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Gymnasterones, Novel Cytotoxic Metabolites Produced by a Fungal Strain from a Sponge

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Abstract : Gymnasterones A and B, produced by a strain of *Gymnasella dankaliensis* from the sponge *Halichondria japonica*, are novel ergostanoids with cytotoxicity against tumour cells in culture. Their structures have been established on the basis of spectral analyses.

We previously reported that cytotoxic compounds, gymnastatins A-C,[1] were produced by a strain of *Gymnasella dankaliensis* which was isolated from the sponge *Halichondria japonica*, and their structures were established. Further investigation has led to the isolation of two new cytotoxic ergostanoids, gymnasterones A (**1**) and B (**2**).

According to the method reported previously,[1] the MeOH extract from the mycelium of the fungal strain cultured in a medium of 60 l was purified to afford gymnasterones A (**1**) (10 mg) and B (**2**) (8 mg).

Gymnasterone A (**1**)[2] had the molecular formula $C_{45}H_{67}NO_5$ established by HREIMS. The ^1H-^1H COSY analysis and coupling relationships for the functional groups established by the ^1H and ^{13}C NMR spectral data of **1** led to four partial structural units (Fig. 1) which were supported by HMBC correlations. The geometry of the double bonds in the units was deduced from ^1H-^1H coupling constants, NOEs and a chemical shift of ^{13}C NMR signals of the allylic methyl group.[1] The connection of these units and the remaining functional groups was deduced from HMBC correlations (Fig. 1). The connection between C6 and C7 was deduced from the fact that C6 (δ_C 186.83) is a ketone in a cross-conjugated cyclohexadienone system. Thus the planar structure for gymnasterone A was concluded to be **1** .

The observation of NOEs from H19 to H1β, H2β and H11β, and from H9 to H1α suggested that H19 is arranged *trans* to H9 and the A-ring exists in a twist-chair conformation. The coupling constants from H3 to H2α and H2β (6.0 and 8.0 Hz, respectively) implied that 3-OH is arranged pseudoaxial. NOEs

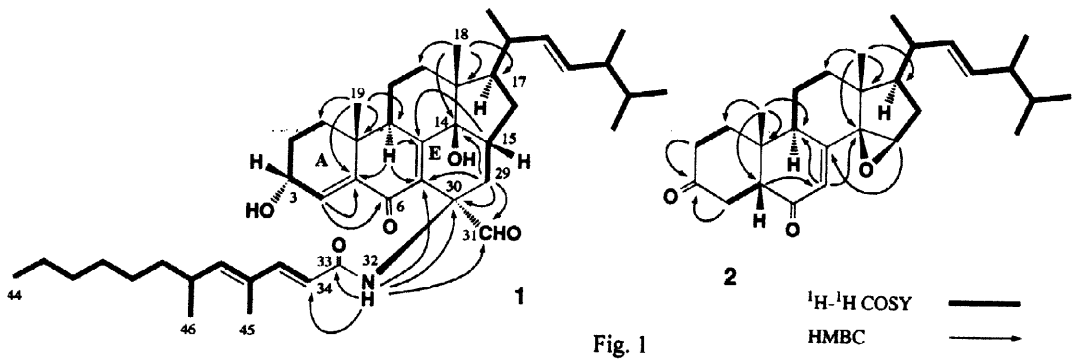

Fig. 1

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from H18 to H12β and H15, and from H9 to H12α and H17, and the coupling constant (13 Hz) between H9 and H11β indicated that the C-ring exists in a twist-boat conformation, and H9 is arranged *cis* to H17 and *trans* to H18 which is oriented *cis* to H15 and 14-OH. In addition, the observation of NOEs from H31 to H16α, H29α and H32 suggested that the E-ring exists in a twist-chair conformation with the formyl group in a pseudoaxial arrangement and, consequently, with the amide bond in a pseudoequatorial arrangement. The protons in the side chain (C20–C28) showed complicated NOEs, perhaps implying rotation at the C17-C20 bond or another axis.

Gymnasterone B (**2**)[3] had the molecular formula $C_{28}H_{40}NO_5$ established by HREIMS. The ^1H and ^{13}C NMR spectra of **2** revealed that the formyl groups, the Δ^4-olefin, the E-ring and the one side chain (C32–C48) in **1** were absent and the two hydroxy groups in **1** were replaced by a ketone and an epoxide ($J_{CH\ (15)}$ 183 Hz) in **2**. This consideration was confirmed by ^1H-^1H COSY and HMBC correlations (Fig.1). The stereochemistry for **2** was established by NOESY data. The observation of NOEs from H19 to H1α, H1β and H5 and from H9 to H2α and H4α suggested that the A-ring exists in a chair conformation with H19 in *cis* and *trans* arrangements to H5 and H9, respectively. NOEs from H9 to H12α and H15, from H18 to H12β, and from H17 to H12α implied that the C-ring exists in a chair conformation and that H9 is arranged *cis* to H15, and H18 is *trans* to H9, H15 and H17. The protons in the side chain showed complicated NOEs as observed in **1** and, therefore, the configuration of C20 and C24 was not established.

Gymnasterones A (**1**) and B (**2**) exhibited moderate to weak cytotoxic activity in the P388 lymphocytic leukemia test system in cell culture (ED_{50} 10.1 and 1.6 μg/ml, respectively).

References and Notes

1. A. Numata, T. Amagata, K. Minoura and T. Ito, *Tetrahedron Lett.*, **38**, 5675 (1997).
2. **1**: Pale yellow oil, $[\alpha]_D$ −110.7° (*c* 1.44, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 270 (4.22). IR ν_{max} (film) cm^{-1}: 3339, 1729, 1658, 1613. HREIMS *m/z*: 701.5028 [M^+] ($C_{45}H_{67}NO_5$ requires 701.5016). ^1H NMR (500 MHz, $CDCl_3$) δ 1.52 (m; H1α), 1.90 (m; H1β), 2.10 (m; H2α), 1.54 (m; H2β), 4.34 (ddd, 8.0, 6.0 and 2.0 Hz; H3), 6.72 (t, 2.0 Hz; H4), 2.45 (dd, 13.0 and 3.8 Hz; H9), 1.50 (m; H11α), 2.14 (m; H11β), 1.69 (m; H12α), 1.76 (m; H12β), 2.48 (m; H15), 1.10 (m; H16α), 1.59 (m; H16β), 1.26 (m; H17), 1.14 (s; H18), 1.05 (s; H19), 2.13 (m; H20), 1.03 (d, 6.8 Hz; H21), 5.06 (dd, 15.1 and 8.7 Hz; H22), 5.24 (dd, 15.1 and 8.0 Hz; H23), 1.83 (d quintet, 8.0 and 6.8; H24), 1.46 (octet, 6.8 Hz; H25), 0.80 (d, 6.8 Hz; H26), 0.82 (d, 6.8 Hz; H27), 0.89 (d, 6.8 Hz; H28), 1.74 (dd, 15.0 and 3.2 Hz; H29α), 2.52 (dd, 15.0 and 6.0 Hz; H29β), 9.18 (s; H31), 7.12 (s; H32), 5.72 (d, 15.1 Hz; H34), 7.11 (d, 15.1 Hz; H35), 5.60 (d, 9.6Hz; H37), 2.48 (m; H38), 1.21 (m; H39A), 1.33 (m; H39B), 1.20 (m; H40 or H41), 1.22 (m; H41 or H40 and H42 or H43), 1.26 (m; H43 or H42), 0.87 (t, 6.8 Hz; H44), 1.72 (s; H45), 0.95 (d, 6.8 Hz; H46), 6.19 (s; 14-OH). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 34.47 (C1), 27.96 (C2), 67.69 (C3), 137.32 (C4), 142.35 (C5), 186.83 (C6), 129.42 (C7), 162.23 (C8), 48.41 (C9), 38.58 (C10), 17.50 (C11), 36.70 (C12), 44.32 (C13), 76.96 (C14), 38.71 (C15), 33.60 (C16), 51.77 (C17), 18.74 (C18), 19.14 (C19), 40.28 (C20), 21.65 (C21), 134.39 (C22), 133.59 (C23), 42.83 (C24), 33.16 (C25), 19.91 (C26), 19.62 (C27), 17.61 (C28), 30.12 (C29), 59.31 (C30), 194.13 (C31), 165.50 (C33), 117.82 (C34), 146.80 (C35), 130.85 (C36), 148.09 (C37), 32.98 (C38), 37.26 (C39), 27.43 (C40 or C41), 29.38 (C41 or C40), 22.61 (C42 or C43), 31.80 (C43 or C42), 14.08 (C44), 12.45 (C45), 20.54 (C46).
3. **2**: Colourless powder, mp 197–199°C, $[\alpha]_D$ −76.3° (*c* 0.76, $CHCl_3$). UV λ_{max} (EtOH) nm (log ε): 255 (4.13). IR ν_{max} (KBr) cm^{-1}: 1719, 1657, 1625. HREIMS *m/z*: 424.2975 [M^+] ($C_{28}H_{40}O_3$ requires 424.2976). ^1H NMR (500 MHz, $CDCl_3$) δ 2.14 (ddd, 14.0, 6.8 and 3.0 Hz; H1α), 1.61 (td, 14.0 and 4.9 Hz; H1β), 2.53 (ddd, 16.0, 14.0 and 6.8 Hz; H2α), 2.37 (m; H2β), 2.28 (dd, 14.9 and 13.7 Hz; H4α), 2.33 (m; H4β), 2.43 (dd, 13.7 and 5.3 Hz; H5), 6.06 (d, 2.6 Hz; H7), 2.93 (ddd, 7.8, 6.8 and 2.6 Hz; H9), 1.81 (m; H11), 1.68 (m; H12α), 1.84 (m; H12β), 3.18 (d, 2.0 Hz; H15), 2.03 (ddd, 15.2, 10.1 and 2.0 Hz; H16α), 2.09 (dd, 15.2 and 3.7 Hz; H16β), 1.72 (ddd, 15.2, 6.0 and 3.7 Hz; H17), 1.13 (s; H18), 1.08 (s; H19), 2.33 (m; H20), 0.95 (d, 7.4 Hz; H21), 5.28 (dd, 15.6 and 8.0 Hz; H22), 5.20 (dd, 15.6 and 7.8 Hz; H23), 1.92 (d quintet, 7.8 and 6.9; H24), 1.49 (octet, 6.9 Hz; H25), 0.84 (d, 6.9 Hz; H26), 0.86 (d, 6.9 Hz; H27), 0.96 (d, 6.9 Hz; H28). ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 34.82 (C1), 36.90 (C2), 207.86 (C3), 39.62 (C4), 56.12 (C5), 198.19 (C6), 119.77 (C7), 158.64 (C8), 38.96 (C9), 37.43 (C10), 20.54 (C11), 38.99 (C12), 45.60 (C13), 71.91 (C14), 69.01 (C15), 29.48 (C16), 53.22 (C17), 15.81 (C18), 22.68 (C19), 38.30 (C20), 23.03 (C21), 133.54 (C22), 133.31 (C23), 43.12 (C24), 33.09 (C25), 19.74 (C26), 20.04 (C27), 17.74 (C28).