Rapid Paper

Metabolism of GA₉ Methyl Ester in a Culture of Prothallia of Lygodium japonicum

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GA₉ methyl ester (GA₉-Me) fed to a culture of prothallia of Lygodium japonicum was metabolized into three monohydroxy-GA₉-Me after three weeks. One of these was identified as GA₂₀-Me and the structures of the other two were defined to be 12α- and 12β-hydroxy-GA₉-Me by spectroscopic analyses.

Lygodium japonicum has been shown by Takeno et al.¹ to produce substances which induce antheridium formation (antheridiogen) and those which inhibit the archegonial differentiation on prothallia. Yamane et al.² has succeeded in identifying the GA₉ methyl ester (GA₉-Me, 1) as one of the antheridiogens and suggested the occurrence of a more active principle. Successively, they have shown that GA₉ (free acid) fed to the culture media of prothallia of L. japonicum was converted into GA₉-Me very rapidly. Herein, the metabolism of GA₉-Me in the culture of prothallia of L. japonicum is described.

Prothallia of Lygodium japonicum (Thunb.) Sw. were aseptically cultured by the method reported by Yamane et al.² Prothallia formed by germination of spores in a Petri dish were transferred to a 100 ml flask containing 20 ml of a medium (1/10-strength modified Murashige and Skoog’s mineral salt solution) and 660 μg of GA₉-Me was fed to each flask, the prothallia being incubated for three weeks. The combined culture filtrate from 50 flasks was extracted three times with 500 ml of ethyl acetate at pH 3 and the combined ethyl acetate was washed three times with 200 ml of 5% aqueous sodium bicarbonate solution to give an ethyl acetate neutral fraction (NE fraction). The TLC of the NE fraction, using a solvent system of n-hexane–ethyl acetate= 6:4 (v/v) with duplicated development, showed three spots with blue fluorescence under 356 nm UV light after spraying with 70% sulfuric acid and heating at 120°C. Repetitive preparative TLC using the same solvent system gave three pure substances designated X₁ (0.1 mg), X₂ (1.3 mg) and X₃ (1.7 mg) with Rf values of 0.35, 0.33 and 0.28, respectively.

In GC/MS analyses, these compounds showed peaks at tᵣ 2.6 min (X₁), 2.8 min (X₃) and 3.4 min (X₂) under the following conditions: column, 2% OV-1 on Chromosorb W-DMCS (1 m × 3 mm i.d.); column temp., 220°C; He flow, 35 ml/min. All the X₁, X₂ and X₃ showed a molecular ion of m/z 346 and prominent fragment ions of m/z 328 (M⁺−18), 314 (M⁺−32), 300 (M⁺−46), 296 (M⁺−50) and 286 (M⁺−60). This fragmentation pattern is characteristic of hydroxylated gibberellin methyl esters. In GC/MS analyses of their trimethylsilylated derivatives, they showed peaks at tᵣ 3.2 min (X₁), 3.3 min (X₃) and 3.9 min (X₂) under the above conditions, except for a column temp. of 210°C. Since their molecular ions shifted to m/z 418 by trimethylsilylation, they were all monohydroxy-GA₉-Me. The PMR of X₁, X₂ and X₃ are summarized in Table I.

In the PMR of X₁, no carbinyl proton signal was observed, suggesting the presence of a tertiary hydroxyl group, probably at C-13. The tᵣ and mass spectra of X₁ and its trimethylsilyl (TMSi) derivative were identical with those of GA₂₀-Me and the TMSi ether of GA₂₀-Me (GA₂₀-Me-TMSi), respectively. The PMR of X₁ also supported the identity of X₁ and GA₂₀-Me. Thus, X₁ was identified as GA₂₀-Me (2).

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Table I. PMR Spectra of X₁, X₂ and X₃

Spectra were obtained by JEOL FX-400 FT NMR spectrometer in CDCl₃. Chemical shifts are expressed by the δ-value.

<table>
<thead>
<tr>
<th></th>
<th>C-4 CH₃</th>
<th>C-5 H</th>
<th>C-6 H</th>
<th>C-13 H</th>
<th>C-6 COOCH₃</th>
<th>C-12 H (OH)</th>
<th>C=CH₂ (C-17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>X₁</td>
<td>1.09 (3H, s)</td>
<td>2.53 (1H, d)</td>
<td>2.72 (1H, d)</td>
<td>—</td>
<td>3.72 (3H, s)</td>
<td>—</td>
<td>4.94, 5.23</td>
</tr>
<tr>
<td>X₂</td>
<td>1.08 (3H, s)</td>
<td>2.57 (1H, d)</td>
<td>2.73 (1H, d)</td>
<td>2.64 (1H, d)</td>
<td>3.65 (3H, s)</td>
<td>Approx. 3.7ₐ</td>
<td>4.99, 5.10 (broad two s)</td>
</tr>
<tr>
<td>X₃</td>
<td>1.10 (3H, s)</td>
<td>2.56 (1H, d)</td>
<td>2.70 (1H, d)</td>
<td>2.83 (1H, t)</td>
<td>3.72 (3H, s)</td>
<td>4.08 (1H, m)</td>
<td>5.11, 5.16 (broad two s)</td>
</tr>
</tbody>
</table>

ₐ The signal overlapped that of OCH₃ in the methoxycarbonyl group.

Fig. 1. Mass Spectra of Trimethylsilylated X₂ (a) and X₃ (b).
As shown in the Fig. 1, the mass spectra of trimethylsilylated X₂ and X₃ were very similar, except for the relative intensity of the fragment ion of M⁺−15. A rather intense peak of m/z 328 (M⁺−90) and the presence of carbinyl proton signals at δ 2.63 and 2.83 are indicative of a secondary hydroxyl group.

X₂ and X₃ were oxidized with Jones reagent to give an identical keto-GA₉-Me (3), showing M⁺ at m/z 344 in its mass spectrum. This indicates that X₂ and X₃ were epimeric to each other at the carbon to which a hydroxyl group is attached. The keto-GA₉-Me showed a UV absorption maximum at 286 nm (ε 414) and CD 

\[[\theta]_{\text{EtOH}} - 1.18 \times 10^4 (294 \text{ nm})\]

which are indicative of the presence of β, γ-unsaturation of a ketone group in the appropriate molecular geometry.³ Similar UV absorption \(\lambda_{\text{max}}\) 288 nm, ε 280) and CD⁴⁻⁵ \[[\theta]_{\text{EtOH}} - 8.97 \times 10^3 (294 \text{ nm})\] have been observed in 12-keto-GA-Me such as GA₂₀-Me (6) due to the trans-annular interaction between the C-12 ketone and the C-16 exocyclic methylene group. Thus, the hydroxyl group in X₂ and X₃ must exist at C-12 and the keto-GA₉-Me should have the structure 3. The stereochemistry of the C-12 hydroxyl group of X₂ and X₃ was decided by careful analyses of their PMR.

The chemical shifts of C-12, C-13 and the exocyclic methylene protons in GAs and their derivatives with a hydroxyl group at C-12 are summarized in Table II, together with those of X₂ and X₃. The chemical shifts and splitting pattern of X₂ are very similar to those of GA₃₀ (9), GA₃₁ (10)⁵ and the 2,3-diacetate of GA₉-Me (8),⁴ which contain the 12z-hydroxyl
Table II. Chemical Shifts of Protons in the Vicinity of the C-12 Hydroxyl Group in PMR of 12-OH GAs and Their Derivatives

<table>
<thead>
<tr>
<th>Configuration of C-12 OH</th>
<th>C-12 H (OH)</th>
<th>C-13 H</th>
<th>C=CH₂ (C-17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA₃₀ (Acetone-d₆)⁵</td>
<td>α</td>
<td>3.70 (m)</td>
<td>2.59 (d)</td>
</tr>
<tr>
<td>GA₃₁ (Acetone-d₆)⁵</td>
<td>α</td>
<td>3.70 (m)</td>
<td>?</td>
</tr>
<tr>
<td>2,3-Diacetate of GA₄₉-Mc (CDCl₃)⁹</td>
<td>β</td>
<td>4.13 (m)</td>
<td>2.85 (t)</td>
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<tr>
<td>2,3-Diacetate of GA₄₉-Me (CDCl₃)⁹</td>
<td>α</td>
<td>Approx. 3.7⁹</td>
<td>2.64 (d)</td>
</tr>
<tr>
<td>X₂</td>
<td>α</td>
<td>Approx. 3.7⁹</td>
<td>2.64 (d)</td>
</tr>
<tr>
<td>X₃</td>
<td>β</td>
<td>4.08 (m)</td>
<td>2.83 (t)</td>
</tr>
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</table>

⁵ PMR of these compounds, which were supplied by Dr. Yokota in our laboratory, were obtained by a JEOL FX-400 FT NMR spectrometer.
⁹ The signals overlapped those of OCH₃ in the methoxycarbonyl groups.

group, while those of X₃ were similar to the 2,3-diacetate of GA₄₈-Me (7)⁴ with the 12β-hydroxyl group. This comparison clearly indicates that X₂ and X₃ should have the 12α- and the 12β-hydroxyl group respectively. Thus, the structure 12α-hydroxy-GA₉-Me (4) was assigned to X₂ and 12β-hydroxy GA₉-Me (5) to X₃. Now it has been shown that GA₉-Me is metabolized to monohydroxy-GA₉-Me, namely, GA₂₀-Me, 12α- and 12β-hydroxy-GA₉-Me, in the culture of prothallia of L. japonicum.

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REFERENCES