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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 1341–1344

An anti-herpes simplex virus-type 1 agent from *Xylaria mellisii* (BCC 1005)

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Received 30 October 2004; revised 16 December 2004; accepted 21 December 2004
Available online 12 January 2005

Abstract—A structurally unique polyketide, mellisol (**1**) and 1,8-dihydroxynaphthol 1-*O*-α-glucopyranoside (**3**), were isolated from the fungus *Xylaria mellisii* (BCC 1005). The relative stereostructure of **1** was determined on the basis of X-ray crystallographic data. Compounds **1** and **3** exhibited activity against herpes simplex virus-type 1 with IC_{50} values of 10.50 and 8.40 μg/mL, respectively. They also showed cytotoxic activity against vero cells at the concentration of 40–50 μg/mL.

Fungi of the genus *Xylaria* have been shown to be potential sources of novel secondary metabolites and many of these possess very useful biological activities.[1–4] For example, the antifungal xylarin and multiplolides were isolated from *X. longipes*[2] and *X. multiplex*, respectively,[3] and acetylcholine esterase inhibitors, xyloketals A–E, from *Xylaria* sp. collected from the South China Sea coast.[4] The structural diversity of chemical constituents within the genus *Xylaria* should also be noted[5–7] and in many cases, due to the difficulty and confusion regarding the identity of *Xylaria* species, these metabolite mixtures were used as markers for their taxonomic identification.[7] Herein we report another example of a novel metabolite from *Xylaria mellisii*.

As part of our on-going search for bioactive substances from microorganisms[8,9] we noted that the crude extract of *X. mellisii* showed activity against herpes simplex virus-type I, therefore further investigation was persued to identify the active ingredient(s). *X. mellisii* (BCC 1005) was collected from Kaeng Krachan National Park, Phetchaburi, and identified by Dr. S. Thienhirun. The organism was deposited at the BIOTEC Culture Collection (BCC), Thailand (registration no. BCC 1005). *X. mellisii* BCC 1005 was cultured on potato dextrose broth and, after 25 days, transferred into a malt extract media (1:10 v/v) containing an additional 6% glucose. After cultivation under stationary conditions for eight weeks the mycelium was removed by filtration and the medium was extracted with EtOAc to yield a semi-solid gum (2.9 g). The crude product was purified initially by chromatography using silica gel, followed by Sephadex LH-20 to yield mellisol (1, 56.9 mg), 1,8-dihydroxynaphthol 1-*O*-α-glucopyranoside (3, 0.72 g), (−)-5-carboxymellein (31.5 mg), cytochalasin C (82.9 mg) and cytochalasin D (0.59 g). The chemical and physical properties of (−)-5-carboxymellein, cytochalasins C and D were identical to those published in the literature.[10–13]

Compound **1**[14] was obtained as colourless needles and its HRMS revealed a prominent ion at *m/z* 281 $[M+Na]^+$ corresponding to the molecular formula $C_{12}H_{18}O_6$. The IR spectrum showed absorptions at ν_{max} 3448 (OH) and 1735 cm^{-1} (C=O) while the ^{13}C and DEPT-135 NMR spectral data showed 12 carbons including one methyl, four methylenes, four methines and three quaternary carbons. Five carbons at δ_C 64.3 (C-11), 77.2 (C-10), 79.9 (C-5), 99.3 (C-2) and 104.2 (C-6) suggested their connections to oxygens, two of which (δ_C 79.9

Keywords: *Xylaria mellisii*; Anti-HSV-1; Mellisol; 1,8-Dihydroxynaphthol 1-*O*-α-glucopyranoside.
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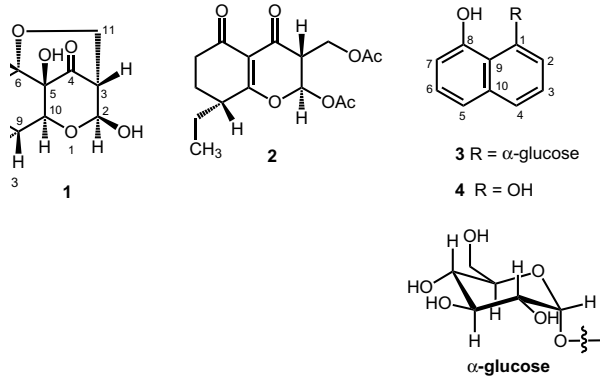
doi:10.1016/j.tetlet.2004.12.110

and 104.2) were quaternary carbons. The HMQC experiment showed respective correlations C-11, C-10 and C-2 to the non-equivalent methylene protons at δ_H 4.29 (H_a-11) and 4.57 (H_b-11) and the two methine protons at δ_H 4.76 (H-10) and 6.03 (H-2). The ^1H–^1H COSY spectrum not only indicated the connectivity of the non-equivalent methylene protons at C-11 to the chiral proton at δ_H 3.10 (H-3) but it also revealed correlations for H-2 to H-3; H-7 to H-8; H-8 to H-7 and H-9; H-9 to H-8 and H-10; H-12 to H-9 and H-13. The HMBC spectrum showed cross peaks from H-2 to C-4, C-10 and C-11; H-3 to C-2, C-4 and C-5; H-7 to C-6 and C-8; H-8 to C-9; H-9 to C5; H-10 to C-4, C-5, C-6, C-8 and C-9; H-11 to C-2, C-4 and C-6; H-12 to C-9 and finally H-13 to C-9 and C-12. The NOESY spectrum indicated spatial correlation from H-13 to H-2, H_a-11 to H-2 and the hydroxyl proton at δ_H 4.97 also correlates to two hydroxyls at δ_H 6.90 and 8.20. The latter indicated that the three hydroxyl groups share the same space.

The physical information described above together with the data obtained from X-ray crystallography (Fig. 1) established the structure of mellisol.

Acetylation of compound **1** with acetic anhydride and pyridine gave a yellow oil **2**[15] (ν_{max} at 1766 and 1742 cm^{-1}; C=O), which showed two additional methyl groups, at δ_H 2.12 and 2.32, in its ^1H NMR spectrum. The H_b-proton of the C-11 methylene moiety (originally resonated at δ_H 4.57 (H_b-11) in **1**) shifted to δ_H 5.03 and the H-2 methine proton (at δ_H 6.03 in **1**) was observed at δ_H 6.57 in **2**. The absence of the original carbonyl carbon at δ_C 207.2 and the presence of two new carbonyl absorptions at δ_C 173.6 and 181.9 in compound **2** pointed to ring opening of the ketal group. Also the absence of the methine proton (originally at δ_H 4.76, H-10 in **1**) together with the appearance of absorptions at δ_C 133.0 and 164.6 pointed to the formation of a double bond as part of an α,β-unsaturated carbonyl moiety. This was confirmed by the IR absorption at ν_{max} 1686 cm^{-1}. 1D and 2D NMR spectral data fully supported the proposed structure of the diacetyl compound **2**.

Compound **3**[16] was obtained as a brown solid. HRMS revealed the molecular formula to be $C_{16}H_{18}O_7$ where the mass ion was observed at *m/z* 321 $[M-H]^-$. The IR spectrum showed a broad stretch at ν_{max} 3386 cm^{-1} (OH) and absorptions at ν_{max} 1629, 1607 and 1587 cm^{-1} indicated the existence of an aromatic component. The ^1H and ^{13}C NMR spectra indicated the presence of a glucose moiety with characteristic absorptions observed at δ_H 3.20–5.50 and δ_C 60.8–101.2. The four hydroxyl groups of the sugar unit (δ_H 4.70, 5.10, 5.27, 6.09) and the OH at δ_H 9.66 disappeared after D_2O addition. Six protons resonated in the aromatic region at δ_H 6.78–7.53 ppm and 10 carbons situated in the range of δ_C 110–160 ppm could be assigned to a 1,8-disubstituted naphthalene moiety where two quaternary carbons attached to oxygens resonated at δ_C 153.6 and 154.4. The HMBC spectrum indicated that the anomeric proton of the glucose moiety (at δ_H 5.50) correlated to the carbon at 154.4, also the coupling constant of 3.46 Hz for this proton suggested that the naphthol moiety was attached to the glucose unit at the α-position.[17] All evidence, including detailed ^1H–^1H COSY and HMQC experiments on compound **3** and its acid hydrolysis products, from which product **4** together with D-glucose were identified, indicated that compound **3** was 1,8-dihydroxynaphthol 1-*O*-α-glucopyranoside. Compound **3** is synthetically known[17] and a recent report described the isolation of 1,8-dihydroxynaphthalene monoglucoside from *Sclerotinia sclerotiorum*.[18] However, the reported physical data are different from that of compound **3** obtained in this study, which suggests that they are not the same compound, presumably due to the stereochemical difference at the linkage between the glucose unit and the naphthol moiety in these two compounds.



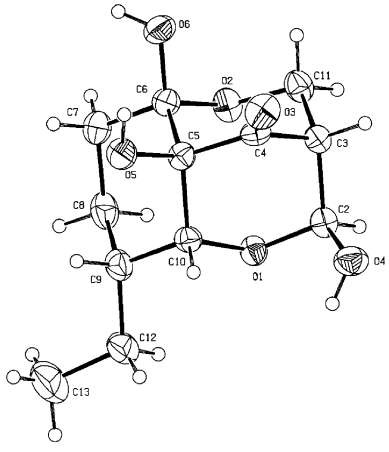


Figure 1. X-ray structure of **1**.

Antiviral activity was evaluated against the herpes simplex virus-type 1 (ATCC VR-260) employing a modified plaque reduction assay[19] and the colorimetric method described by Skehan et al.[20] Under the screening conditions, the reference compound, acyclovir, exhibited an antiviral HSV-1 activity with IC_{50} of 2–5 μg/mL. The cytotoxicity assay against BCA (human breast cancer cells), KB (human epidermoid carcinoma of the mouth; ATCC CCL-17) and the vero cell line was performed employing the colorimetric method using ellipticine as reference (IC_{50} of 0.1–0.4 μg/mL for both BCA and KB cells and 0.4–0.9 μg/mL for vero cells).

Compounds **1** and **3** exhibited anti-HSV-1 activity with IC_{50}'s of 10.5 and 8.4 μg/mL, respectively, and displayed

cytotoxicity against the vero cell line at concentrations of 39.4 and 45.8 μg/mL. However, both were inactive against BCA and KB cell lines at a concentration of 20 μg/mL. Cytochalasins C, D and (−)-5-carboxymellein showed no activity against the HSV-1 virus. (−)-5-Carboxymellein was earlier reported to be the antimalarial principle from the marine fungus *Halorosellinia oceanica*.[9]

The polyketide mellisol represents a new structural entity. The isolation of mellisol also demonstrates the wide diversity of compounds produced by the genus *Xylaria*.

Acknowledgements

We are grateful for the financial support from the Biodiversity Research and Training Program (BRT). We also acknowledge the help of BIOTEC's fermentation technology and biological activity screening laboratories for large-scale fermentation and bioactivity screening. S.P. thanks the Postgraduate Education and Research Program in Chemistry for his scholarship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.12.110.

References and notes

1. Schneider, G.; Anke, H.; Sterner, O. *Nat. Prod. Lett.* **1995**, *7*, 309–316.
2. Schneider, G.; Anke, H.; Sterner, O. *Z. Naturforsch.* **1996**, *51c*, 802–806.
3. Boonphong, S.; Kittakoop, P.; Pittayakhajonwut, D.; Tatichareon, M.; Thebtaranonth, Y. *J. Nat. Prod.* **2001**, *64*, 965–967.
4. Lin, Y.; Wu, X.; Feng, S.; Jiang, G.; Luo, J.; Zhou, S.; Vrijmoed, L. L. P.; Jones, E. B. G.; Krohn, K.; Steingrover, K.; Zsila, F. *J. Org. Chem.* **2001**, *66*, 6252–6256.
5. Edwards, R. L.; Maitland, D. J.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. 1* **1991**, 1411–1417.
6. Dagne, E.; Gunatilaka, A. A. L.; Asmellash, S.; Abate, D.; Kingston, D. G. I.; Hofmann, G. A.; Johnson, R. K. *Tetrahedron* **1994**, *50*, 5615–5620.
7. Edwards, R. L.; Maitland, D. J.; Pittayakhajonwut, P.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. 1* **2001**, 1296–1299.
8. Isaka, M.; Jaturapat, A.; Kladwang, W.; Punya, J.; Lertwerawat, Y.; Tanticharoen, M.; Thebtaranonth, Y. *Planta Med.* **2000**, *66*, 473–475.
9. Chinworrungsee, M.; Kittakoop, P.; Isaka, M.; Rungrod, A.; Tanticharoen, M.; Thebtaranonth, Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1965–1970.
10. Anderson, J. R.; Edwards, R. L.; Whalley, A. J. S. *J. Chem. Soc., Perkin Trans. 1* **1983**, 2185–2192.
11. Claydon, N.; Grove, J. F.; Pople, M. *Phytochemistry* **1985**, *24*, 937–943.
12. Okuno, T.; Oikawa, S.; Goto, T.; Sawai, K.; Shirahama, H.; Matsumoto, T. *Agric. Biol. Chem.* **1986**, *50*, 997–1001.
13. Merifield, E.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1999**, 3269–3283.
14. Compound **1** (mellisol) was obtained as colourless needles (acetone/hexane); mp 125–127 °C; $[\alpha]_D^{28}$ +141.91 (*c* 0.27, EtOH); UV (EtOH) λ_{max} (log ε) 205 (3.49), 267 (2.65) nm; IR (KBr) ν_{max} 3448 (br), 2961, 2932, 2905, 1735, 1467 (w), 1459 (w), 1389, 1341, 1273, 1211,1198, 1153, 1123, 1092, 993, 966, 935, 916, 857, 812, 702 cm^{-1}; ^1H NMR (C_5D_5N, 500 MHz): δ 0.82 (3H, td, *J* = 7.43, 1.53 Hz, H-13), 1.42 (1H, quint, *J* = 7.43 Hz, H_a-12), 1.56–1.63 (2H, m, H_a-8 and H_b-12), 2.11 (1H, dt, *J* = 12.95, 9.72, 1.24 Hz, H_a-7), 2.15–2.20 (1H, m, H-9), 2.32 (1H, ddt, *J* = 12.60, 3.60, ≪1 Hz, H_b-8), 2.46 (1H, ddd, *J* = 12.95, 9.72, 1.24 Hz, H_b-7), 3.10 (1H, d, *J* = 1.19 Hz, H-3), 4.29 (1H, d, *J* = 11.05 Hz, H_a-11), 4.57 (1H, dd, *J* = 11.05, 1.19 Hz, H_b-11), 4.76 (1H, s, H-10), 4.97 (1H, s, OH), 6.03 (1H, s, H-2), 6.90 (1H, s, OH), 8.20 (1H, s, OH); ^{13}C NMR (C_5D_5N, 125 MHz): δ 11.6 (CH_3, C-13), 24.9 (CH_2, C-8), 25.0 (CH_2, C-12), 34.6 (CH_2, C-7), 36.8 (CH, C-9), 56.7 (CH, C-3), 64.3 (CH_2, C-11), 77.2 (CH, C-10), 79.9 (C, C-5), 99.3 (CH, C-2), 104.2 (C, C-6), 207.2 (C, C-4); HRMS: *m/z* calcd for $C_{12}H_{18}O_6Na$ (M+Na): 281.0684; found: 281.0684.
X-ray data for mellisol: X-ray data were collected on a Bruker–Nonius kappaCCD diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). The crystal structure was solved by direct methods using SIR-97, and all atoms except hydrogen atoms were refined anisotropically on F^2 using SHELXL-97 to give a final *R*-factor of 0.0470 and *wR* = 0.1243 (all data). $C_{12}H_{18}O_6$, MW = 258.27, colourless crystal, crystal system: orthorhombic, space group: *P*2(1)2(1)2(1), cell parameters: *a* = 6.0917(1), *b* = 12.7543(2), *c* = 31.5832(8) Å, *V* = 2453.87(8) $Å^3$, *Z* = 8, D_{calcd} = 1.398 Mg/m^3. A total of 2623 unique reflections (2263 observed, $|F_o| > 4\sigma|F_o|$) were measured at room temperature from a $0.25 \times 0.15 \times 0.15$ mm^3 colourless crystal. Atomic coordinates, bond lengths, bond angles and thermal parameters have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, England (CCDC 251036).
15. Purification of the crude product obtained from treatment of **1** with acetic anhydride/pyridine by Sephadex LH-20 using 100% MeOH as an eluent gave mellisol diacetate **2** (2.29 mg) as a yellow oil; UV (EtOH) λ_{max} (log ε) 203 (3.44), 273 (3.58) nm; IR ($CHCl_3$) ν_{max} 2963, 2929, 1766, 1742, 1686, 1623, 1540, 1456, 1373, 1219, 1146, 1036, 962, 935 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 0.88 (3H, t, *J* = 7.48 Hz, H-13), 1.45–1.65 (2H, m, H-12), 1.90–2.05 (1H, m, H_a-8), 2.12 (3H, s, CH_3CO), 2.18–2.23 (1H, m, H_b-8), 2.30–2.40 (2H, m, H_a-7 and H-9), 2.32 (3H, s, CH_3CO), 2.43–2.53 (1H, m, H_b-7), 2.75 (1H, m, H-3), 4.29 (1H, dd, *J* = 11.30, 3.01 Hz, H_a-11), 5.03 (1H, dd, *J* = 11.30, 1.0 Hz, H_b-11), 6.57 (1H, s, H-2); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 11.8 (CH_2, C-13), 20.3 ($COCH_3$), 20.7 ($COCH_3$), 24.9 (CH_2, C-12), 29.6 (CH_2, C-8), 33.1 (CH_2, C-7), 41.1 (CH, C-9), 48.4 (CH, C-3), 60.7 (CH_2, C-11), 92.3 (CH, C-2), 133.0 (C, C-5), 164.6 (C, C-10), 168.3 ($COCH_3$), 168.5 ($COCH_3$),173.6 (C, C-6), 181.9 (C, C-4); HRMS: *m/z* calcd for $C_{16}H_{20}O_7Na$ (M+Na): 347.1107; found 347.1114.
16. Compound **3** was obtained as a brown amorphous (EtOAc); mp 205–207 °C (dec); $[\alpha]_D^{31}$ +127 (*c* 0.32, EtOH); UV (EtOH) λ_{max} (log ε) 226 (4.89), 302 (4.09), 317 (4.04), 332 (4.01) nm; IR (KBr) ν_{max} 3386 (br), 2936, 1629 (w), 1607, 1587, 1397, 1298, 1247, 1110, 1087, 1045, 998, 814, 754 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz): δ 3.20–3.25 (1H, s, H-4′), 3.52–3.60 (4H, m, H-2′, H-3′, H-5′, H-6′), 3.72–3.75 (1H, m, H-6′), 4.70 (1H, t, *J* = 5.79 Hz, OH), 5.10 (1H, d, *J* = 6.14 Hz, OH), 5.27 (1H, d, *J* = 4.61 Hz, OH), 5.50 (1H, d, *J* = 3.46 Hz, H-1′), 6.09 (1H, d,

J = 3.87 Hz, OH), 6.78 (1H, dd, J = 3.39, 5.38 Hz, H-2), 7.33–7.36 (3H, m, H-3, H-4, H-6), 7.44 (1H, dd, J = 7.65, 0.85 Hz, H-7), 7.53 (1H, dd, J = 8.28, 0.85 Hz, H-5), 9.66 (1H, s, OH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 60.8 (CH_2, C-6′), 69.5 (CH, C-4′), 71.0 (CH, C-3′), 73.6 (CH, C-5′), 74.2 (CH, C-2′), 101.2 (CH, C-1′), 110.7 (CH, C-2), 110.9 (CH, C-7), 115.5 (C, C-9), 118.7 (CH, C-4), 122.8 (CH, C-5), 126.3 (CH, C-6), 127.4 (CH, C-3), 136.3 (C, C-10), 153.6 (C, C-8), 154.4 (C, C-1); HRMS: *m/z* calcd for $C_{16}H_{17}O_7$ (M−H): 321.0983; found 321.0984.

17. Cameron, D. W.; Craik, J. C. A. *J. Chem. Soc. (C)* **1968**, 3068–3072.
18. Starratt, A. N.; Ross, L. M.; Lazarovits, G. *Can. J. Microbiol.* **2002**, *48*, 320–325.
19. Abou-Karam, M.; Shier, W. T. *J. Nat. Prod.* **1990**, *53*, 340–344.
20. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.