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AN ANTHERIDIOGEN, 13-HYDROXY-GA_{73} METHYL ESTER (GA_{109}), FROM THE FERN *LYGODIUM CIRCINNATUM*

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

Abstract—The structure of a new gibberellin-like antheridiogen from gametophytes of the fern *Lygodium circinnatum* has been confirmed as the methyl ester of 9,11-didehydro-GA_{20} by synthesis of an authentic sample from gibberellic acid (GA_3). Comparative bioassays of the synthetic compound as an antheridium inducing substance in *Lygodium japonicum* have demonstrated that it is highly potent, showing activity down to 10^{-14} M concentrations, and that the high level of activity is correlated with the incorporation of the 9,11-alkene bond. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Recent studies on a number of antheridiogens isolated from gametophytes of the fern *Lygodium circinnatum* have led to the identification of several gibberellin (GA) derivatives based on 9,11-didehydro-gibberellin A_9 methyl ester, including the parent molecule, GA_{73}-Me (**1**) and three hydroxylated analogues: GA_{88}-Me (**2**), 3-epi-GA_{88}-Me (**3**) and GA_{96}-Me (**4**) [1]. GC-MS data indicated that three further antheridiogens from this species were also monohydroxylated GA_{73} methyl esters, one of which appeared to be the 13-hydroxy analogue (**15**). We have now undertaken the conversion of GA_3 (**5**) into **15** with a view to confirming the tentative structural assignment and to studying the efficacy of **15** as an antheridiogen in *Lygodium japonicum* as compared with similar GAs.

RESULTS AND DISCUSSION

An extensive study on the synthesis of GA_{73} methyl ester (**1**) had established reliable procedures for the preparation of this type of gibberellin [2]. We expected that it should therefore be straightforward to adapt this methodology to the synthesis of the 13-hydroxy analogue (**15**). Thus, GA_3 (**5**) was converted into the known iodolactone (**8**) as described earlier [3] and the elements of HI eliminated by treatment with 1,8-diazabicylo[5.4.0]undec-7-ene (DBU) to give the allylic lactone (**9**). Treatment of this product with zinc bromide in moist ether then led to fission of the lactone ring with formation of the corresponding 1,9-diene 19-carboxylic acid functionality and with concomitant deprotection of the 13-methoxymethyl (MOM) ether to afford (**10**). The loss of the protecting group was somewhat unexpected, as gibberellin 13-MOM ethers are normally stable to these conditions. Presumably, the deprotection was assisted by chelation of the Zn^{2+} ions to the 16-keto function. Hydrogenation of **10** was uneventful, giving the hydroxy acid (**11**) in excellent yield. To access the target molecule **15**, all that was now required was to introduce the $\Delta^{9,11}$ and $\Delta^{16,17}$-olefinic bonds. In the synthesis of GA_{73} methyl ester (**1**), the equivalent goal had been achieved by iodolactonisation of the 13-desoxy acid corresponding to **11**, followed by DBU induced elimination of HI, then methylenation of the 16-norketone. It was not possible to utilise this sequence in the present study, however, as attempted iodolactonisation of **11** under either aqueous or anhydrous conditions gave a mixture of intractable materials. We assumed that the sensitive α-hydroxy ketone functionality in **11** was too labile, even for the relatively mild reaction conditions that were employed, and therefore elected to restore the 17-methylene function before re-attempting the iodolactonisation. Methylenation could be carried out directly on **11**, but low yields were obtained and it was found to be more effective to protect **11** as its *bis*-MOM analogue before executing the Wittig reaction,

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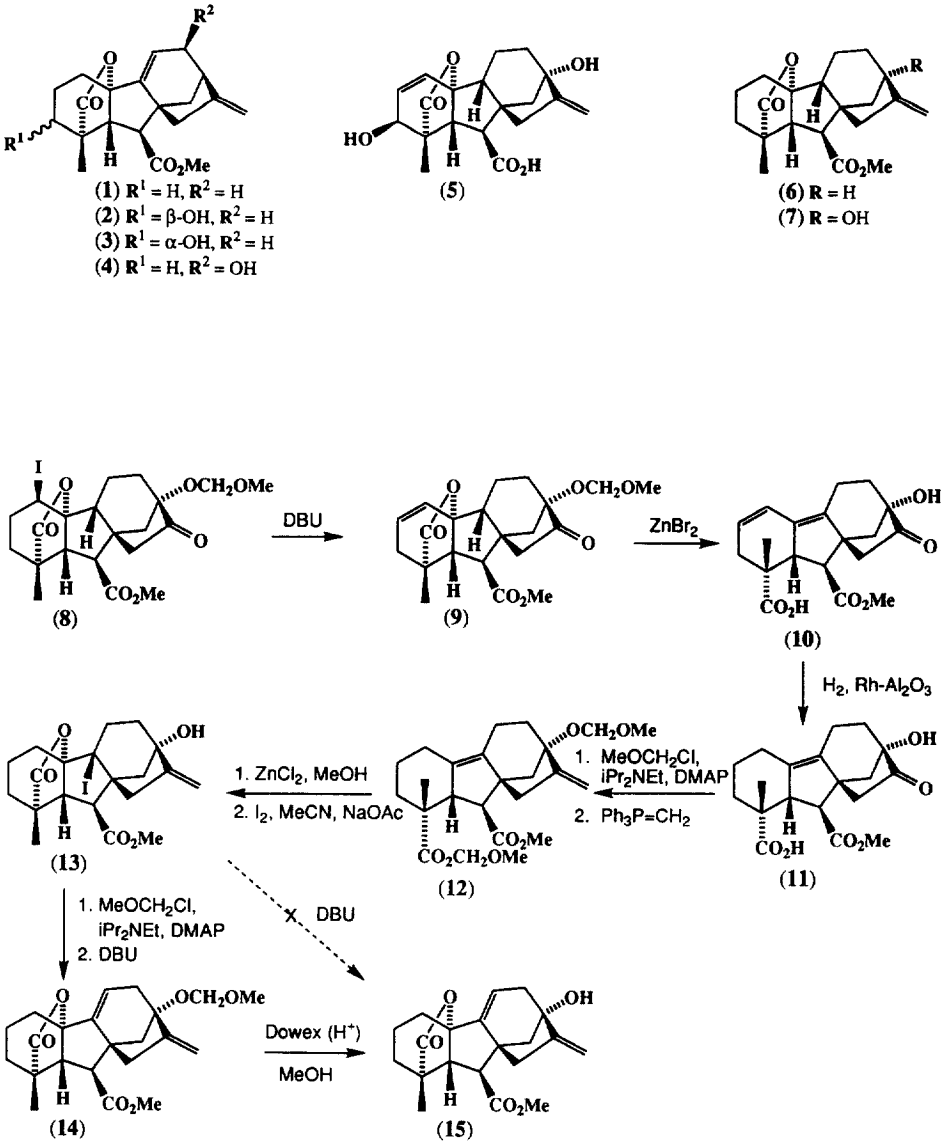

thereby affording **12**. Deprotection of the MOM-ester in **12** was required next, to enable iodolactonisation to be carried out. Unfortunately, it proved impossible to remove the MOM-ester from **12** without removing the 13-MOM ether function as well. Although iodolactonisation proceeded smoothly on the free 13-carbinol to give the desired 9-iodide (**13**), attempted elimination of HI from **13** afforded a mixture of intractable products, and it was found to be essential to reprotect the 13-hydroxy group as its MOM-ether before DBU promoted formation of the $\Delta^{9,11}$-olefin. Having now established a viable route to **14**, removal of the 13-MOM protecting group [4] was straightforward, affording 13-hydroxy-GA_{73} methyl ester (**15**) in good yield. Direct GC-MS comparison of the TMS-ether of synthetic **15** with that of the natural antheridiogen showed that the two samples were identical. According to convention [5], the parent acid corresponding to **15** is now designated as GA_{109} (GA_{108} = 11β-hydroxy-9β,15β-cyclo-GA_9 [6, 7]). Clearly, this synthetic route could be improved by the use of a more robust protecting group for the 13-hydroxy function, but nonetheless, sufficient material was obtained for full characterisation and to assay the effectiveness of **15** as an antheridium inducing substance, i.e. antheridiogen.

The bioassays [8] were conducted on *Lygodium japonicum* protonemata with four reference GAs across an extensive range of concentrations ($10^{-14} \rightarrow 10^{-8}$ M) as summarised in Fig. 1. GA_{109}-Me (**15**) displayed activity down to the 10^{-14} M level, but with approximately one third of the potency of GA_{73}-Me (**1**) at the same concentration. The 13-hydroxy derivative **15** was significantly more potent than the 12β-hydroxy analogue **4**, which was only slightly more active than GA_9-Me (**6**). It was especially interesting to see that the elevation of activity associated with the

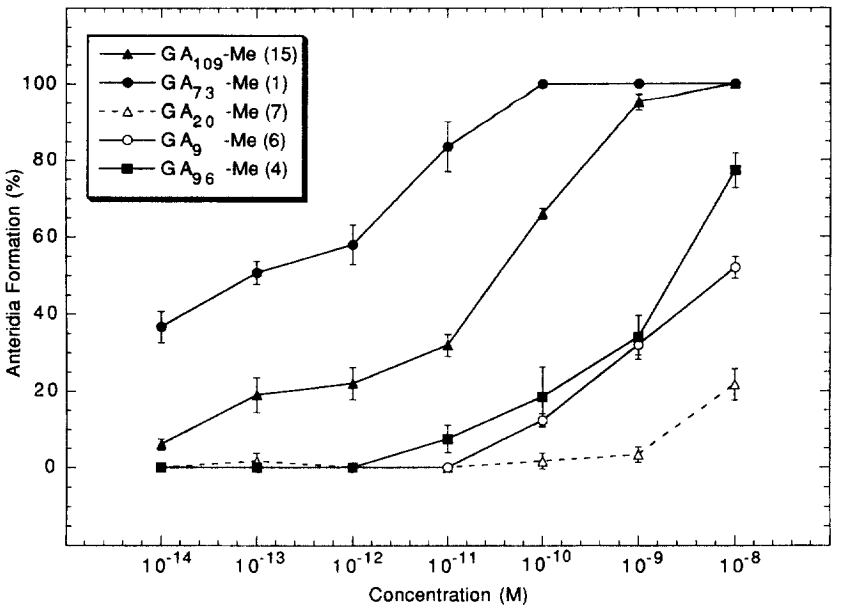

Fig. 1. Antheridium induction in protonemata of the fern *Lygodium japonicum*.

incorporation of the $\Delta^{9(11)}$-alkene bond into GA_{20}-Me (**7**) was equivalent to that observed for GA_{73}-Me (**1**) relative to GA_9-Me (**6**).

EXPERIMENTAL

Antheridial formation assay. Spores of *Lygodium japonicum* were sterilised (0.6% NaOCl, 5 min) and aseptically imbibed with shaking (120 rpm) at 25° C for 5 days in a 15 ml plastic tube containing 10 ml of 1/10-strength Skoog's [9] mineral salts soln. The imbibed spores, suspended in 3 ml of the fresh medium were aseptically sown onto a Petri dish (9 cm diameter) containing 12 ml of the fresh medium solidified with 0.5% agar and irradiated for 24 hr with red light [6] to stimulate germination. A sample containing *ca.* 150 of the germinated spores was transferred onto a well of a 24-well plastic microplate containing 1 ml of the fresh medium solidified with 0.5% agar and a test compound at the indicated concentration, and incubated at 25° for 7 days. The resulting protonemata were observed under a microscope to score antheridial formation.

ent-13-*Methoxymethoxy*-16-*oxo*-17,20-*dinorgibberell*-1-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**9**). A soln of iodide (**8**) (950 mg, 1.83 mmol) in dry CH_2Cl_2 (25 ml) was treated with DBU (2.7 ml, 18.3 mmol) and heated at reflux under a nitrogen atmosphere for 17 hr. The soln was cooled, diluted with CH_2Cl_2 (75 ml), and washed with HCl (2 M, 25 ml), brine (25 ml), dried ($MgSO_4$), filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc 2:1, then 1:1) gave the allylic lactone as a white solid (617 mg, 86% yield), mp 125–127° (EtOAc-hexane). IR ν_{max}^{KBr} cm^{-1}: 1780, 1740, 1630. ^1H NMR (300 MHz, $CDCl_3$): δ 6.16 (1H, *ddd*, J = 9.3, 2.1, 2.1 Hz, H-1), 5.90 (1H, *ddd*, J = 9.2, 3.3, 3.3 Hz, H-2), 4.80 (1H, *d*, J = 7.5 Hz, OCH_2O), 4.64 (1H, *d*, J = 7.4 Hz, OCH_2O), 3.76 (3H, *s*, CO_2Me), 3.36 (3H, *s*, OMe), 2.98 (1H, *d*, J = 10.6 Hz, H-5), 2.81 (1H, *d*, J = 10.5 Hz, H-6), 2.40–2.20 (5H, *m*), 2.20–2.04 (2H, *m*), 2.04–1.9 (2H, *m*), 1.9–1.7 (2H, *m*), 1.23 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 214.7 (C-16), 178.4 (C-19), 171.6 (C-7), 130.8 (C-1), 128.8 (C-2), 92.1 (OCH_2O), 89.2 (C-10), 82.3 (C-13), 56.0 (C-5), 55.3 (OMe), 52.0 (C-9), 51.9 (OMe), 50.8 (C-6), 48.1, 47.5 (C-4, C-8), 47.0 (C-15), 37.6, 37.4 (C-12, C-14), 30.9 (C-3), 17.1 (C-18), 16.4 (C-11). EI-MS *m/z* (rel. int.): 390 $[M]^+$ (10), 359 (65), 341 (20), 330 (29), 317 (32), 302 (52), 285 (73), 271 (93), 257 (77), 241 (81), 225 (33), 213 (94), 197 (100), 183 (56), 171 (41), 155 (82), 143 (72), 129 (51), 105 (43), 91 (55), 71 (35). HREI-MS *m/z* calcd for $[M]^+$, $C_{21}H_{26}O_7$; 390.1679; found 390.1680.

ent-13-*Hydroxy*-16-*oxo*-17,20-*dinorgibberell*-1,9-*diene*-7,19-*dioic acid* 7-*methyl ester* (**10**). A soln of the allylic lactone (**9**) (1.2 g, 3.1 mmol) in moist Et_2O (120 ml) was treated with zinc bromide (10.4 g, 46.2 mmol) and the resulting suspension stirred at room temp, unprotected from atmospheric moisture, for 6 hr. The now homogenous soln was diluted with Et_2O (150 ml), then washed with ice cold HCl (1 M, 50 ml), brine (50 ml), dried ($MgSO_4$), filtered and evaporated to dryness. CC on silica gel (EtOAc–petrol, 1:1, plus 1% AcOH) gave the hydroxy acid **10** (0.94 g, 88%) as a white solid, mp 80–81° (EtOAc-hexane). IR ν_{max}^{KBr} cm^{-1}: 3250, 1750, 1695, 1650. ^1H NMR (300 MHz, $CDCl_3$): δ 6.26 (1H, *ddd*, J = 9.8, 2.4, 2.4 Hz, H-1), 5.82 (1H, *m*, H-2), 3.74 (3H, *s*, CO_2Me), 3.43–3.32 (2H, *m*), 2.80–2.63 (2H, *m*), 2.37 (1H, *dd*, J = 0.8, 2.1 Hz), 2.30–2.09 (3H, *m*), 2.09–1.92 (3H, *m*), 1.92–1.75 (2H, *m*), 1.25 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$):

δ 217.9 (C-16), 181.7 (C-19), 174.3 (C-7), 133.9 (C-10), 128.85 (C-9), 128.8 (C-1), 121.5 (C-2), 80.2 (C-13), 54.1 (C-5), 53.5 (C-4), 51.9 (OMe), 50.0 (C-6), 49.4 (C-15), 44.6 (C-8), 44.5 (C-14), 38.2 (C-3), 36.1 (C-12), 23.9 (C-18), 19.6 (C-11). EI-MS *m/z* (rel. int.): 346 $[M]^+$ (94), 328 (10), 314 (47), 300 (82), 286 (69), 271 (57), 256 (37), 241 (73), 225 (34), 213 (100), 195 (72), 181 (51), 169 (67), 155 (98), 141 (69), 128 (61), 115 (57), 105 (30), 91 (41), 77 (32), 65 (15). HREI-MS *m/z* calcd for $[M]^+$, $C_{19}H_{22}O_6$; 346.4616; found 346.1418.

ent-13-*Hydroxy*-16-*oxo*-17,20-*dinorgibberell*-9-*ene*-7,19-*dioic acid* 7-*methyl ester* (**11**). A soln of diene acid (**10**) (0.94 g, 2.71 mmol) in EtOAc (100 ml) was treated with Rh-Al_2O_3 (5% Rh; 5 mol%, *ca* 300 mg) and stirred under an atmosphere of hydrogen for 42 hr. The resulting suspension was filtered, concentrated, and chromatographed on silica gel (EtOAc–petrol, 2:1, plus 1% AcOH), to give the ene acid **11** as a white solid (842 mg, 89%), mp 68–70° (EtOAc-hexane). IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1700. ^1H NMR (300 MHz, $CDCl_3$): δ 3.72 (3H, *s*, CO_2Me), 3.38 (1H, *d*, $J = 6.8$ Hz, H-6), 3.01 (1H, *m*, H-5), 2.6–2.4 (2H, *m*), 2.27 (1H, *d*, $J = 10.7$ Hz), 2.2–2.05 (5H, *m*), 1.95–1.7 (6H, *m*), 1.5–1.4 (2H, *m*), 1.22 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 217.8 (C-16), 182.5 (C-19), 174.8 (C-7), 131.5, 130.8 (C-9, C-10), 80.3 (C-13), 56.9 (C-5), 53.0 (C-4), 51.8 (OMe), 50.7 (C-6), 49.4 (C-15), 47.4 (C-8), 44.9 (C-14), 37.9 (C-3), 36.2 (C-12), 25.0 (C-18), 24.7 (C-1), 22.0 (C-2), 19.7 (C-11). EI-MS *m/z* (rel. int.): 348 $[M]^+$ (19), 330 (5), 316 (10), 302 (53), 288 (100), 274 (36), 259 (26), 243 (29), 227 (19), 215 (43), 202 (18), 183 (7), 169 (13), 159 (19), 149 (32), 135 (27), 114 (53), 91 (25), 77 (15). MREI-MS *m/z* calcd for $[M]^+$, $C_{19}H_{24}O_6$; 348.1573; found 348.1571.

ent-13-*Methoxymethoxy*-16-*oxo*-17,20-*dinorgibberell*-9-*ene*-7,19-*dioic acid* 7-*methyl ester* 19-*methoxymethyl ester*. A soln of hydroxy acid **11** (785 mg, 2.25 mmol) in dry CH_2Cl_2 (60 ml) was cooled to 0°, and treated successively with *di*isopropylethylamine (4 ml, 22.5 mmol) and 4-dimethylaminopyridine (70 mg, 0.6 mmol). Chloromethyl methyl ether (1.5 ml, 20.3 mmol), was added over 1 min, and the resulting soln allowed to warm to room temp, stirring in total for 48 hr. The soln was then diluted with CH_2Cl_2 (150 ml), washed with HCl (2 M, 50 ml) and brine (50 ml), dried ($MgSO_4$), filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc, 2:1) gave the *bis*-protected compound as a colourless oil (818 mg, 83%). IR ν_{max}^{film} cm^{-1}: 1740, 1720. ^1H NMR (300 MHz, $CDCl_3$): δ 5.20 (1H, *d*, $J = 6.0$ Hz, $C(O)OCH_2O$), 5.17 (1H, *d*, $J = 6.1$ Hz, $C(O)OCH_2O$), 4.80 (1H, *d*, $J = 7.7$ Hz, OCH_2O), 4.64 (1H, *d*, $J = 7.1$ Hz, OCH_2O), 3.68 (3H, *s*, CO_2Me), 3.42 (3H, *s*, OMe), 3.33 (3H, *s*, OMe), 3.30 (1H, *d*, $J = 6.8$ Hz, H-6), 2.95 (1H, *m*, H-5), 2.60–2.48 (1H, *m*), 2.50 (*d*, $J = 10.4$ Hz), 2.48–2.34 (1H, *m*), 2.23 (1H, *d*, $J = 10.9$), 2.14–1.97 (3H, *m*), 1.97–1.60 (5H, *m*), 1.50–1.21 (2H, *m*), 1.17 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, APT) $CDCl_3$: δ 215.4 (C-16), 174.3, 173.9 (C-7, C-19), 131.3, 130.2 (C-9, C-10), 91.9, 89.6 (OCH_2O), 84.1 (C-13), 57.0 (OMe), 56.9 (C-5), 55.2 (OMe), 52.6 (C-4), 51.4 (OMe), 50.6 (C-6), 47.0 (C-8), 45.7, 45.5 (C-14, C-15), 37.4 (C-3), 34.1 (C-12), 24.5 (C-18), 24.3 (C-1), 21.6 (C-2), 19.2 (C-11). EI-MS *m/z* (rel. int.): 436 $[M]^+$ (34), 405 (8), 391 (54), 347 (51), 331 (100), 317 (52), 301 (66), 285 (74), 271 (68), 241 (82), 227 (68), 213 (68), 199 (48), 185 (32), 171 (41), 157 (58), 143 (45), 129 (48), 117 (35), 105 (28), 92 (43), 77 (21), 69 (16), 59 (22). HREI-MS *m/z* calcd for $[M]^+$, $C_{23}H_{32}O_8$; 436.2097; found 436.2101.

ent-13-*Methoxymethoxy*-16-*oxo*-20-*norgibberell*-9,16-*diene*-7,19-*dioic acid* 7-*methyl ester* 19-*methoxymethyl ester* (**12**). Potassium *tert*-butoxide (530 mg, 4.7 mmol) was added in one portion to a stirred suspension of methyltriphenylphosphonium bromide (1.76 g, 4.9 mmol) in dry THF (50 ml) at room temp. The resultant yellow ylide was stirred at room temp for 0.5 hr, then a soln of the ketone prepared above (430 mg, 1 mmol) in dry THF (40 ml) was added *via* cannula. The soln was stirred at room temp for 19 hr, then quenched by the addition of water (30 ml). The soln was extracted with CH_2Cl_2 (3×70 ml), and the organic layer washed (brine, 30 ml), dried over $MgSO_4$, filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc, 9:1, then 1:1) gave the alkene **12** as a colourless oil (422 mg, 98%). IR ν_{max}^{film} cm^{-1}: 1740, 1660. ^1H NMR (200 MHz, $CDCl_3$): δ 5.21 (2H, *s*, $C(O)O\text{-}CH_2OMe$), 5.06 (1H, *m*, H-17), 4.98 (*br s*, 1H, H'-17), 4.85 (1H, *d*, $J = 7.1$ Hz, OCH_2O), 4.64 (1H, *d*, $J = 7.1$ Hz, OCH_2O), 3.69 (3H, *s*, CO_2Me), 3.44 (3H, *s*, OMe), 3.39 (3H, *s*, OMe), 3.25 (1H, *d*, $J = 7.1$ Hz, H-6), 2.93 (1H, *m*, H-5), 2.50–2.13 (4H, *m*), 2.13–1.57 (8H, *m*), 1.50–1.30 (2H, *m*), 1.17 (3H, *s*, 4-Me). ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.4, 174.3 (C-7, C-19), 151.6 (C-16), 133.7, 128.5 (C-9, C-10), 105.8 (C-17), 91.7, 89.9 (OCH_2O), 84.4 (C-13), 57.4, 57.3, 57.3, 57.2 (C-5, $3 \times$ OMe), 51.6 (C-4), 50.5 (C-6), 48.5 (C-8), 47.4 (C-14), 39.9 (C-15), 38.6, 38.0 (C-3, C-12), 25.1 (C-18), 24.6 (C-1), 22.0 (C-2), 20.4 (C-11). EI-MS *m/z* (rel. int.): 434 $[M]^+$ (16), 389 (36), 372 (100), 357 (57), 340 (89), 318 (59), 311 (27), 296 (50), 283 (59), 269 (47), 253 (26), 239 (24), 223 (49), 197 (19), 183 (21), 155 (22), 142 (13), 129 (21), 105 (15), 85 (13), 71 (17). HREI-MS *m/z* calcd for $[M]^+$, $C_{24}H_{34}O_7$; 434.2305; found 434.2309.

ent-13-*Hydroxy*-16-*oxo*-20-*norgibberell*-9,16-*diene*-7,19-*dioic acid* 7-*methyl ester*. A soln of ester **12** (740 mg, 1.7 mmol) was treated with a soln of $ZnCl_2$ in MeOH (1 M, 34 ml, 34 mmol) and stirred at 70° for 72 hr. The mixture was then cooled, diluted with EtOAc, and washed (20% $Na_2H_2PO_4$, 30 ml. brine, 30 ml). The aq. phase was back extracted (EtOAc, 100 ml), and the combined organic layers dried over $MgSO_4$, filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc, 1:1, plus 1% AcOH) gave the title compound as an off white solid (281 mg, 48%), mp 65–68° (EtOAc-hexane). IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 2860, 1735, 1695, 1660. ^1H NMR (300 MHz, $CDCl_3$): δ 8.80 (1H, *v.br*, CO_2H), 5.08 (1H,

br s, H-17), 4.90 (1H, *br s*, H′-17), 3.68 (3H, *s*, OMe); 3.29 (1H, *d*, $J = 6.7$ Hz, H-6), 2.92 (1H, *br t*, H-5), 2.50–2.30 (2H, *m*), 2.30–2.13 (2H, *m*), 2.13–1.98 (3H, *m*), 1.98–1.51 (4H, *m*), 1.51–1.27 (2H, *m*), 1.27–1.18 (1H, *m*), 1.14 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$) APT: δ 182.9 (C-19), 175.6 (C-7), 153.9 (C-16), 133.6, 128.8 (C-9, C-10), 105.4 (C-17), 79.4 (C-13), 57.1 (C-5), 55.5 (C-4), 52.3 (C-8), 51.6 (OMe), 50.4 (C-6), 47.4 (C-14), 39.6 (C-15), 39.6, 38.1 (C-3, C-12), 25.0 (C-18), 24.6 (C-1), 22.1 (C-2), 20.6 (C-11). EI-MS *m/z* (rel. int.): 346 $[M]^+$ (61), 328 (66), 314 (58), 300 (75), 286 (100), 268 (47), 257 (40), 241 (81), 223 (55), 202 (45), 184 (25), 171 (40), 157 (58), 143 (45), 129 (52), 115 (49), 91 (57), 77 (41). HREI-MS *m/z* calcd for $[M]^+$, $C_{20}H_{26}O_5$; 346.1708; found 346.1776.

ent-9α-*Iodo*-13-*hydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**13**). A soln of the hydroxy acid prepared above (100 mg, 290 μmol) in MeCN (5 ml) was treated successively with NaOAc (28.4 mg, 350 μmol) and iodine (110 mg, 430 μmol). This suspension was stirred at room temp for 21 hr, then diluted with Et_2O (40 ml), and washed ($Na_2S_2O_3$, 5 ml), dried over $MgSO_4$, filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc, 3:1) gave the iodo lactone (**13**) as a white solid (93.3 mg, 69%), mp 135–137 (EtOAc-hexane). IR ν_{max}^{KBr} cm^{-1}: 1775, 1730, 1660. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.23 (1H, *br s*, H-17), 4.93 (1H, *br s*, H′-17), 3.72 (3H, *s*, OMe); 3.06 (1H, *br d*, $J = 17.0$ Hz, H-15β), 2.98 (1H, *d*, $J = 8.4$ Hz, H-5), 2.75–2.55 (1H, *m*), 2.67 (1H, *d*, $J = 8.4$ Hz, H-6), 2.40 (1H, *br d*, $J = 10.9$ Hz, H-14β), 2.25–1.45 (8H, *m*), 1.63 (1H, *d*, $J = 11.4$ Hz, H-14α), 1.12 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$) APT: δ 178.7 (C-19), 172.5 (C-7), 155.8 (C-16), 105.8 (C-17), 96.6 (C-10), 77.0 (C-13), 71.9 (C-8), 56.6 (C-5), 55.8 (C-9), 53.5 (C-6), 52.3 (OMe), 50.5 (C-15), 50.2 (C-4), 46.4 (C-14), 36.8 (C-12), 34.1, 33.8, 33.8 (C-1, C-3, C-11), 20.5 (C-2), 17.1 (C-18). EI-MS *m/z* (rel. int.): 472 $[M]^+$ (26), 441 (4), 413 (39), 345 (62), 327 (11), 313 (85), 285 (64), 267 (53), 241 (100), 223 (13), 207 (25), 157 (32), 143 (30), 129 (39), 91 (51). HREI-MS *m/z* calcd for $[M]^+$, $C_{20}H_{25}O_5I$; 472.0747; found 472.0746.

ent-9α-*Iodo*-13-*methoxymethoxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone*. A soln of alcohol **13** (85 mg, 180 μmol) in dry CH_2Cl_2 (10 ml) was cooled to 0° and treated successively with diisopropylethylamine (160 μl, 0.9 mmol) and 4-dimethylaminopyridine (5 mg, 45 μmol). Chloromethyl methyl ether was then added (90 μl, 0.88 mmol), and the resulting soln warmed to room temperature and stirred for 4 days. The soln was diluted (CH_2Cl_2, 35 ml), washed (brine, 5 ml), dried (Mg_2SO_4), filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc, 3:1) gave the title compound as an oil (78.4 mg, 84%). IR ν_{max}^{film} cm^{-1}: 1780, 1740, 1655. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.11 (*br s*, 1H, H-17), 5.02 (1H, *br s*, H′-17), 4.76 (1H, *d*, $J = 7.3$ Hz, OCH_2O), 4.51 (1H, *d*, $J = 7.3$ Hz, OCH_2O), 3.72 (3H, *s*, CO_2Me), 3.34 (3H, *s*, OMe), 3.02 (1H, *br d*, $J = 17.0$ Hz, H-15β), 2.98 (1H, *d*, $J = 8.4$ Hz, H-5), 2.69 (1H, *d*, $J = 8.4$ Hz, H-6), 2.78–2.52 (2H, *m*), 2.33 (1H, *dd*, $J = 11.4, 3.4$ Hz, H-14β), 2.27–2.05 (3H, *m*), 1.97 (1H, *d*, $J = 11.4$ Hz, H-14α), 1.95–1.66 (2H, *m*), 1.65–1.45 (2H, *m*), 1.12 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$) APT: δ 178.7 (C-19), 172.5 (C-7), 151.9 (C-16), 106.3 (C-17), 96.6 (C-10), 91.9 (OCH_2O), 82.0 (C-13), 71.7 (C-8), 56.6 (C-5), 55.4 (OMe), 53.5 (C-6), 52.2 (OMe), 51.2 (C-15), 50.2 (C-4), 42.9 (C-14), 36.9 (C-12), 33.8, 33.8, 33.7 (C-1, C-3, C-11), 20.5 (C-2), 17.0 (C-18). EI-MS *m/z* (rel. int.): 516 $[M]^+$ (9), 501 (12), 485 (6), 456 (3), 411 (3), 389 (60), 372 (8), 357 (100), 329 (57), 299 (62), 269 (59), 239 (50), 223 (38), 157 (33), 129 (37), 91 (42). HREI-MS *m/z* calcd for $[M]^+$, $C_{22}H_{29}O_6I$; 516.1009; found 516.1018.

ent-13-*Methoxymethoxy*-20-*norgibberell*-9(11),16-*diene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**14**). A soln of the iodide prepared above (57 mg, 110 μmol), in dry CH_2Cl_2 (6 ml) was treated with DBU (165 μl, 1.1 mmol) and heated at reflux for 43 hr. The solvent was then removed *in vacuo* and the residue chromatographed on silica gel (petrol–EtOAc, 4:1, then 2:1), affording the diene **14** as a colourless gum (22.1 mg, 52%). IR ν_{max}^{film} cm^{-1}: 1770, 1735, 1660. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.86 (1H, *dd*, $J = 3.4, 3.4$ Hz, H-11), 5.17 (1H, *s*, H-17), 5.02 (1H, *s*, H′-17), 4.84 (1H, *d*, $J = 7.2$ Hz, OCH_2O), 4.63 (1H, *d*, $J = 7.2$ Hz, OCH_2O), 3.73 (3H, *s*, CO_2Me), 3.39 (3H, *s*, OMe), 2.75 (1H, *dd*, $J = 17.2, 2.9$ Hz, H-12), 2.74 (1H, *d*, $J = 11.4$ Hz, H-6), 2.58 (1H, *d*, $J = 11.4$ Hz, H-5), 2.47–2.00 (4H, *m*), 2.00–1.80 (2H, *m*), 1.80–1.47 (4H, *m*), 1.30–1.15 (1H, *m*), 1.09 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.6 (C-19), 172.1 (C-7), 151.9 (C-16), 146.5 (C-9), 123.4 (C-11), 108.5 (C-17), 92.0 (OCH_2O), 88.4 (C-10), 83.7 (C-13), 57.8 (C-5), 55.4 (OMe), 52.2 (OMe), 51.3 (C-4), 49.0 (C-6), 48.4 (C-8), 43.2, 43.2, 42.8 (C-12, C-14, C-15), 34.9 (C-3), 30.4 (C-1), 19.6 (C-2), 17.0 (C-18). EI-MS *m/z* (rel. int.): 388 $[M]^+$ (45), 370 (16), 356 (95), 343 (72), 326 (100), 311 (92), 299 (75), 283 (99), 267 (60), 255 (47), 239 (94), 223 (60), 209 (37), 197 (46), 181 (36), 167 (37), 155 (51), 141 (46), 129 (50), 115 (43), 91 (52), 69 (55). HREI-MS *m/z* calcd for $[M]^+$, $C_{22}H_{28}O_6$; 388.1886; found 388.1887.

ent-13-*Hydroxy*-20-*norgibberell*-9(11),16-*diene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**15**). A soln of the MOM ether **14** (22.1 mg, 57 μmol) was heated under reflux in MeOH (3 ml) containing Dowex resin (H^+ form) (10 mg) and H_2O (100 μl), for 24 hr. The soln was then cooled, diluted with EtOAc (50 ml) and filtered through Celite. The filtrate was washed (brine, 10 ml), dried over $MgSO_4$, filtered and evaporated to dryness. Chromatography on silica gel (petrol–EtOAc, 1:1) gave 13-hydroxy-GA_{73} Me ester (**15**) (GA_{109}-Me). IR ν_{max}^{film} cm^{-1}: 1765, 1730, 1680. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.87 (1H, *dd*, $J = 3.8, 2.9$ Hz, H-11), 5.25 (1H, *dd*, $J = 2.75, 2.70$ Hz, H-17), 4.96 (1H, *br s*, H′-17), 3.73 (3H, *s*, CO_2Me), 2.77 (1H, *d*, $J = 11.4$ Hz, H-6), 2.63 (1H, *dd*, $J = 17.2, 2.9$ Hz, H-12), 2.60 (1H, *d*, $J = 11.4$ Hz, H-6), 2.45–2.10 (6H,

m), 1.95–1.80 (2H, *m*), 1.80–1.5 (4H, *m*), 1.09 (3H, *s*, 4-Me). ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.7 (C-19), 172.1 (C-7), 155.2 (C-16), 146.6 (C-9), 123.5 (C-11), 107.5 (C-17), 88.4 (C-10), 78.8 (C-13), 57.7 (C-5), 52.1 (OMe), 51.4 (C-4), 48.9 (C-6), 48.4 (C-8), 47.2 (C-14), 43.6, 42.3 (C-12, C-15), 34.8 (C-3), 30.4 (C-1), 19.5 (C-2), 17.1 (C-18). EI-MS *m/z* (rel. int.): 344 $[M]^+$ (67), 326 (46), 312 (64), 300 (100), 284 (65), 270 (30), 256 (34), 241 (97), 227 (15), 217 (45), 197 (54), 183 (44), 171 (31), 155 (40), 141 (43), 131 (26), 115 (45), 105 (34), 91 (54), 77 (38), 65 (21). HREI-MS *m/z* calcd for $[M]^+$, $C_{20}H_{24}O_5$; 344.1624; found 344.1619. GA_{109}-Me TMSi ether: EI-MS *m/z* (rel. int.): 416 $[M^-]$ 416 (84), 401 (20), 385 (14), 372 (100), 357 (35), 344 (8), 313 (92); KRI 2520.

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