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STRUCTURE OF AN ANTHERIDIOGEN FROM THE FERN *ANEMIA MEXICANA*

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Key Word Index—*Anemia mexicana*; Schizaeaceae; fern; gametophyte; structure; nuclear magnetic resonance; antheridiogen; gibberellin.

Abstract—A new antheridiogen isolated from gametophytes of the fern *Anemia mexicana* has been determined to have the structure *ent*-1α,10β-dihydroxy-9α,15α-cyclo-20-norgibberell-16-ene-7,19-dioic acid 10,19-lactone.

INTRODUCTION

Following the discovery [1, 2] of an antheridium inducing substance in gametophytes of the bracken fern, *Pteridium aquilinum*, several discrete compounds, for which the term antheridiogen has been coined, have been isolated from a number of other fern species [3]. These compounds appear to play an important role in promoting cross fertilization and thereby maintaining genetic diversity. The very small quantities which have been obtained (nanograms to micrograms in most cases) have rendered structural analysis extremely difficult, however. An important clue to the constitution of these intriguing substances in the case of the family Schizaeaceae was the observation that they possessed gibberellin-like properties [4] and conversely, that gibberellins had antheridium inducing properties [5, 6]. Sufficient material was eventually acquired from *Anemia phyllitidis* to allow the structure of the major antheridium inducing factor in this species to be established as antheridic acid, **1a**. Nakanishi *et al.* had arrived at formula **1b** [7] but this was later refined to **1a** [8] following the total synthesis of the racemates of **1a** and the methyl ester of **1b** [9]. Further proof of the structure and the absolute configuration was provided by a partial synthesis of **1a** from gibberellin A_7(GA_7) (**2**) [10]. Antheridic acid has also been shown to be a natural antheridiogen in further members of the *Anemia* genus, i.e. *A. hirsuta* [11], *A. rotundifolia* and *A. flexuosa* [12] whereas the structure of the more potent antheridiogen in *Lygodium japonicum* (also belonging to the Schizaeaceae) has very recently been found to be 9,11-didehydro-GA_9 methyl ester (GA_{73}-Me) (**3**) [13, 14].

It was not possible to detect compound **1a** in *Anemia mexicana*, despite a thorough computer search based on selected major ions of the mass spectrum, but a further antheridiogen with molecular formula $C_{19}H_{22}O_5$ was obtained [15] demonstrating that structural diversity existed down to the species level. The new substance was shown from its general chemical properties, gas-phase fourier transform IR spectroscopy, and mass spectra to be, like **1a**, a gibberellin-like molecule possessing a γ-lactone function and a non-conjugated carboxy group, but only a single hydroxyl. It was clear from mass spectra, however, that the new compound was quite unlike antheridic acid or any known gibberellin. Following the disclosure of these preliminary structural studies, it has been possible to accumulate sufficient material to measure a ^{1}H NMR spectrum, and to arrive at a plausible hypothesis for the structure of this substance. The details of this study are provided in this paper.

RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of the *A. mexicana* antheridiogen displayed, *inter alia*, a pair of AB doublets (J = 9 Hz) at δ2.70 and 2.85, highly reminiscent of the signals associated with H(5) and H(6) in a large number of gibberellins. Broad singlet resonances at δ4.74 and 4.77 attributable to a 17-methylene group were also observed (although at frequencies which were slightly upfield from the normal range for gibberellins) [16], but no further resonances which could be assigned to an olefinic CH. It was therefore not possible to reconcile these observations with either a simple gibberellane or an antheridane based system in the light of the molecular formula, which would have required a second (tetrasubstituted) double bond to be incorporated into either of these skeletons.

It was concluded provisionally, therefore, that the skeleton of the new antheridiogen incorporated an additional ring, and after taking into account the likely biosynthetic relationships involved in these compounds, that it might be formulated as a 9,15-cyclogibberellane, i.e. correspond to structure **4**. Consistent with this hypothesis was the presence of a further singlet resonance at δ2.40 in the NMR spectrum which could be attributed to the cyclopropyl proton, assuming that anisotropic deshielding from one or more proximal functional groups could be invoked to explain the unusually low chemical shift. Apart from a strong singlet at δ1.09 arising from the

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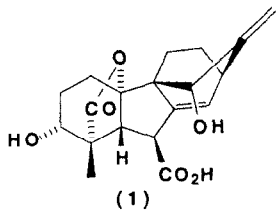
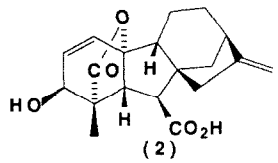


(1)



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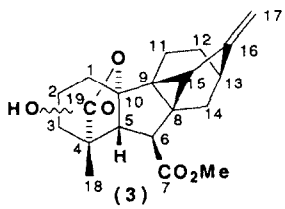
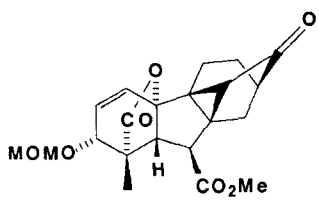
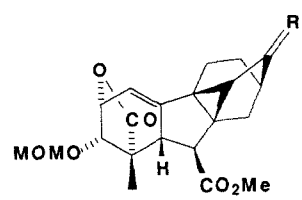


(3)

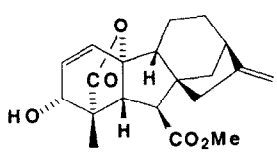
a 1β-OH
b 2α-OH



(4)



(5) R = O
(6) R = CH_2



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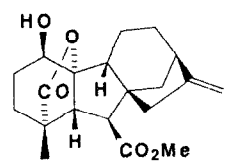


(8)

presumed 4-methyl substituent, the only remaining NMR signal that could be assigned with confidence was a narrow envelope at δ4.15; i.e. this clearly arose from a methine proton associated with the hydroxy function. The most common location for such groups in gibberellins is at C(3), but this possibility may be rejected on the basis of the chemical shift and the absence of a significant peak at *m/z* 129 in the mass spectrum of the methyl ester TMSi ether [17]. Although it was difficult to identify much fine structure in the methine resonance, the narrow signal width ($W_{1/2}$ 8 Hz), severely restricts the choice of possible locations. Molecular modelling [18] of the vicinal dihedral angles in ring C and application of the Karplus equation [19] indicated that a methine proton located at C(11) or C(12) should give rise to a more extensively coupled signal. The possibility that the hydroxyl might be attached to C(14) could not be discounted, although hydroxylation at this position in a gibberellin has never been observed [20]. It therefore seemed most likely that the methine proton was associated with an axial hydroxyl [21] in the A-ring, i.e. either 1β, as in **4a**, or 2α as in **4b**. A fragment ion at *m/z* 116 in the mass spectrum of the silylated methyl ester was consistent with the 1β assignment [22], and it seemed that the 1β hydroxyl would also account for the deshielding of the cyclopropyl H(15). However, the associated de-shielding of H(5) which would have been expected, was not apparent [H(5) shows a chemical shift of δ3.15 in GA_{61} methyl ester (**5b**), for example] [22]. With less than 50 μg of material available there was little prospect of gathering further evidence in support of structure **4a**, much less arriving at a definitive result, but we fortuitously had in hand from the conversion of GA_7 (**2**) into antheridic acid (**1a**) [10] samples of the cyclopropyl ketones **6** and **7**. There were also sufficient quantities of the latter compound to prepare the useful reference sample **8** by means of a Wittig methylenation.

Comparison of NMR data for these three compounds, as well as 3-*epi*-GA_7 methyl ester (**9**) [23] and GA_{61} methyl ester (**5b**) [22] with those of the antheridiogen (Table 1) enabled us to conclude that the provisional structural assignment **4a** was indeed plausible. The chemical shifts of H(6) and the cyclopropyl H(15) fell within an acceptable range, given the variations in A-ring structure, while those of H (17) and H(17′) for **4a** and **8** were not only an excellent match, but distinct from the values normally observed for gibberellins [16]. Comparisons between spectra of **6** and **9** showed that H(5) in **6** was shielded significantly (0.53 ppm) by the neighbouring cyclopropyl ring, and when this increment was subtracted

Table 1. Selected ^{1}H NMR data (δppm)

Compound	H(5)	H(6)	H(15)	H(17,17')
2*	3.25	2.73		4.83, 4.95
4a†	2.70	2.85	2.40	4.74, 4.77
5b‡	3.15	2.73		4.87, 5.00
6§	2.39	3.00	2.17	
7§	2.96	2.96	2.30	
8†	3.00	3.00	2.40	4.74, 4.78
9†	2.92	2.78		4.86, 4.99

*Ref. [16].
†This study.
‡Ref. [22].
§Ref. [10].

from the chemical shift observed for H(5) in GA_{61} methyl ester (**5b**), a value of *ca* δ2.62 could be deduced for H(5) in structure **4a**, reasonably close to the observed shift of δ2.70. Thus, the proximity of the cyclopropyl ring satisfactorily accounts for the *apparent* lack of deshielding by the 1β-hydroxyl (relative to the corresponding gibberellins). The spatial relationships between the 1β-hydroxyl and the neighbouring H(5) and H(15) are illustrated in Fig. 1, which also depicts the location of H(5) within the shielding zone of the cyclopropyl ring.

The above considerations allowed structure **4a** to be assigned to the *Anemia mexicana* antheridiogen with sufficient confidence for us to embark upon a synthesis of **4a** from GA_7 (**2**). This has now been successfully accomplished and will be described elsewhere [24]. A direct comparison has not been possible, but the synthetic and natural material gave rise to virtually identical ^{1}H NMR spectra,* while the derived methyl esters and their TMSi ethers afforded identical mass spectra.† The TMSi ethers also had the same Kovats Retention Index [25].

The range of gibberellin structural types occurring naturally among the higher plants is surprisingly limited [20]. The basic skeleton is constant apart from the presence or absence of C(20), and even then the C-20

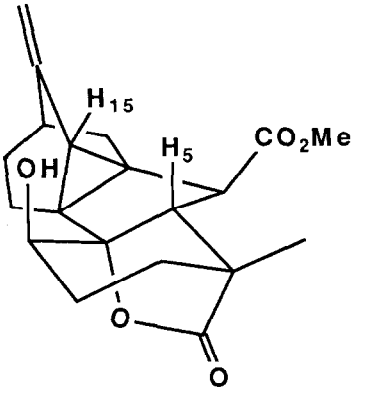
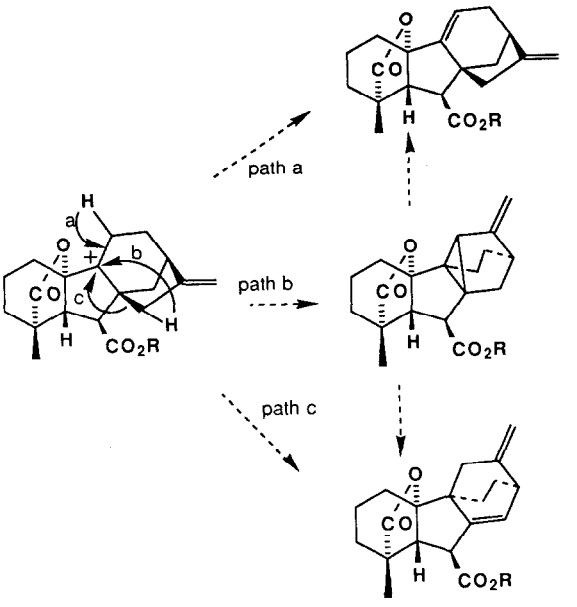


Fig. 1.

*^{1}H NMR spectral measurements and comparisons were carried out by Dr C. L. Willis (University of Bristol).

†Mass spectral measurements and comparisons were carried out by Dr P. Gaskin (University of Bristol).



Scheme 1. Speculations on antheridiogen biosynthesis

gibberellins are present primarily as antecedents to the biologically operative C-19 derivatives. It is as though the standard C-19 skeleton with the associated γ-lactone function has evolved as the optimal arrangement, with variations in the patterns of functionality associated with the number and location of hydroxy groups as well as the occasional additional olefinic bond in the A-ring providing a degree of fine tuning for specific functions. The occurrence of **1b** in several *Anemia* species, of **3** in *Lygodium japonicum*, and now of **4a** in *Anemia mexicana*, leads to a number of fascinating conjectures concerning the evolutionary aspects of the biosynthesis and biological roles of the gibberellins in the plant kingdom. This new collection of compounds provides a major departure from the structural homogeneity associated with the higher plant gibberellins, although it might reasonably be assumed that the new group has a relatively cohesive biogenetic origin based on the generation of an electron deficient centre at C(9) (Scheme 1). Each of the structural types could arise by means of three divergent pathways. Alternatively, the biosynthetic pathways may be more linear, with derivatives of one or more of these compound types serving as an intermediate for another. We have initiated experiments to probe these questions. The most exciting prospect, however, stems from the possibility that this structural variability will be extended amongst other fern species and elsewhere in the plant kingdom.

EXPERIMENTAL

^{1}H NMR spectra were measured on $CDCl_3$ solutions (unless indicated otherwise) on a Varian XL-300 spectrometer (synthetic samples) or a JEOL GX-400 spectrometer [natural material **4a** and comparison (CD_3OD solutions) with synthetic **4a**], with TMS as ref. (δ0.0 ppm).

Antheridiogen **4a**. The isolation and purification of **4a** from *Anemia mexicana* has been reported earlier [15]. Preparation of the synthetic material **4a**, needles, mp 213–214°, $[\alpha]_D^{21}$ −72°

(MeOH; *c* 0.013) has been described elsewhere [24]. The synthetic material gave ^{1}H NMR (300 MHz) 4.82 (1H, *s*, H-17), 4.78 (1H, *s*, H-17), 4.30 (1H, *m*, H-1), 2.99 (1H, *d*, $J_{6,5}$ 8.9 Hz, H-6), 2.70 (1H, *d*, $J_{5,6}$, 8.9 Hz, H-5), 2.44 (1H, *m*, H-13), 2.38 (1H, *s*, H-15), 2.24–2.10 (2H, *m*, H-11α and H-14α), 1.96 (1H, *m*, H-11β), 1.87–1.70 (3H, *m*, H-2β, H-3α and H-3β), 1.67 (1H, *d*, $J_{14\alpha,14\beta}$, 11.3 Hz, H-14α), 1.68–1.53 (3H, *m*, H-12α, H-12β and H-2α), 1.17 (3H, *s*, 4-Me); ^{1}H NMR (300 MHz, CD_3OD), 4.77 (1H, *s*, H-17), 4.74 (1H, *s*, H-17'), 4.15 (1H, *m*, H-1), 2.85 (1H, *d*, $J_{6,5}$, 8.8 Hz, H-6), 2.70 (1H, *d*, $J_{5,6}$, 8.8 Hz, H-5), 2.40 (1H, *s*, H-15), 2.39 (1H, *m*, H-13), 2.16–1.92 (3H, *m*, H-11α, H-14β and H-11β), 1.83–1.66 (3H, *m*, H-2β, H-3α and H-3β), 1.65 (1H, *d*, $J_{14\alpha,14\beta}$, 11.1 Hz, H-14α), 1.66–1.56 (2H, *m*, H-12α and H-12β), 1.51 (1H, *m*, H-2α), 1.09 (3H, *s*, 4-Me).

Lactone **8**. A soln of ketone **7** (5 mg) [10] in dry tetrahydrofuran (0.5 ml) at 0° was treated dropwise with a soln of methylidenetriphenylphosporane until TLC analysis indicated that reaction was complete (10 min). Saturated NH_4Cl soln was then added and the product extracted into Et_2O. After drying ($MgSO_4$) and removal of solvent, the residue was chromatographed on Merck Kieselgel 60. Lactone **8** (4 mg, 80%) was eluted in Et_2O–hexane (1:2). ^{1}H NMR (300 MHz, $CDCl_3$) 5.63 (1H, *dm*, $J_{1,2}$, 5.4 Hz, H-1), 4.78 (1H, *br s*, H-17), 4.74 (2H, *m*, H-2 + H-17'), 4.42, 4.40 (2 × 1H, AB*d*, J = 7.0 Hz, $OC\underline{H}_2OMe$), 3.92 (1H, *d*, $J_{3,2}$, 5.0 Hz, H-3), 3.70 (3H, *s*, CO_2Me), 3.39 (3H, *s*, OCH_2OMe), 3.00 (2H, *s*, H-5 + H-6), 2.40 (1H, *e*, H-13), 2.38 (1H, *s*, H-15), 1.18 (3H, *s*, 4-Me).

3-epi-*Gibberellin* A_7 *methyl ester* (**9**). This compound was prepared by hydride reduction of the 3-oxo derivative as described in ref. [26]. ^{1}H NMR (300 MHz) 6.23 (1H, *dd*, $J_{1,2}$, 9.33 Hz, $J_{1,3}$, 1.9 Hz, H-1), 5.86 (1H, *dd*, $J_{2,1}$, 9.33 Hz, $J_{2,3}$, 2.54 Hz, H-2), 4.99 (1H, *br s*, H-17), 4.86 (1H, *br s*, H-17'), 4.27 (1H, *br s*, H-3), 3.72 (3H, *s*, OMe), 2.92 (1H, *d*, J = 10.94 Hz, H-5), 2.78 (1H, *d*, J = 10.94 Hz, H-6), 2.64 (1H, *m*, H-13), 1.30 (3H, *s*, 4-Me).

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