GRISEOLIC ACIDS B AND C, AN INHIBITOR OF CYCLIC ADENOSINE 3',5'-MONOPHOSPHATE PHOSPHODIESTERASE

Sir:

In the previous paper, we reported the structure 1 of griseolic acid A (previously reported as griseolic acid1) produced by Streptomyces griseoaurantiacus SANK 63479. In this paper, we report the isolation procedure and structural elucidation of griseolic acids B2) and C3).

Griseolic acids B and C were isolated from the mother liquid of griseolic acid A by Sephadex G-10 column chromatography. The mother liquid was dissolved in water and applied on a Sephadex G-10 column and developed with water. Two active fractions (griseolic acids B and C) were individually chromatographed on a column of DEAE-Sephadex A-25. After washing with water, the column was eluted with 0.3 m NaCl. The active eluate was then adsorbed on a Diaion HP-20 column. The column was washed with water and eluted with 60% Me2CO. The eluate was concentrated in vacuo and stored at 4°C overnight to give colorless crystals.

Griseolic acid B (2): C14H13N5O7; mp 160°C (dec); [α]D° +13.2° (c 1.1, DMSO); fast atom bombardment mass spectra (FAB-MS) m/z 364 (M+H)+, and griseolic acid C (3): C14H15N5O7; mp 160°C (dec); [α]D° -50.7° (c 1.0, DMSO); FAB-MS m/z 366 (M+H)+ were both crystalline. Griseolic acids B (2) and C (3) were both attributed to have a N-9 substituted adenine moiety in view of the UV absorption at max 256 nm in 0.01N HCl and 260 nm in 0.01N NaOH, 1H NMR and 13C NMR spectra, which included signals at 8.21 and 8.33 ppm for 1H NMR spectra of 2 and 13.48 ppm in 2 and 155.8 (s, C-6), 152.8 (d, C-2), 148.4 (s, C-4), 139.5 (d, C-8) and 118.6 (s, C-5) in 3. On comparison of the 1H NMR spectra of 2 with that of 1, it was noted that the singlet at 4.7 ppm in 1 (representing a C-7' proton) was not present in 2, and the doublet doublets of AB type (2.78 and 3.08 ppm) appeared instead. This observation suggested that the structure of griseolic acid B (2) corresponded to 7'-deoxygriseolic acid A. In the 13C NMR spectrum of griseolic acid C (3), sp2 carbons at 97.3 and 157.7 ppm of 2 disappeared and sp2 carbons at 85.6 (or 77.9) and 35.7 ppm newly appeared as a doublet and triplet. A C-5' olefinic proton at 5.08 ppm in the 1H NMR spectrum of 2 was absent in 3; this indicated the presence of a methylene group (2.3 ppm) and a methine bearing oxygen (4.46 ppm) in 3. These spectral properties suggested that the structure of griseolic acid C (3) corresponded to dihydrogriseolic acid B. The structure of griseolic acid C (3) was deduced by X-ray analysis. Crystal data are as follows: C14H13N5O7·2H2O, MW 401.4, monoclinic, P21, a=13.660(2), b=8.509(1), c=7.881(2) Å, β=106.6(1)°, U=887.9 Å³, Z=2, Dcalc=1.52 gcm⁻³, μ(CuKα)=1.1 mm⁻¹. The structure was solved by MULTAN⁴) and refined.

Table 1. 1H NMR data of griseolic acids.

<table>
<thead>
<tr>
<th></th>
<th>C-1'</th>
<th>C-2'</th>
<th>C-3'</th>
<th>C-4'</th>
<th>C-5'</th>
<th>C-7'</th>
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<tbody>
<tr>
<td>Griseolic acid A (1)</td>
<td>6.67 (s)</td>
<td>4.77 (d)</td>
<td>5.79 (q)</td>
<td>5.31 (d)</td>
<td>4.70 (s)</td>
<td></td>
</tr>
<tr>
<td>Griseolic acid B (2)</td>
<td>6.49 (s)</td>
<td>4.60 (d)</td>
<td>6.0 (q)</td>
<td>5.08 (d)</td>
<td>2.78, 3.08 (AB, q)</td>
<td></td>
</tr>
<tr>
<td>Griseolic acid C (3)</td>
<td>6.20 (s)</td>
<td>4.46 (ov)</td>
<td>4.46 (ov)</td>
<td>2.30 (AB, q)</td>
<td>2.94 (AB, q)</td>
<td>4.46 (ov)</td>
</tr>
</tbody>
</table>

s: Singlet, d: doublet, q: quartet, ov: overlap.
Inhibitory activities of griseolic acids A, B, C and papaverine against cyclic adenosine 3',5'-monophosphate phosphodiesterase from rat brain [EC 3.1.4.17] expressed in terms of 50% inhibition (IC50, μM) were 0.16, 0.16, 0.12 and 3.5, respectively.

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References