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Dykellic acid, a novel apoptosis inhibitor from *Westerdykella multispora* F50733

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Abstract

A novel apoptosis inhibitor, dykellic acid (**1**), was isolated from the fermentation broth of *Westerdykella multispora* F50733. The structure of **1** was elucidated by spectral and X-ray crystallographic data. Compound **1** inhibited the etoposide-induced apoptosis of human monocytic leukemia U937 cells.

Apoptosis is an intrinsic cell suicide system that is required for the normal development and homeostasis of organisms.[1] The pivotal role of apoptosis in such diverse pathological processes as tumor growth, the immune response and neurodegeneration suggests that the compounds which modulate this apoptotic process might be promising therapeutic agents. Caspases, a family of cystein proteases, play a critical role in execution of apoptosis and are responsible for many of the biochemical and morphological changes associated with apoptosis.[2] From this point of view, we have employed the etoposide-induced caspase induction assay[3] as a primary screening method for identifying apoptosis modulators in microbial products. As a result of screening, we found a novel fungal metabolite, dykellic acid (**1**) from the culture filtrate of *Westerdykella multispora* F50733, inhibited the etoposide-induced apoptosis in U937 cells. In this report, the structural elucidation and biological activities of **1** are described.

Dykellic acid (**1**)[4] was obtained as white powder and its molecular formula, $C_{14}H_{16}O_4$, was obtained form HRFAB-MS ($[M+H]^+$ *m/z* 249.1131; calcd 249.1127). The IR absorptions of **1** at 2927 and 1712 cm^{-1} indicated C–H bonds and α,β-unsaturated δ-lactone, respectively. The ^{13}C NMR spectrum,[5] HMQC and DEPT experiments revealed the presence of fourteen carbon signals, which were attributed to one methyl, three methylenes, six methines, two quaternary and two carbonyl carbons. The ^1H–^1H COSY data showed the couplings among 1′-H and 2′-H, 1″-H and 2″-H, 2″-H and 3″-H, 3″-H and 4″-H, 4″-H and 5″-H, respectively. The long-range couplings from the protons at 1′-H and 2′-H to a carboxyl carbon at C-3′ showed the presence of a propanoic acid moiety. Correlation of the methylene

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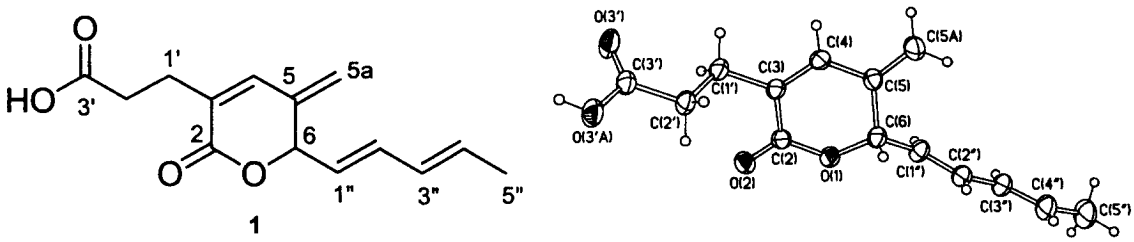

Figure 1. Structure and ORTEP view of dykellic acid (**1**)

5a-H to C-4, C-6 and C-1″, the methine 4-H to C-2, C-1′, C-5a and C-6, the methine 6-H to C-2, C-5, C-5a and C-1″, together with the methine 1″-H to C-5 and C-6 established the planar structure of **1**. The *E* configurations of 1″- and 3″-methine were deduced by ^1H–^1H coupling constants (J=15.0 Hz) and NOESY data. Furthermore, X-ray crystallographic analysis[6] confirmed the above structure and also indicated that compound **1** is racemic. Fig. 1 is an ORTEP drawing of one possible enantiomer of **1**.

Naturally occurring α-pyrones are an interesting group of biologically active natural products widely distributed in both fungi and plants. It is of great biological interest that **1**, a novel α-pyrone, has been isolated for the first time as a new apoptosis inhibitor in leukemia cells. Dykellic acid (**1**) blocked the etoposide-induced apoptotic cell death of human monocytic leukemia U937 cells dose dependently with an IC_{50} value of 4.6 μg/ml.

References

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4. Dykellic acid: mp 128–130°C. $[\alpha]_D^{25}$ +2.0 (*c* 1.0, MeOH). UV λ_{max} nm (ε) in MeOH 229 (30400), 265 (9000). IR (KBr) ν_{max} (cm^{-1}) 2927, 1712, 1382, 1207, 1103, 1024, 991.
5. ^{13}C NMR (125 MHz, CD_3OD): δ 18.2 (C-5″), 27.3 (C-1′), 33.7 (C-2′), 82.4 (C-6), 119.7 (C-5a), 127.4 (C-1″), 130.9 (C-3), 131.4 (C-3″), 133.4 (C-4″), 136.1 (C-2″), 140.2 (C-5), 140.6 (C-4), 167.2 (C-2), 176.4 (C-3′). ^1H NMR (600 MHz, CD_3OD): δ 1.81 (3H, dd, J=6.8, 1.2 Hz, 5″-H), 2.52 (2H, t, J=7.2 Hz, 2′-H), 2.62 (2H, m, 1′-H), 5.32, 5.41 (2H, s, 5a-H), 5.50 (1H, d, J=7.3, 6-H), 5.65 (1H, dd, J=15.0, 7.3 Hz, 1″-H), 5.82 (1H, dq, J=15.0, 6.8 Hz, 4″-H), 6.10 (1H, dd, J=15.0, 10.5 Hz, 3″-H), 6.30 (1H, dd, J=15.0, 10.5 Hz, 2″-H), 7.02 (1H, s, 4-H).
6. Dykellic acid (**1**) was crystallized from MeOH–hexane to afford colorless blocks. The crystals of 0.3×0.5×0.4 mm in size were monoclinic ($P2_1/c$) with lattice parameters a=12.7894(19) Å, b=6.8439(8) Å, c=15.508(2) Å, α=90°, β=91.305(7)0°, γ=90°, Z=4, V=1356.8(3) $Å^3$, Dc=1.215 Mg/m^3, μ=0.089 mm^{-1}. All reflections (θ range: 2.63 to 27.49°) were collected at 18°C with a Siemens P4 X-ray diffractometer. Full-matrix least-squares refinement converged to a final R factor of 0.0505 for the 3103 reflections. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center.