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BIOLOGICAL ACTIVITY OF ANTHERIDIC ACID, AN ANTHERIDIOGEN OF *ANEMIA PHYLLITIDIS*

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Key Word Index—*Anemia phyllitidis*; Schizaeaceae; antheridium formation; dark spore germination; gibberellin activity; antheridiogen; antheridic acid.

Abstract—The biological activities of synthetic antheridiogen of *Anemia phyllitidis*, (±)-antheridic acid, and naturally derived antheridic acid with regard to induction of antheridial formation and dark spore germination in *A. phyllitidis* were closeiy similar. The activity of (±)-3-*epi*-antheridic acid was weaker than that of (±)-antheridic acid in inducing these phenomena. (±)-Antheridic acid was active in inducing elongation growth in the dwarf rice bioassay system, although its activity was weaker than that of GA_3. In this bioassay system, (±)-3-*epi*-antheridic acid showed higher activity than did (±)-antheridic acid.

INTRODUCTION

Since Döpp first found antheridiogen, a hormonal substance which induced antheridial formation in *Pteridium aquilinum* [1], 10 fern species have so far been reported to produce antheridiogens [2]. The antheridiogen of *Anemia phyllitidis* has been identified as 1 [3] and that of *Lygodium japonicum* as the methyl ester of gibberellin A_9 [4]. However, the structures of those in other fern species are still unknown. Recently (±)-1 and its 3-epimer (2) were prepared by total synthesis and the former was shown by careful comparison to be identical chromatographically and spectroscopically with natural *Anemia* antheridiogen [5, 6]. The *Anemia* antheridiogen has been termed antheridic acid.

In the present work, the biological activities of the synthetic (±)-antheridic acid and native antheridic acid were compared with one another and with the synthetic 3β-epimer of (±)-antheridic acid. Since antheridic acid possesses a rearranged *ent*-gibberellane structure and since GA can also induce antheridial formation [7], (±)-antheridic acid was also studied for its GA-like activity in the bioassay system used for GA.

RESULTS

Induction of antheridial formation

Antheridial formation rates in gametophytes of *A. phyllitidis* treated with natural antheridic acid and synthetic (±)-antheridic acid and (±)-3-*epi*-antheridic acid are shown in Table 1. Natural antheridic acid and synthetic (±)-antheridic acid were active in inducing antheridial formation at 0.003 and 0.01 ppm, respectively. The activity of (±)-3-*epi*-antheridic acid was one order weaker than that of (±)-antheridic acid. Under the same conditions, GA_3 induced antheridial formation only at 0.1 ppm and higher (data not shown).

Induction of dark spore germination

It has been reported that antheridic acid induces dark spore germination as well as antheridial formation in *A. phyllitidis* [8]. Consequently, the natural antheridic acid and synthetic (±)-antheridic acid were assayed for such activity (Table 2). Both natural antheridic acid and synthetic (±)-antheridic acid induced dark spore germination at 0.0003 ppm and higher concentrations, whereas (±)-3-*epi*-antheridic acid showed this activity at 0.003 ppm and higher. GA_3 was active in inducing dark spore germination at 0.3 ppm and higher under the same conditions (data not shown).

GA-like activity in dwarf rice assay

The synthetic (±)-antheridic acid and (±)-3-*epi*-antheridic acid were tested for GA-like activity in the dwarf rice bioassay system [9] (Table 3). Both (±)-antheridic acid and (±)-3-*epi*-antheridic acid apparently stimulated elongation growth of the second leaf sheath in two dwarf rice varieties, although the activities were weaker than that of GA_3. The activity of (±)-antheridic acid in both Tan-ginbozu and Waito-C was four orders lower than that of GA_3, whereas the activities of (±)-3-*epi*-antheridic acid in Tan-ginbozu and Waito-C were three and two orders lower than that of GA_3, respectively. It is important to note that the activity of (±)-3-*epi*-antheridic acid was 10–100 times higher than that of (±)-antheridic acid.

Table 1. Activity of natural antheridic acid and synthetic (±)-antheridic acid and (±)-3-*epi*-antheridic acid to induce antheridial formation in *Anemia phyllitidis*

	Antheridial formation (%)					
	Concentration (ppm)					
Sample	0	0.001	0.003	0.01	0.03	0.1
Natural antheridic acid	0±0	0±0	21.4±4.7	90.1±2.1	95.6±1.8	92.8±2.6
Synthetic (±)-antheridic acid	0±0	0±0	0±0	71.5±5.8	91.5±1.1	98.0±0.9
Synthetic (±)-3-*epi*-antheridic acid	0±0	0±0	0±0	0±0	38.1±1.5	94.8±0.2

Table 2. Activity of natural antheridic acid and synthetic (±)-antheridic acid and (±)-3-*epi*-antheridic acid to induce dark spore germination in *Anemia phyllitidis*

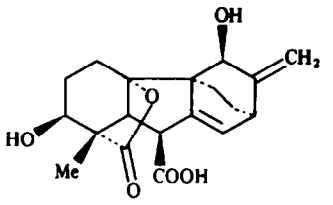
	Germination (%)						
	Concentration (ppm)						
Sample	0	0.0003	0.001	0.003	0.01	0.03	0.1
Natural antheridic acid	0±0	3.2±1.1	17.4±2.0	57.8±5.6	89.5±0.9	92.3±0.8	86.1±2.2
Synthetic (±)-antheridic acid	0±0	0.2±0.1	0.9±0.1	15.0±1.2	65.6±1.0	88.1±2.1	94.1±1.6
Synthetic (±)-3-*epi*-antheridic acid	0±0	0.1±0.2	0.1±0.1	0.9±0.1	37.5±2.7	85.4±2.3	90.7±3.1

Table 3. Activity of (±)-antheridic acid, (±)-3-*epi*-antheridic acid and GA_3 in a bioassay system using *Oryza sativa*, var. Tan-ginbozu and Waito-C

		Second leaf sheath length (mm)					
		Dosage (ng/seedling)					
Variety	Sample	0	0.1	1.0	10	100	1000
Tan-ginbozu	(±)-antheridic acid	15.7±0.6	15.5±0.5	15.7±0.5	14.4±0.6	17.0±0.6	21.0±0.9
	(±)-3-*epi*-antheridic acid		15.8±1.4	16.2±1.1	17.4±0.8	21.8±0.3	40.6±1.6
	GA_3		20.6±1.1	30.0±0.6	44.0±1.2	56.1±1.3	54.9±1.5
Waito-C	(±)-antheridic acid	16.0±0.1	15.2±0.4	15.2±0.2	16.1±1.0	16.1±0.3	23.4±1.0
	(±)-3-*epi*-antheridic acid		15.7±0.4	15.2±0.6	17.5±0.3	24.3±1.6	48.6±3.4
	GA_3		18.3±0.8	31.4±2.3	44.4±1.8	53.1±1.1	54.9±1.2

DISCUSSION

The level of biological activity was found to be almost the same for natural antheridic acid and synthetic (±)-antheridic acid in terms of induction of antheridial formation and of dark spore germination in *A. phyllitidis*. The natural compound was slightly more active than the synthetic racemate, suggesting that the unnatural enantiomer in the synthetic preparation is inactive. The activity of (±)-3-*epi*-antheridic acid was weaker than that of (±)-antheridic acid in the induction of these phenomena. These results indicate that formula 2 is the correct structural designation of antheridic acid supporting the results of chemical studies [6]. The results of the assay of GA-like activity of (±)-antheridic acid confirmed the report by Sharp *et al.* [10] that naturally obtained *Anemia* antheridiogen is weakly active in three bioassay systems. (±)-Antheridic acid possesses a 3α-hydroxyl group as well as an unique C, D ring system. Since the activity of GA derivatives with a 3α-hydroxyl group is low but measurable in most of bioassay systems [11], the low activity of (±)-antheridic acid in the dwarf rice bioassay system may be partly due to the 3α-hydroxyl group. However, the C, D ring system of (±)-antheridic acid may also be responsible for the low growth promoting activity in this assay because even (±)-3-*epi*-antheridic acid showed lower activity than that of GA_3. In contrast to the results of the assay for antheridial formation and dark spore germination, the results of the dwarf rice assay showed that the activity of (±)-antheridic acid was lower than that of GA_3. This fact suggests that the structure of the receptor to which antheridic acid and GA_3 bind is different for rice and fern.



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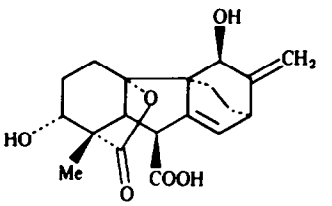

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EXPERIMENTAL

Preparation of natural antheridic acid. The acidic EtOAc fraction obtained from the medium on which prothallia of *A. phyllitidis* L. Sw. were cultured was purified by three steps of HPLC to provide natural antheridic acid [6]. The synthesis of (±)-antheridic acid and (±)-3-*epi*-antheridic acid have been reported previously [5]. Comparison of naturally obtained antheridic acid and synthetic (±)-antheridic acid was done by TLC, HPLC and GC-MS as reported in ref. [6].

Assay of antheridial formation. Spores of *A. phyllitidis* were aseptically inoculated on the surface of 1/10-strength of Murashige and Skoog's [12] mineral salts solution solidified with 0.3% agar and containing the test compounds at the desired concn. Incubation was carried out at 25° under continuous white light. The resulting gametophytes were observed under a microscope to score antheridial formation rates after incubation for 20 days [8].

Assay of dark spore germination. Spores of *A. phyllitidis* were inoculated in the same manner as above but were incubated in total darkness. The cultures were observed under a microscope to score spore germination rates 20 days after inoculation.

Assay with dwarf rice seedlings. One μl of the test compounds as 50% aq Me_2CO soln at the desired concn was applied between coleoptile and the first leaf of 3-day-old seedlings of *Oryza sativa* cv Tan-ginbozu and Waito-C. Seedlings were incubated at 30° under continuous white light (*ca* 2800 lx). The length of the second leaf sheath was measured four days after sample application [7].

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