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GIBBERELLIN A_{117} METHYL ESTER, A NEW ANTHERIDIOGEN FROM *LYGODIUM CIRCINNATUM*

GRAHAM WYNNE, LEWIS N. MANDER,* NOBUYUKI GOTO,† HISAKAZU YAMANE† and TOSHIO OMORI†

Research School of Chemistry, Institute of Advanced Studies, Australian National University, Canberra, A.C.T. 0200, Australia; †Biotechnology Research Center, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

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Key Word Index—*Lygodium circinnatum*; Schizaeaceae; fern; gametophyte; synthesis; structure; bioassay; antheridiogen; gibberellin.

Abstract—The structure of a new gibberellin-like antheridiogen from gametophytes of the fern *Lygodium circinnatum* has been confirmed as the methyl ester of 12α-hydroxy gibberellin A_{73} (GA_{117}) by synthesis of an authentic sample from the 17-nor-16-one derived from GA_{73}. Comparative bioassays of the synthetic compound as an antheridium inducing substance in *Lygodium japonicum* showed that it is highly potent, with activity in the picomolar range, and that it is considerably more active than the 12β-epimer (GA_{96}).

INTRODUCTION

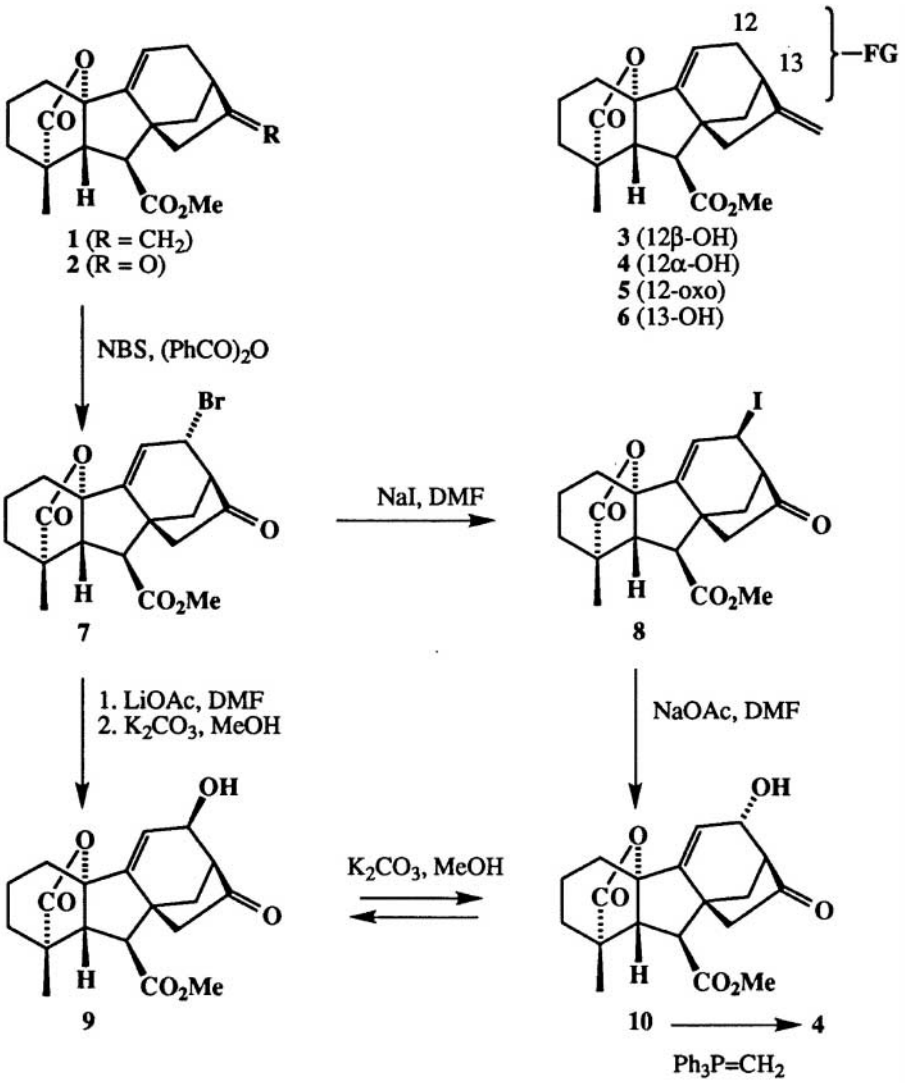
Recent studies on a number of antheridiogens isolated from gametophytes of the fern *Lygodium circinnatum* have led to the identification of several 9,11-didehydro-gibberellin A_9 (GA_{73}) methyl ester derivatives, including the parent **1**, and its 3α-, 3β-, 12β-, and 13-hydroxy derivatives [1]. GCMS data indicated that a further antheridiogen from this species was epimeric with the 12β-hydroxy analogue **3** [2], i.e. that the new compound possessed structure **4** [1]. In order to confirm this tentative assignment and to obtain sufficient material for more extensive biological studies, we have undertaken a synthesis of **4** from bromo ketone **7**, an advanced intermediate [2] in the preparation of **1** and **3**. The details are reported in this paper.

RESULTS AND DISCUSSION

To prepare the methyl ester (**3**) of GA_{96}, ketone **2** had been converted into bromo ketone **7**, reaction of which with lithium acetate, had proceeded with inversion to afford the 12β-acetate. The sequence had then been completed by Lombardo methylenation [3], followed by hydrolysis of the acetate function. An attempt to transform **3** into its 12α-epimer **4** by hydride reduction of ketone **5** was unsuccessful. In the present study, therefore, we decided to pursue an alternative approach based on a double inversion at C-12. Thus, bromide **7** [2] was converted into the unstable iodide **8** with sodium iodide in methyl ethyl ketone, and this product immediately treated with sodium acetate in moist dimethylformamide (DMF) to afford the 12α-hydroxy ketone **10** (Scheme 1). Wittig methylenation of **10** then afforded the target gibberellin **4** as a 5:1 mixture with the 12β-epimer **3**. This partial isomerisation was assumed to occur prior to methylenation as a consequence of a retro-aldol/aldol process, catalysed by the basic conditions of the Wittig reaction. To explore this aspect further, the 12β-acetate derived from the reaction of bromide **7** with lithium acetate in anhydrous DMF, was treated with potassium carbonate in aqueous methanol for an extended period (48 h). This experiment resulted in a 1:1 mixture of the hydroxy ketones **9** and **10**, the vicinal couplings for H-11, H-12 and H-13 in the respective ^{1}H NMR spectra being consistent with the stereochemical assignments, and could be of value for the synthesis of 12-hydroxy gibberellins in the future.

Direct GC-MS comparison of the TMS-ether of synthetic **4** with that of the natural antheridiogen showed that the two samples were identical. According to convention [4], the parent acid corre-

*Author to whom correspondence should be addressed.



Scheme 1.

sponding to **4** is now designated as GA_{117} ($GA_{116} = 12\beta$-hydroxy-GA_{24} [5]).

A bioassay for antheridium induction [6] was conducted on *Lygodium japonicum* protonemata with **1** and **3** as reference GAs across an extensive range of concentrations ($10^{-14} \rightarrow 10^{-8}$ M). The data are summarised in Fig. 1 and show that **4** is active at concentrations as low as 10^{-13} M. Although **4** was less active than GA_{73}–Me (**1**) at equivalent concentrations, with a level of activity similar to that determined previously for the 13-hydroxy isomer **6** [7], it was significantly more potent than the 12β-epimer **3**. This result is not all that surprising, given that the 12α-hydroxy function in **4** occupies a similar spatial location as that of the 13-hydroxyl in **6**.

EXPERIMENTAL

Antheridial formation assay [6]

Spores of *Lygodium japonicum* were sterilised (0.6% NaOCl, 5 min) and sown onto Petri dishes (3 cm diameter) containing 5 ml of fresh 1/10 strength Murashige and Skoog's mineral salts solution [8] solidified with 0.5% agar, and allowed to be imbibed in darkness at 25° for 5 days. The imbibed spores were irradiated for 24 h with red light (0.65 W m^{-2}) to stimulate germination, then immediately *ca.* 140 of the germinated spores were transferred onto a Petri dish (3 cm diameter) containing 5 ml of the fresh medium solidified with 0.5% agar and a test compound at the indicated

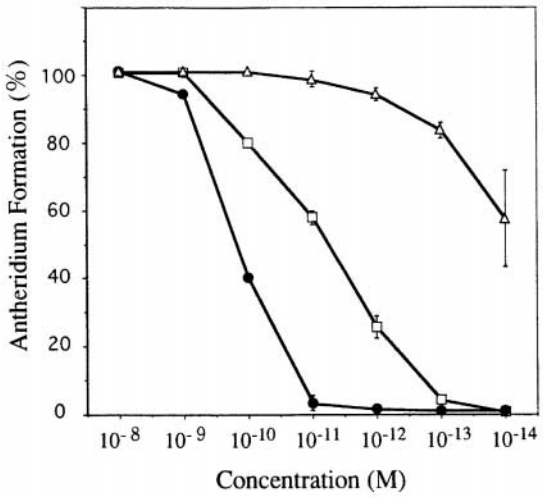


Fig. 1. Antheridium formation activity of GA_{73}–Me (△), GA_{117}–Me (□), GA_{96}–Me (●) on dark–grown protonemata of *Lygodium japonicum*. Each value represents the mean ± SE of the results from two replicates

concentration, and incubated at 25° for 7 days. The resulting protonemata were observed under a microscope to score antheridial formation.

Ent-10*β*-*hydroxy*-12*α*-*iodo*-16-*oxo*-17,20-*dinorgibberell*-9(11)-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester* (**8**)

A soln of bromide **7** (15 mg) in methy ethyl ketone (5 ml) was treated with NaI (156 mg) and the mixture stirred at room temp for 36 h. After dilution with EtOAc, the mixture was washed with aq. $Na_2S_2O_5$, brine, and dried (Na_2SO_4). After removal of solvent, iodide **8** (14 mg), was obtained as a yellow gum which was treated directly with LiOAc as described below. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.91 (1H, *d*, *J* = 3.6 Hz, H-11), 4.24 (1H, *br s*, H-12) 3.76 (3H, *s*, OMe), 2.92 (1H, *m*, H-13), 2.80 (1H, *d*, *J* = 11.3 Hz, H-6), 2.53 (1H, *s*, *J* = 11.3Hz, H-5), 1.14 (3H, *s*, 4-Me).

Ent-12*β*,10*β*-*dihydroxy*-16-*oxo*-17,20-*dinorgibberell*-9(11)-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester* (**10**)

A soln of crude iodide **8** (14 mg) in undried DMF (5 ml) was treated with NaOAc (150 mg) and the mixture stirred at 45° for 92 h. After the solvent was removed under high vacuum, water was added to the residue and the product extracted into EtOAc. This solution was washed with brine and dried (Na_2SO_4) and reduced to dryness. After chromatography on silica gel (EtOAc/hexane, 1:1) hydroxy ketone **10** (6.9 mg) was obtained as a colourless gum. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.91 (1H, *dd*, *J* = 3.7, 1.4 Hz, H-11), 4.26 (1H, approx. *t*, *J* = 3.2 Hz, H-12) 3.77 (3H, *s*, OMe), 2.92 (1H, *m*, H-13), 2.81 (1H, *d*, *J* = 11.7 Hz, H-6), 2.53 (1H, *s*, *J* = 11.7 Hz, H-5), 1.14 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 214.1 (C-16), 178.1 (C-19), 171.4 (C-7), 151.2 (C-9), 124.6 (C-11), 88.2 (C-10), 66.9 (C-12), 57.3 (C-5), 54.8 (C-13), 52.4 (OMe), 51.8 (C-8), 49.8 (C-6), 48.3 (C-4), 46.4 (C-15), 34.8 (C-3), 34.0 (C-14), 30.0 (C-1), 19.4 (C-2), 17.0 (C-18). EI-MS *m*/*z* (rel. int.): 346 $[M]^+$ (28), 315 (30), 304 (41), 302)32), 286 (29), 274 (13), 260 (100), 243 (30), 227 (29), 215 (19), 200 (29), 201 (44), 199 (48), 185 (31), 183 (35), 171 (24), 159 (28), 157 (33), 145 (38), 130 (28), 129 (29), 115 (30), 91 (31), 77 (27). HREI-MS *m*/*z* calcd for $[M]^+$, $C_{19}H_{22}O_6$; 346.1416; found 346.1415.

Ent-12*α*,10*β*-*dihydroxy*-16-*oxo*-17,20-*dinorgibberell*-9(11)-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester* (**9**)

Bromide **7** was converted into the 12*β*-acetate as described previously [3]. A portion of this material (21 mg) was dissolved in MeOH (2 ml) and treated with a stock soln of K_2CO_3/$KHCO_3$ [2 ml, from K_2CO_3 (12.5 g), $KHCO_3$ (2.5 g), H_2O (25 ml)]. After stirring at room temp for 48 h the mixture was diluted with EtOAc, washed with brine and dried. After chromatography on silica gel (EtOAc/hexane, 1:1) the 12*α*-epimer **10** (5 mg, 27%) was obtained as a colourless gum followed by the 12*β*-isomer **9** (6 mg, 32%). Ketol **9**: ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.86 (1H, *dd*, *J* = 2.7, 1.2 Hz, H-11), 4.69 (1H, *dd*, *J* = 5.6, 2.6 Hz, H-12) 3.77 (3H, *s*, OMe), 2.92 (1H, *dd*, *J* = 5.6, 5.9 H-13), 2.77 (1H, *d*, *J* = 11.7 Hz, H-6), 2.60 (1H, *s*, *J* = 11.7Hz, H-5), 1.14 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$): *δ* 214.3 (C-16), 178.2 (C-19), 171.4 (C-7), 148.9 (C-9), 125.9 (C-11), 88.0 (C-10), 70.7 (C-12), 58.0 (C-5), 52.8 (C-6), 52.4 (OMe), 51.5 (C-8), 51.3 (C-15), 49.5 (C-13), 48.4 (C-4), 39.5 (C-14), 34.8 (C-3), 29.9 (C-1), 19.4 (C-2), 17.0 (C-18). EI-MS *m*/*z* (rel. int.): 346 $[M]^+$ (15), 315 (18), 302 (46), 260 (100), 242 (16), 215 (19), 210 (23), 201 (57), 200 (40), 199 (37), 185 (25), 171 (14), 159 (20), 157 (20), 145 (33), 130 (21), 129 (23), 115 (19), 91 (30), 77 (22). HREI-MS *m*/*z* calcd for $[M]^+$, $C_{19}H_{22}O_6$; 346.1416; found 346.1407.

Ent-10*β*,12*β*-*dihydroxy*-17,20-*dinorgibberell*-9(11),16-*diene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester* (**4**)

A soln of ketone **10** (5 mg) in THF (2.5 ml) was treated dropwise with a solution of ylide generated from equimolar amounts of methyltriphenylphosphonium bromide and KO*t*Bu until the yellow colour persisted for more than 5 min. After 6 h, water was added and the product extracted into CH_2Cl_2. After washing with brine the mixture was chromatographed on silica gel (EtOAc/Hexane, 1:1) with a 5:1 mixture of diene **4** and **3** (2.2 mg, 40%)

being eluted first, followed by starting material (3.0 mg). The two dienes were separated by HPLC (Waters Prep NovaPak HR C18 6 μm column (7.8 × 300 mm)-isocratic elution with methanol/water, 65:35). Diene **4**: ^{1}H NMR (300 MHz, $CDC1_3$): δ 5.86 (1H, *dd*, J = 3.7, 1.4 Hz, H-11), 5.14 (1H, *br s*, H-17), 5.0 (1H, *s*, H′-17), 3.99 (1H, *t*, J = 3.4 Hz, H-12), 3.75 (3H, *s*, OMe), 3.02 (1H, *m*, H-13), 2.78 (1H, *d*, J = 11.3 Hz, H-6), 2.56 (1H, *d*, J = 11.3 Hz, H-5), 1.12 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDC1_3$): δ 178.7 (C-19), 171.2 (C-7), 150.5 (C-9), 125.7 (C-11), 88.8 (C-10), 71.5 (C-12), 57.2 (C-5), 53.4 (C-8), 52.1 (OMe), 49.2 (C-6), 48.3 (C-4), 48.2 (C-13), 40.4 (C-15), 35.4 (C-14), 34.9 (C-3), 30.2 (C-1), 19.5 (C-2), 17.1 (C-18). EI-MS m/z (rel. int.): 344 $[M]^+$ (90), 326 (54), 313 (63), 300 (100), 298 (62), 284 (61), 267 (49), 255 (51), 241 (65), 223 (47), 211 (52), 209 (58), 199 (41), 183 (41), 169 (36), 155 (40), 145 (36), 129 (38), 128 (412), 115 (38), 105 (32), 91 (43), 77 (35). HREI-MS m/z calcd for $[M]^+$, $C_{20}H_{24}O_5$; 344.1624; found 344.1625. EI-MS m/z (rel. int.) (12-trimethylsilyl ether-methyl ester): 416 $[M]^+$ (15), 401 (10), 385 (12), 372 (100), 357 (12), 313 (14), 267 (16), 223 (18), 141 (10), 129 (18); KRI: 2522 (lit. [1]: 2521).

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