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CONSTITUTION OF ANTHERIDIUM-INDUCING FACTOR OF *ANEMIA PHYLLITIDIS*

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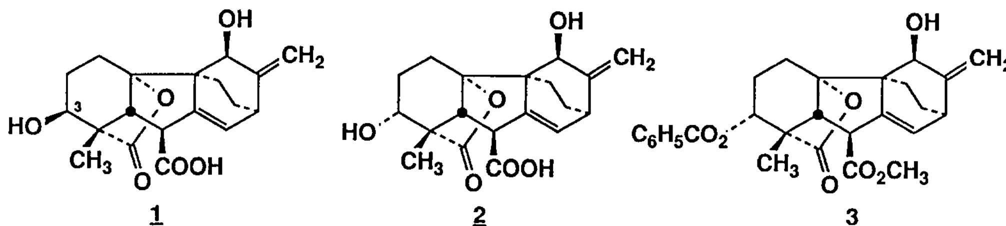
Summary: Careful comparison of naturally derived and synthetic samples of the antheridium-inducing factor of *Anemia phyllitidis*, A_{An}, by TLC, HPLC, NMR and GC-MS measurements allow unambiguous assignment of structure **2** to this substance, designated herein as antheridic acid.

A hormonal substance which stimulates sex-organ development (antheridium formation) and spore germination in certain species of ferns was isolated several years ago from the fern *Anemia phyllitidis* and designated antheridiogen-An (A_{An}).[1] Subsequently, it was proposed on the basis of spectral studies that A_{An} possessed the rearranged gibbane structure 1.[2] Recently, **1** was prepared by total synthesis and found to have a pmr spectrum different from that of naturally derived A_{An}.[3] On the other hand, the pmr spectra of A_{An} and synthetic **2** were in excellent correspondence, as was also the case for the methyl ester and methyl ester 3-benzoate derivatives.[3] Although this constitutes strong evidence for the assignment of structure **2** to A_{An}, final proof was not obtained for lack of an authentic sample of A_{An} since none was available from the earlier work. A rigorous comparison of native A_{An} with synthetic material has now been performed with the result that A_{An} can unambiguously be formulated as **2**.



A sample of authentic A_{An} was obtained by extraction of the culture medium of *Anemia phyllitidis*[1,2,4] and purification by reversed phase HPLC (3 steps). The material thus obtained was homogeneous when analyzed as the trimethylsilyl (TMS) ester-bis TMS ether by GC-MS (monitoring total ion current or m/e 562 (M^+)). A sample of *ca.* 100 μg was

used for comparison with synthetic (±)-**2**. Thin layer chromatography on silica gel (tlc-sg) of synthetic **2** and native A_{An} using the solvent system 16:3:1 methylene chloride-methanol-triethylamine revealed identical mobilities, R_f 0.45. Comparison of the methyl esters of synthetic **2** and A_{An} (formed using CH_2N_2 in ethyl acetate) by tlc-sg also indicated identity (R_f 0.51 in 1:1 trimethylpentane-isopropyl alcohol) under conditions which distinguish the methyl ester of **2** from **1** methyl ester (R_f 0.57). The methyl ester 3-benzoate derivative of **2** and of A_{An} were also indistinguishable by tlc-sg (R_f 0.29 in 3:2 hexane-ethyl acetate). In addition, the methyl ester 3-benzoate derivative (**3**) of synthetic **2** and of native A_{An} were identical by HPLC analysis using a Du Pont Zorbax silica column and three different solvent systems (3:1 hexane-t-butyl methyl ether, R_V 22 ml; 33:1 hexane-isopropyl alcohol, R_V 15 ml; 17:3 hexane-tetrahydrofuran, R_V 18 ml).

The 500 MHz pmr spectra of the methyl ester 3-benzoate derivatives (**3**) of synthetic **2** and native A_{An}, measured in 17:1 CCl_4-C_6D_6, also confirmed structural identity and were completely consistent with formula **3**.

Finally, the GC-MS data obtained for the tris TMS derivatives of synthetic **2** and native A_{An} corresponded both with respect to GC retention time (5 min on a 2% OV-1 column of 1 m x 3 mm size at 230° C with a flow rate of 40 ml/min of N_2) and principal MS peaks (562, 534, 416, 367, 311, 283, 220, 180, 147, 129, and 73 m/e).

Bioassay of synthetic (±)-A_{An} (**2**) and native A_{An} revealed similar activities both with respect to dark germination and antheridial formation; high activity was observed at concentrations of even 0.05 ppm of **2** under which conditions gibberellic acid or controls showed no activity; details will be given in a separate paper.

These results leave no doubt as to the constitution (**2**) of A_{An}. The fern hormone A_{An} is quite unique in terms of structure not only because it possesses the rearranged gibbane skeleton, but also because of the 3α-hydroxyl function. None of the more than 50 known gibberellins contains a 3α-oriented hydroxyl group. Because of the structural uniqueness of this hormone, the awkwardness of the designation A_{An}, and the possibility that **2** may play a broader role in fern biology, it seems desirable to replace A_{An} by a more conventional name. We therefore propose that **2** be designated as antheridic acid, rather than A_{An}, and that the parent hydrocarbon be called antheridane.[5,6]

References and Notes

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