Tetrahedron Letters,Vol.27,No.42,pp 5083-5084,1986 0040-4039/86 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

# CONSTITUTION OF ANTHERIDIUM-INDUCING FACTOR OF *ANEMIA PHYLLITIDIS*

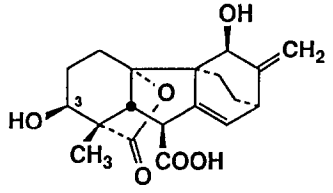
E. J. Corey and Andrew G. Myers
Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

Nobutaka Takahashi and Hisakazu Yamane
Department of Agricultural Chemistry, University of Tokyo, Bunkyo-ku, Tokyo

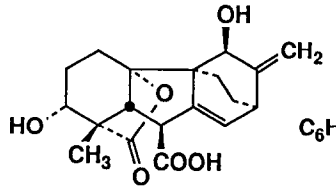
Helmut Schraudolf
Abt. Botanik, Universität Ulm, D-7900 Ulm, W. Germany

*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.

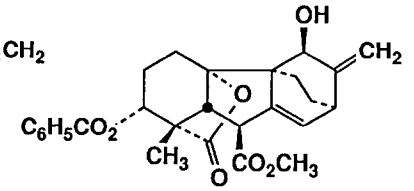
A hormonal substance which stimulates sex-organ development (antheridium formation) and spore germination in certain species of ferns was isolated several years ago from the fern *Anemia phyllitidis* and designated antheridiogen-An ($A_{An}$).[1] Subsequently, it was proposed on the basis of spectral 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**.




**1**




**2**




**3**

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


1. U. Näf, K. Nakanishi and M. Endo, *Bot.Rev.*, **41**, 315 (1975).
2. K. Nakanishi, M. Endo, N. Näf, and L.F. Johnson, *J. Am. Chem. Soc.*, **93**, 5579 (1971).
3. E. J. Corey and A. G. Myers, *J. Am. Chem. Soc.*, **107**, 5574 (1985).
4. M. Endo, K. Nakanishi, U. Näf, W. McKeon and R. Walker, *Physiol. Plant*, **26**, 183 (1972).


5. We are grateful to Drs. Ulrich Näf and Koji Nakanishi for their active interest and advice.
6. This work was assisted financially by a grant from the National Science Foundation.

(Received in USA 17 June 1986)