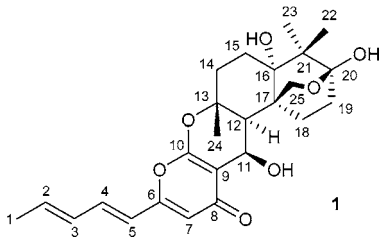
ABSTRACT



(1)

Hesseltin A (1), a novel compound of mixed polyketide-terpenoid origins was isolated from the filamentous fungus *Penicillium hesseltinei*. The structure and stereochemistry were determined from extensive one- and two-dimensional NMR and mass spectral data.

During ongoing investigations of secondary metabolites from filamentous fungi[1,2] of unusual habitats, an extract of a new species, *Penicillium hesseltinei*,[3] isolated from the cheek pouch of a kangaroo rat (*Dipodomys spectabilis*) in New Mexico, USA, has attracted attention. A family of compounds, all with UV spectra previously unseen in any extracts of our comprehensive collection of *Penicillium* species, were detected. These metabolites were isolated and purified by UV-guided fractionation to yield the novel compound hesseltin A (**1**) as the major metabolite.



1

We report here the isolation and structure elucidation of **1**. The structures of the minor analogues will be reported shortly. *P. hesseltinei* (IBT12396)[3] was cultured on full strength YES agar at 25 °C for 14 days and then extracted with ethyl acetate. The agar plate extract (988 mg) was chromatographed on flash reverse-phase (rp) columns using a sharp, stepped gradient from water through methanol. The fraction that eluted with 75% methanol was purified on two Waters Prep Nova-Pak Porasil cartridges (25 × 100 mm, 6 μm, 60 Å) connected in series, using 20 mL/min H_2O-CH_3CN (starting at 70:30, increasing to 50:50 over 20 min) as the mobile phase to yield **1** (5.2 mg).

Hesseltin A (**1**) was obtained as a pale yellow powder. The UV spectrum of **1** displayed absorption maxima at 234.5 and 308.5 nm, with the fine structure suggesting a polyene chain. The ES positive ion mass spectrum of **1** showed strong

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(1) Larsen, T. O.; Frydenvang, K.; Frisvad, J. C.; Christophersen, C. *J. Nat. Prod.* **1998**, *61*, 1154−1157.

(2) Larsen, T. O.; Franzyk, H.; Jensen, S. R. *J. Nat. Prod.* **1999**, *62*, 1578−1580.

(3) Culture was determined by Prof. Jens C. Frisvad to be a previously undescribed species and was assigned the name *Penicillium hesseltinei* (#56A = IBT 12396) and placed in *Penicillium* subgenus *Penicillium* section *Viridicata* series *Viridicata*. Subcultures have been deposited at the IBT culture collection, BioCentrum-DTU, Technical University of Denmark, Kgs. Lyngby, Denmark. The strain of *P. hesseltinei* was originally isolated from a cheek pouch of a live trapped kangaroo rat (*Dipodomys spectabilis*) in Seviletta National Wildlife Refuge, Socorro County, New Mexico, USA, in March 1988, by Lauraine Hawkins (#56A). The combination of penicillic acid, terrestric acid, cyclopeptin, dehydrocyclopeptin, cyclopenol, cyclopenin, viridicatol produced by *P. hesseltinei* sets this species apart from any other species in series *Viridicata* (Frisvad, J. C.; Samson, R. A., *Stud. Mycol. (Utrecht)* **2004**, *49*, 1−173).

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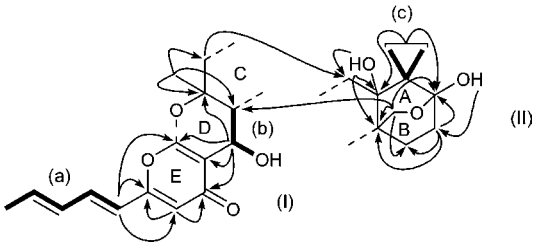


Figure 1. Important HMBC correlations (from ^{1}H to ^{13}C) for fragments **I** and **II** of hesseltin A in DMSO-d_6.

M + H$^+$ and M + CH$_3$CN + Na$^+$ peaks at *m*/*z* 445 and 518, respectively. High-resolution mass measurement on the [M + H]$^+$ (*m*/*z* 445.2254) in the ESI mass spectrum, in combination with ^{1}H and ^{13}C NMR data,[4] supported a molecular formula $C_{25}H_{32}O_7$ (10 double-bond equivalents).

The ^{1}H NMR spectrum of **1**[4] showed three exchangeable protons OH11, OH16, and OH20, seven methines (five of which were vinylic), five methylenes (one heteroatom-substituted), and four methyl signals. The ^{13}C NMR experiment[4] identified 25 carbon signals, comprising four CH_3, five CH_2, six CH, and 10 quaternary carbon signals. One carbonyl (C8) was observed and several other carbons, C2, C3, C4, C5, C6, C7, C9, and C10, resonated in the vinylic/aromatic region with C6 and C10 oxygenated. Five mid-range resonances C11, C13, C16, C20, and C25 were assigned as oxygenated carbons, with C20 assigned as an anomeric carbon. Four methyl signals C1, C22, C23, and C24 were observed, and so were four aliphatic methylene resonances, C14, C15, C18, and C19.

A series of COSY, TOCSY, and HSQC experiments established three partial connectivities, defining the spin systems **a**−**c** (Figure 1 in bold). A further five methylene groups were also identified, but further connectivities between four of these groups were unclear due to signal overlap in the aliphatic region. The assignments of the remaining signals were made from a detailed analysis of HMBC experimental data.

Protons H4 and H5 correlated to an oxygenated vinyl carbon, C6, with H5 also correlating to C7. H7 showed correlations to C8 and a high-field vinyl carbon C9. H11 correlated to C8, C9, and C10, all part of the vinylic polyene system. A weak, but definite $^4J_{CH}$ correlation from H5 was also seen to C10 to close ring E (Figure 1). Correlations to C13 were seen from H11, H14b, and H24, with the chemical shift of C13 indicating oxygenation at a constrained center. H24 also correlated to C12 and C14, placing C24 on C13 to give fragment I (Figure 1). The HMBC correlations for those protons resonating around 2.00 ppm were very complex due to signal overlap, and therefore only limited information about the HMBC correlations were able to be determined. The methyl protons H22 and H23 both correlated to C16, C20, and C21, as well as each other. It has already been established from long-range coupling in the COSY spectrum that the two methyl groups were attached through a quaternary carbon, which was assigned as C21. The hydroxyl proton OH16 correlated to C15, C16, C17, and C21, and the hydroxyl proton OH20 correlated to C19, C20, and C21. H19b, an isolated aliphatic proton, correlated to C17, another aliphatic carbon C18, and with correlations from H25a and H25b to C16, C17, C18, and C20 closed rings A and B (Figure 1). The final correlations from H25a and H25b to C12, and a second isolated aliphatic proton, H14b, to C15 linked fragments **I** and **II** together, creating ring C. This accounted for all but one degree of unsaturation required by the molecular formula. A link from the oxygen on C13 was made to the only remaining available site, C10, to complete the degrees of unsaturation allowing the assignment of hesseltin A as **1**.

NOESY experiments[4] on **1** enabled the relative stereochemistry to be determined. There are six stereogenic centers in hesseltin A: C11, C12, C13, C16, C17, and C20. The H22 methyl protons correlated to H25a, placing these protons on the same side. C18 and C19 must therefore be on the opposite side to assign C17 as R*. The carbons C17 and C20 are bridgehead atoms of a bicyclo[2,2,2]octane, consequently the configuration at C20 is locked to C17 and was assigned as *S**. Correlations from OH11 and H24 to H25a placed them on the same side as H25a, assigning C11 and C13 as *R** and *S** respectively. Correlations from H23 to OH16 assigned C16 as *R**.

No correlations were observed from H12 to H24 or H25a and b, but a correlation was seen to H11, indicating that C12 was in the *S** configuration. Important NOESY correlations and the w-coupling are shown in Figure 2. This configuration also enables the w-coupling seen between H25b and H18b to occur. On the basis of the large coupling constants for H2 and H4, the vinyl bonds in the diene side chain were both assigned (*E*).

The recently described compounds decaturin A (**2**) and B (**3**)[5] contain sesquiterpene moieties very similar to that observed in **1**. Only minor differences in the ^{13}C NMR data for the bicyclo[2,2,2]octane moiety were observed between **1**[4] and **2**.[6] These differences were attributed to the use of

(4) **1** was obtained as a pale yellow powder: $[\alpha]^{20}_D$ +82.7° (*c* 0.52, MeOH); UV (MeOH) λ_{max} (log ϵ) 234.5 (4.06), 308.5 (4.21); HRESMS 445.2254 (M + H$^+$) (calcd for $C_{25}H_{33}O_7$, 445.2226); ^{1}H NMR (DMSO-d_6, 800 MHz) δ 6.92 (1H, dd, 10.6, 15.5, H4), 6.25 (1H, dd, 10.6, 14.3, H3), 6.22 (1H, d, 15.5, H5), 6.16 (1H, dq, 14.3, 6.7, H2), 6.10 (1H, s, H7), 5.40 (1H, s, OH20), 5.00 (1H, d, 4.8, OH11), 4.76 (1H, dd, 4.3, 4.8, H11), 4.57 (1H, d, 9.7, H25a), 4.21 (1H, s, OH16), 3.87 (1H, dd, 2.1, 9.7, H25b), 2.06 (1H, m, H14a), 2.03 (1H, m, H15a), 2.00 (1H, d, 4.3, H12), 2.00 (1H, m, H18a), 1.99 (1H, m, H19a), 1.81 (3H, d, 6.7, H1), 1.70 (1H, m, H14b), 1.63 (2H, m, H15b, H18b), 1.56 (1H, m, H19b), 1.52 (3H, s, H24), 0.96 (3H, s, H23), 0.88 (3H, s, H22); ^{13}C NMR (DMSO-d_6, 150.9 and 201.1 MHz) δ 178.2 (C8), 161.6 (C10), 157.6 (C6), 136.7 (C2), 135.1 (C4), 130.4 (C3), 120.0 (C5), 111.2 (C7), 102.5 (C9), 96.9 (C20), 85.2 (C13), 73.6 (C16), 66.5 (C25), 57.6 (C11), 46.5 (C12), 46.2 (C21), 41.7 (C17), 35.0 (C14), 28.9 (C19), 27.3 (C18), 26.8 (C15), 21.5 (C24), 21.1 (C22), 20.2 (C23), 18.4 (C1). NOE correlations: H1/H3, H2/H4, H3/H5, H5/H7, H11/H12, H11/H18b, H25a/H18a, OH11/H25a, H24/H25b, H25b/H22, H24/H14b, H14a/OH16, H22/OH20, H23/OH16, H22/H15a, H25b/H15a, H19a/OH20, H19b/OH20.

(5) Zhang, Y.; Li, C.; Swenson, D. C.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Org Lett.* **2003**, *5*, 773−776.

(6) $[\alpha]_D$ +32° (*c* 0.2, CH_2Cl_2); ^{13}C NMR data ($CDCl_3$, 100 MHz) for the bicyclo[2,2,2]octane moiety of (**2**) (atoms numbered as in **1** for comparison) δ 98.1 (C20), 75.0 (C16), 67.7 (C25), 46.3 (C21), 39.6 (C17), 29.4 (C19), 28.7 (C18), 20.6 (C22), 19.2 (C23).

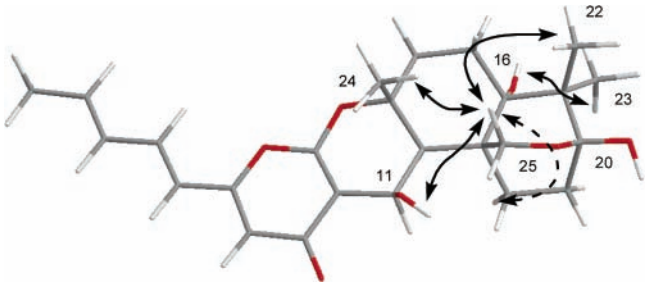
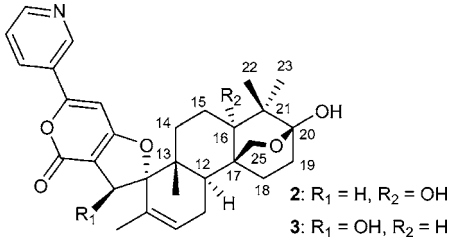


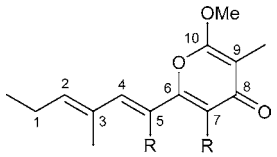
Figure 2. Three-dimensional energy-minimized model (MM2) showing key NOESY correlations (−) and w-coupling (- -) for hesseltin A in DMSO-d_6.

different solvents for NMR data acquisition, as well as differences in the remainder of the molecule.



The absolute stereochemistry of **1** was inferred from the $[\alpha]_D$ values for **1**,[4] **2**,[6] and **3**[7] with strong positive $[\alpha]_D$ values seen for each compound. The absolute stereochemistry of **3** was determined by X-ray crystallography.[5] Compounds **1**−**3** all have stereogenic centers at C12, C13, C16, C17, C20, and C21. If one of the sesquiterpene moieties of **1**, **2**, or **3** was of opposing absolute stereochemistry to the other two compounds, a strong negative $[\alpha]_D$ value is to be expected. Compound **1** was therefore assigned as (11*R*,12*S*,13*R*,16*S*,-17*R*,20*S*)-hesseltin A.

Compound **1** is a unique mixed sesquiterpene pentaketide metabolite containing a very unusual chromophoric structure. Propionate-derived metabolites with a similar chromophoric group have previously been found in marine ascoglossans, *Cyerce* species.[8−10] The ^{13}C NMR data for cyercene A (**4**)[9] and cyercene B (**5**)[9] are a very good match to the NMR data of **1**,[4] with the slight differences attributed to the presence of the extra methyl groups. The UV spectra for **1**, however, varied significantly from that reported for **4**[11] and **5**.[12] It has previously been noted that more methyl groups on the diene side chain lowered the observed λ_{max}.[9] The methyl groups were preventing the molecule from assuming a planar conformation. The lack of methyl groups in **1** allows the molecule to assume a planar conformation, resulting in a higher observed λ_{max}.



4: R = CH_3

5: R = H

Biological studies on compounds **2**−**5** have shown a range of different activities. The cyercenes (**4** and **5**) have indicated a potent ichthyotoxic effect, with cyercene A (**2**) also showing regenerative properties,[9] whereas decaturin A (**2**) shows antiinsectant activity against the fall armyworm (*Spodoptera frugiperda*).[5]

Compound **1** exhibited antiviral activity toward HSV-1. A 25−50% inhibition of viral growth was observed when tested at 300 μg, but more significantly **1** did not show any cytotoxicity. Work on establishing the structures of several analogues of hesseltin A is in progress.

Acknowledgment. We thank Lauraine Hawkins for the *Penicillium* strain. The support of The Danish Research Councils (Grant 9901295) and the Center for Advanced Food Studies are acknowledged. The 800 MHz spectra were obtained using the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules.

Supporting Information Available: Detailed description of experimental procedures and ^{1}H, ^{13}C, and HMBC NMR spectra for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) $[\alpha]_D$ +124° (*c* 0.2, CH_2Cl_2).

(8) Roussis, V.; Pawlik, J. R.; Hay, M. E.; Fenical, W. *Experientia* **1990**, *46*, 327−329.

(9) Vardaro, R. R.; Di Marzo, V.; Crispino, A.; Cimino, G. *Tetrahedron* **1991**, *47* (29), 5569−5576.

(10) Vardaro, R. R.; Di Marzo, V.; Marin, A.; Cimino, G. *Tetrahedron* **1992**, *48* (43), 9561−9566.

(11) **4** UV λ_{max} (log ϵ) 264 (4.14); ^{13}C NMR ($CDCl_3$, 125.8 MHz, atoms numbered as in **1** for comparison) δ 181.5 (C8), 161.9 (C10), 159.1 (C6), 135.8 (C2), 139.4 (C4), 130.9 (C3), 125.5 (C5), 117.7 (C7), 99.3 (C9), 21.6 (C1).

(12) **5** UV λ_{max} (log ϵ) 239 (4.08), 302 (4.06); ^{13}C NMR ($CDCl_3$, 125.8 MHz, atoms numbered as in **1** for comparison) δ 181.0 (C8), 162.3 (C10), 157.2 (C6), 141.4 (C2), 139.5 (C4), 132.4 (C3), 116.5 (C5), 111.4 (C7), 102.5 (C9), 22.1 (C1).