Dankasterone, a new class of cytotoxic steroid produced by a *Gymnascella* species from a marine sponge

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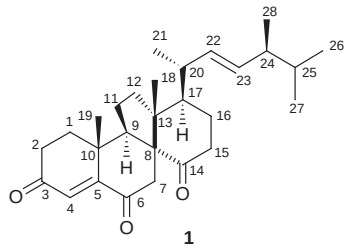
Dankasterone, produced by a strain of *Gymnascella dankaliensis* from the marine sponge *Halichondria japonica*, is a novel class of steroid with significant cytotoxicity against tumour cells in culture.

In our programme devoted to the search for new antitumour metabolites from microorganisms inhabiting the marine environment, we have found a number of antitumour and cytotoxic compounds and elucidated their structures.[1,2] As part of this study, we have previously isolated the cytotoxic gymnastatins[2,3] and gymnasterones[4] from a strain of *Gymnascella dankaliensis* (Castellani) Currah OUPS-N134 originally separated from the sponge *Halichondria japonica*. Further investigation for metabolites of this fungal strain has now led to the isolation of a structurally unique and cytotoxic steroid **1**, designated dankasterone.



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In this experiment, the fungal strain was cultivated in a medium which was prepared by replacement of glucose used for the previous experiment[2–4] by starch. The MeOH extract of the mycelium was purified by bioassay-directed fractionation employing a combination of Sephadex LH-20 and silica gel column chromatography and HPLC to afford dankasterone **1** as prisms.

Dankasterone **1** was assigned the molecular formula of $C_{28}H_{49}O_5$ as deduced from HREIMS. A close inspection of the ^{1}H and ^{13}C NMR spectral data† for **1** from DEPT and ^{1}H–^{13}C COSY experiments revealed the presence of six methyls including four secondary and two tertiary methyls, seven methylenes, five sp^3- hybridised methines, three quaternary sp^3-carbons, one disubstituted and one trisubstituted double bond, and one unconjugated and two conjugated ketones. The ^{1}H–^{1}H COSY analysis for the functional groups led to partial structures A (C-1 and C-2), B (C-9, C-11 and C-12) and C (C-15–C-17 and C-20–C-28), which were supported by HMBC correlations. The geometry of the disubstituted double bond was deduced from the coupling constants ($J_{23,24}$ 15.1 Hz) of the olefinic protons. The connection of the partial structures (A to C) and the remaining functional groups was determined on the basis of HMBC correlations. The typical correlations are as follows; H-1 and H-2 to C-3, H-4 to C-2, C-6 and C-10, H-19 to C-1, C-5, C-9 and C-10, H-7 to C-6, C-8 and C-13, H-9 to C-7, C-8, C-10 and C-14, H-15 to C-14, and H-18 to C-8, C-12, C-13 and C-17. Based on this evidence, the planar structure of **1** was elucidated.

The relative stereochemistry for **1** was established by NOESY experiments, which showed NOEs from 1-H$^\alpha$ to 9-H and 11-H$^\alpha$, 19-H to 1-H$^\beta$ and 11-H$^\beta$, 17-H to 9-H and 12-H$^\alpha$, and 18-H to 7-H$^\alpha$, 7-H$^\beta$ and 12-H$^\beta$. The relative stereostructure of **1** thus expected was cofirmed by X-ray structure analysis‡ on a single crystal of **1** (Fig. 1), which clarified the stereochemistry of the side chain. This compound is an unprecedented steroid with a 13(14→8)*abeo*-8-ergostane skeleton which is considered to have been reconstructed from ergostane through the 1,2-migration of C-13–C-14 bond to the C-8 position.

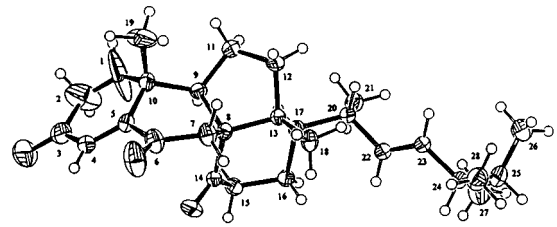

Fig. 1 X-Ray crystal structure for dankasterone **1**.

Dankasterone **1** exhibited significant cytotoxicity (ED_{50} 2.2 μg ml^{-1}) in the P-388 lymphocytic leukemia test system in cell culture. First isolation of this steroid with a unique carbon skeleton consisting of five-membered (C) and six-membered (D) rings has evoked great interest in what sort of biological activity it exhibits, compared with those of usual steroids.

Notes and references

† *Spectral data* for **1**: Prisms, mp 133–134 °C (MeOH), $[\alpha]_D^{26}$ +57.8 (*c* 0.7 in $CHCl_3$); HREIMS *m/z* 424.2988 (M^+), Δ − 1.3 mmu; λ_{max}(EtOH)/nm (log ε) 254 (4.02); ν_{max}(KBr)/cm^{-1} 1695, 1682, 1607; δ_H(500 MHz, $CDCl_3$) 0.81 (3H, d, *J* 6.8, 27-H), 0.84 (3H, d, *J* 6.8, 26-H), 0.91 (3H, d, *J* 6.8, 28-H), 0.98 (3H, s, 18-H), 1.09 (3H, d, *J* 6.8, 21-H), 1.26 (3H, s, 19-H), 1.47 (1H, octet, *J* 6.8, 25-H), 1.48 (1H, dd, *J* 13.2 and 4.2 , 17-H), 1.69 (1H, m, 16-H$^\beta$), 1.71 (1H, m, 12-H$^\alpha$), 1.77 (1H, dt, *J* 13.0 and 7.2, 12-H$^\beta$), 1.85 (1H, m, 11-H$^\beta$), 1.88 (1H, m, 24-H), 1.90 (1H, m, 16-H$^\alpha$), 2.02 (1H, m, 11-H$^\alpha$), 2.04 (1H, m, 1-H$^\beta$), 2.08 (1H, dd, *J* 13.4 and 5.1, 1-H$^\alpha$), 2.42 (1H, m, 20-H), 2.46 (1H, m, 2-H$^\alpha$), 2.48 (2H, m, 15-H), 2.50 (1H, d, *J* 16.8, 7-H$^\beta$), 2.53 (1H, dt, *J* 17.6 and 6.2, 2-H$^\beta$), 2.66 (1H, dd, *J* 16.8 and 1.3, 7-H$^\alpha$), 2.81 (1H, td, *J* 9.0 and 1.3, 9-H), 5.25 (1H, dd, *J* 15.1 and 5.0, 22-H), 5.29 (1H, dd, *J* 15.1 and 3.5, 23-H), 6.36 (1H, s, 4-H); δ_C(125.7 MHz, $CDCl_3$) 17.05 (C-18), 17.58 (C-28), 19.66 (C-27), 20.03 (C-26), 23.16 (C-16), 23.59 (C-21), 23.99 (C-19), 25.10 (C-11), 33.04 (C-25), 34.34 (C-2), 35.99 (C-10), 37.20 (C-20), 37.93 (C-15), 38.30 (C-12), 38.88 (C-1), 40.82 (C-7), 43.21 (C-24), 49.32 (C-17), 49.35 (C-9), 53.97 (C-12), 62.18 (C-8), 126.48 (C-4), 132.31 (C-22), 135.10 (C-23), 156.05 (C-5), 199.10 (C-3), 200.00 (C-6), 214.78 (C-14).

‡ *Crystal data* for **1**: $C_{28}H_{40}O_3$, *M* = 424.60, orthorhombic, $P2_12_12_1$, *a* = 12.667(3), *b* = 23.829(5), *c* = 8.134(4) Å, *V* = 2455.4(14) Å^3, *Z* = 4, d_x = 1.149 g cm^{-3}, *F*(000) = 928, μ(Cu-Kα) = 0.563 mm^{-1}. Data collection was performed on a Rigaku AFC5R using graphite-monochromated radiation (λ = 1.5418 Å); 5117 reflections were collected until θ_{max} = 70.14°, in which 3467 reflections were observed [$I > 2\sigma(I)$]. The crystal structure was solved by direct methods using SHELXS-86 (ref. 5). The structure was refined by full-matrix least-squares methods on F^2 using SHELXL-93 (ref. 6). In the structure refinements, non-hydrogen atoms were refined with anisotropic temperature factors. Hydrogen atoms were calculated on the geometrically ideal positions by the 'ride on' method, and were included in the calculation of structure factors with isotropic temperature factors. In the final stage, *R* = 0.0625, *Rw* = 0.1504 and *S* = 1.036 were obtained. CCDC 182/1288.

1 C. Iwamoto, K. Minoura, S. Hagishita, K. Nomoto and A. Numata, *J. Chem. Soc., Perkin Trans. 1*, 1998, 449 and references cited therein.
2 T. Amagata, M. Doi, T. Ohta, K. Minoura and A. Numata, *J. Chem. Soc., Perkin Trans. 1*, 1998, 3585 and references cited therein.
3 A. Numata, T. Amagata, K. Minoura and T. Ito, *Tetrahedron Lett*., 1997, **38**, 5675.
4 T. Amagata, K. Minoura and A. Numata, *Tetrahedron Lett*., 1998, **39**, 3773.
5 G. M. Sheldrick, SHELXS-86, Program for the Solution of Crystal Structures, University of Göttingen, Göttingen, 1986.
6 G. M. Sheldrick, SHELXS-93, Program for the Refinement of Crystal Structures from Diffraction Data, University of Göttingen, Göttingen, 1993.

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