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Gas chromatographic evidence of the antheridiogen of *Lygodium japonicum* (A_{LY}) (Schizaeaceae)

K. Bürcky, A. Gemmrich and H. Schraudolf[1,2]

Institut für Zuckerrübenforschung, Holtenser Landstrasse 77, D-3400 Göttingen, and Abteilung Allgemeine Botanik, Universität Ulm, Postfach 4066, D-7900 Ulm (Federal Republic of Germany), 5 December 1977

Summary. The antheridiogen in culture medium of *Lygodium japonicum* was separated by GC. It shows different polarity compared with *Anemia* antheridiogens.

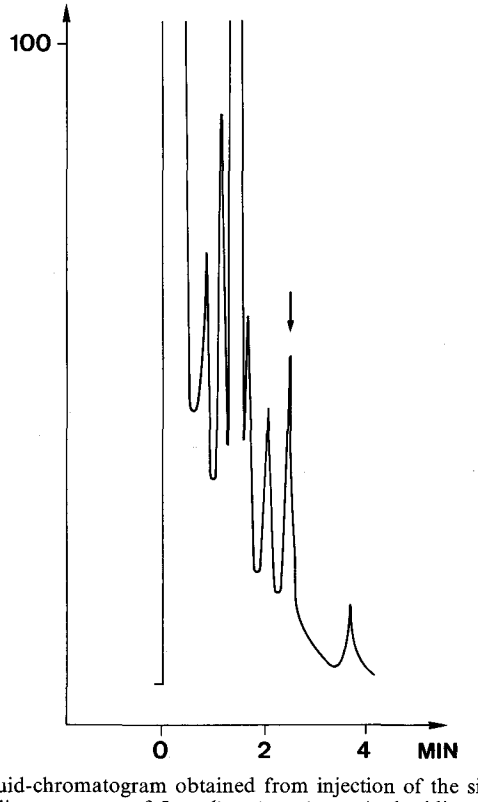
Antheridiogens are phytohormones which are able to induce the male gametangium in ferns. They are synthesized during distinct phases of prothallia development and excreted into the medium. Antheridiogens are described in 3 species of the Polypodiaceae (*Pteridium aquilinum*[3], *Onoclea sensibilis*[4], and *Ceratopteris thalictroides*[5]) and in 2 species of the Schizaeaceae (*Anemia phyllitidis*[6,7], *Lygodium japonicum*[8,9]).

Up till now, qualitative as well as quantitative determinations of fern antheridiogens have been mainly performed by a combination of TLC and biotest. These procedures need at least 10–25 days, depending on the concentration present in the preparation. To improve the analysis, a method for separation and determination of the 2 antheridiogens of *Anemia phyllitidis*[7] by GLC has been worked out in our laboratory[10]. The application of this method to antheridiogens of other species of the Schizaeaceae was obvious, since a combination of GC with mass spectrometric methods should enable the analysis of the as yet unknown chemical structure of these compounds.

Conditions for the culture of the prothallia, the extraction, the TLC and GC methods, as well as the procedure for the biotest of the hydrolyzed TMS-derivatives separated by GC, have already been described[10,11].

The figure shows the GC profile of the TMS-derivatives of the antheridiogen preparation from the culture medium of *Lygodium japonicum*. The arrow indicates the peak which showed antheridiogen activity in the subsequent biotest. The retention time of the TMS-derivative of the *Lygodium* hormone is 0.22 relative to that of TMS-GA_3, whereas the relative retention time of the main hormone of *Anemia* is 1.2.

The result of the present study permits a comparison of the 3 antheridiogens isolated so far from Schizaeaceae (*Anemia* antheridiogen 1 and 2[6,7], A_{Ly}[8,9]) with gibberellic acid. In TLC system these compounds have different R_f values indicating 4 different molecules. The chromatographic properties in TLC, as well as in GC systems, show that the *Lygodium* hormone is less polar than GA_3 or the *Anemia* antheridiogen. The biological and chromatographic properties of A_{Ly} suggest that it also belongs to these biologically active diterpens with a structure comparable to gibberellins. Further studies on structure analysis are in progress.



Gas-liquid-chromatogram obtained from injection of the silylated antheridiogen extract of *Lygodium japonicum*. Antheridiogen fraction (A_{LY}) is marked ↓. Attenuation was 10×256. Equipment: FVT 2400 (Carlo Erba, Mailand); glass column: 2 m, ∅6/2 mm filled with 3% silicone GE SE - 30 on gaschrom Q 100/200 mesh. Temperature: Injector/column/detector = 275/220/260 °C. Carrier: nitrogen, 75 ml/min.

1 We thank Dr P. Macnicol for checking the English version of the manuscript.
2 To whom reprint requests should be addressed. Univ. of Ulm, Dept. of Botany, Postfach 4066, D-7900 Ulm.