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SYNTHESIS AND CONFIRMATION OF STRUCTURE FOR A NEW GIBBERELLIN, 2β-HYDROXY-GA_{12} (GA_{110}), FROM SPINACH AND OIL PALM

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Key Word Index—*Spinacia oleracea*; chenopodiaceae; *Elaeis guineesis*; Palmae; spinach, oil palm; 2β-hydroxy-C_{20}-gibberellins, 2β-hydroxy-GA_{12}, GA_{110}, 2β-hydroxy-GA_{24}.

Abstract—The identity of a new gibberellin (GA) in spinach and oil palm sap has been confirmed as 2β-hydroxy-GA_{12} (GA_{110}) by comparisons of GC-mass spectral data obtained for the trimethylsilyl ether methyl ester derivatives with those of a synthetic sample prepared by means of a 24 step sequence from gibberellic acid; 2β-hydroxy-GA_{24} was also prepared. Experimental details for the latter part of the syntheses are described.

INTRODUCTION

Recently completed syntheses of C_{20}-gibberellins (C_{20}-GAs) have facilitated the identification of several 2β,13-dihydroxy-C_{20} gibberellins in a range of plant species, including the GA_{53} analogue (**1**) (GA_{97}) from spinach, tomato, barley and pea, the GA_{44} derivative (**3**) (GA_{98}) from maize pollen and spinach, and 2β-hydroxy-GA_{19} (**2**) (GA_{99}) from spinach [1, 2]. A new gibberellin (GA) isolated from spinach leaves (*Spinacia oleracea* L.) and oil palm inflorescences (*Elaeis guineesis*) has been tentatively identified as 2β-hydroxy-GA_{12} by comparison of the mass spectra of the Me-TMSi derivatives of the endogenous GAs with a sample derived from compound **17**, prepared originally by incubating *ent*-kaurene-2α,19-diol with resuspended cultures of *Gibberella fujikuroi* B1-41a mutant [3, 4]. With the availability of the bridged ketone **4** [1], used as the precursor to the 2β,13-dihydroxy-C_{20} GAs, we decided to undertake the synthesis of the corresponding 13-deoxy analogues with a view, *inter alia*, to confirming the identity of the putative 2β-hydroxy-GA_{12}.

RESULTS AND DISCUSSION

The methoxymethyl protecting groups were removed [5] from the bridged ketone **4** [1] (available in 16 steps from gibberellic acid), and the 2β-hydroxyl of the resulting diol (**5**) selectively masked to give acetate **6**. The 13-hydroxyl was then removed by treatment of the derived methyl oxalate (**7**) with tri-*n*-butylstannane, following the protocol developed by Dolan and MacMillan [6], and the resulting ketone **8** subjected to oxidative cleavage (oxygenation of the derived potassium enolate) [1, 7] in the expectation that aldehyde **9** would be obtained. Instead, lactone **10** was produced in high yield, apparently as a consequence of the loss of the acetate function followed by an intramolecular Cannizzaro process. Repetition of the sequence with the 2β-benzoate (which was expected to be more stable to the conditions used for enolization of the 19-carbonyl function) resulted in the same outcome. Acetate **8** was therefore hydrolysed and converted into the 2β-methoxymethyl derivative, then subjected to oxidative cleavage, smoothly affording aldehyde **13** in high yield. We expected that there would be difficulty in removing the methoxymethyl function without disturbing the D-ring methylene, but a sufficiently robust protecting group was necessary to survive the rigorous conditions of the next step, namely the Wolf–Kishner reduction to **16** [8]. In the event, treatment of **16** with dimethylboronbromide at −70°, conditions that had proven to be satisfactory for the hydrolysis of the 3β-methoxymethyl group in a synthesis of GA_{36} [7], afforded the target carbinol **17**, but as a 1:2 mixture

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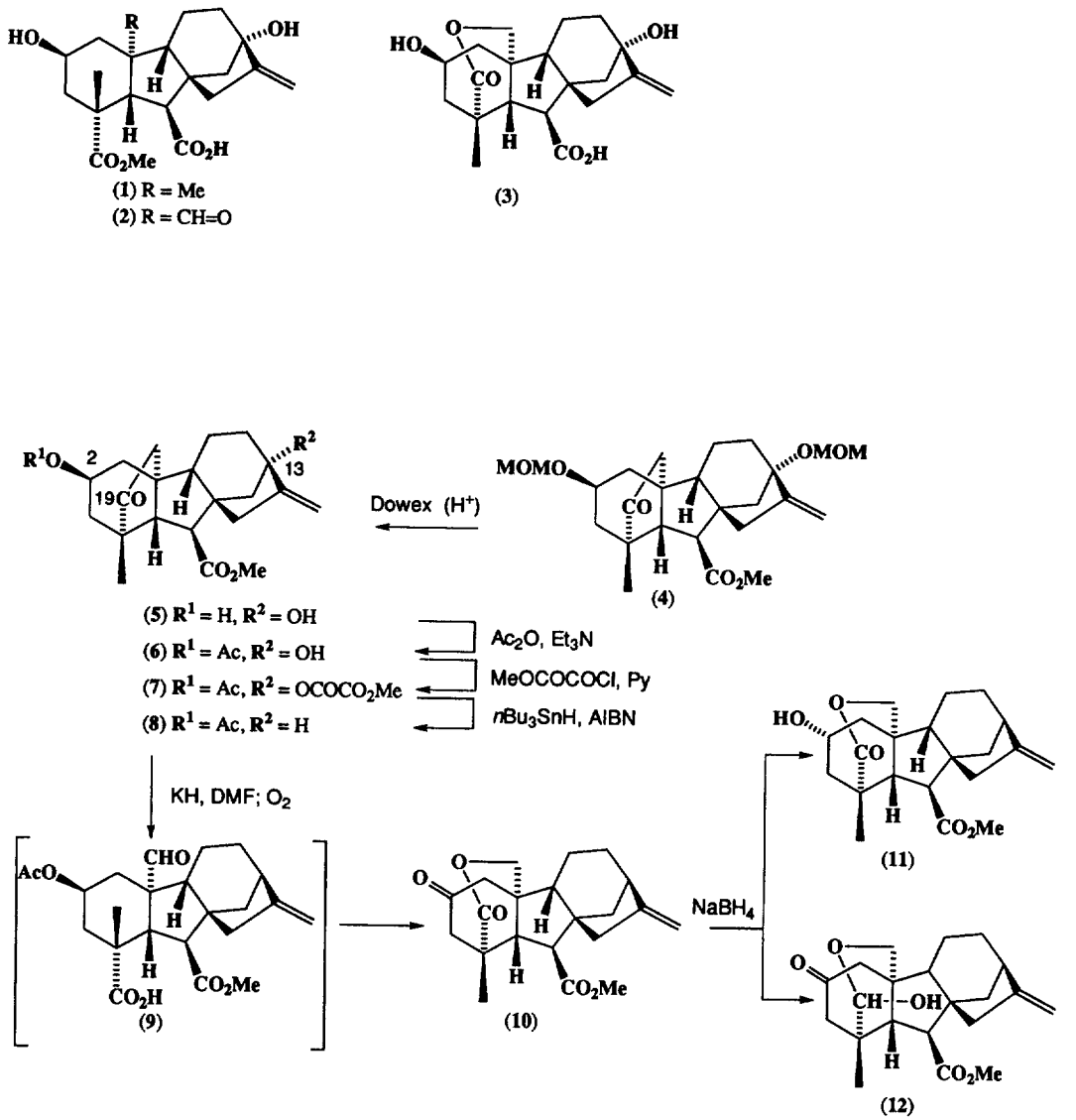
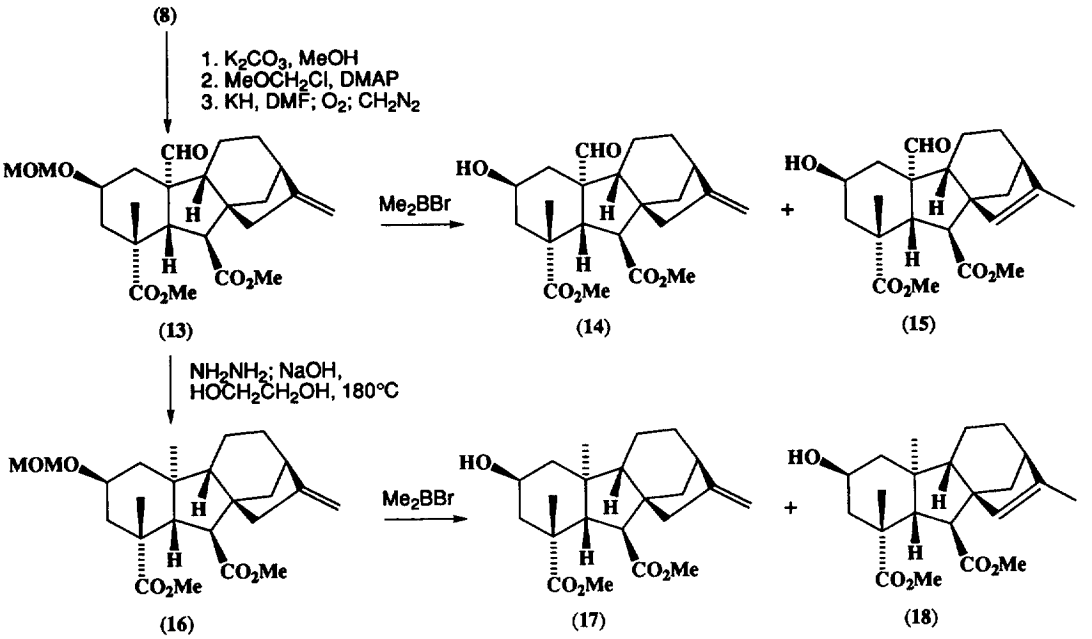


Scheme 1. Attempted preparation of 2β-hydroxy C_{20}-gibberellins.

with the 15-ene isomer **18**. However, the mixture could be resolved on HPLC, and although the overall yield from these two steps was poor, sufficient material was obtained for characterization of the new GA. Deprotection of aldehyde **13** also furnished a 1:2 mixture of 16-ene and 15-ene isomers. An attempt to prepare 2β-hydroxy-GA_{15} by reduction of ketone **10** with sodium borohydride was unsuccessful, a 1:1 mixture of the 2α-hydroxy epimer **11** and the 2-oxo-20,19-hemiacetal **12** being formed; insufficient material was available to attempt an inversion of configuration at C-2.

Comparison of the GC-mass spectral data (TMSi derivative) of the synthetic sample of **17** with those obtained for the new GA from oil palm clearly established that the two GAs were the same. 2β-Hydroxy-GA_{12} was found to be the major component of the oil palm sap, which also contained the 13-hydroxy analogue, i.e. GA_{97} (**1**), 16,17-dihydro-GA_{12}-16,17-diol and 16,17-dihydro-GA_{53}-17-ol (putative). The GAs of the early 13-hydroxylation biosynthetic pathway, namely GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1, GA_8, GA_{29} and GA_{17} were also detected. In addition, direct comparison of the putative 2β-hydroxy-GA_{12} from spinach extracts with the synthetic sample, using full-scan GC-mass spectrometry (Table 1), confirmed the provisional assignment of structure.

According to convention [9], 2β-hydroxy-GA_{12} is now designated as GA_{110} (the previously designated GA number, GA_{109}, has been assigned to 13-hydroxy-GA_{73} [10, 11]). GA_{110} was most abundant in spinach plants grown in short days, and in earlier work, GA_{12} was converted to the 2β-hydroxy derivative by a cell-free system prepared from the leaves [12]. A compound with a mass spectrum similar to that of 2β-hydroxy-GA_{12} was reported in extracts from *Arabidopsis* [13], indicating that this GA may be quite widespread in higher plants. Its co-occurrence with the series of 2β,13-dihydroxy derivatives, GA_{97} (**1**),



Scheme 2. Preparation of 2β-hydroxy GA_{12} dimethyl ester.

Table 1. ^1H NMR spectral data for 2β-hydroxy-GA_{24} dimethyl ester (**14**), 2β-hydroxy-GA_{12} dimethyl ester (**17**), and their 15-ene isomers, **15** and **18**

H	14	15	17	18
2	4.02 *m*	4.02 *m*	4.12 *m*	4.11 *m*
5	2.22 *d* (12.7)	2.25 *d* (12.7)	1.85 *d* (12.5)	1.85 *d* (12.5)
6	3.86 *d* (12.7)	3.83 *d* (12.8)	3.28 *d* (12.5)	3.18 *d* (12.5)
15	2.24 *d* (15.8)	5.49 *br s*	2.19 *d* (15.8)	5.48 *br s*
17	4.85 *br s*	1.66 *s*	4.90 *br s*	1.66 *d* (1.8)
	4.93 *br s*		4.81 *br s*	
18	1.17 *s*	1.17 *s*	1.17 *s*	1.17 *s*
20	9.63 *s*	9.66 *s*	0.69 *s*	0.67 *s*
CO_2CH_3	3.63, 3.73 *s*	3.61, 3.70 *s*	3.66, 3.71 *s*	3.67, 3.70 *s*

GA_{98} (**3**), and GA_{99} (**2**) in spinach leads to speculation about the possibility of an early 2β-hydroxylation pathway in this species.

EXPERIMENTAL

Plant extracts. Spinach (*Spinacia oleracea* L., Savoy Hybrid 612, Harris Seed Co., Rochester, NY) was grown and harvested as described previously [14]. Lyophilized material (25 g) of plants grown in short-day conditions or after exposure to 10 long days was analysed. Extraction, purification and analysis of gibberellins (GAs) by GC-MS was as described [15], except that the gas chromatograph was equipped with a DB-5MS capillary column (30 m × 0.32 mm × 0.25 μm film, J and W Scientific). GA_{110} was located in HPLC fr. 26. EI-MS *m/z* (rel. int.) (Me-TMSi) 448, $[M]^+$ (6), 433 (8), 416 (33), 388 (56), 373 (5), 358 (3), 326 (5), 316 (8), 298 (100), 283 (64), 272 (40), 258 (17), 257 (20), 239 (72), 223 (23), 197 (9), 145 (20). R_I 2570; R_I of synthetic sample: 2568. The higher R_I values (relative to the archive value of 2537 [4] (see below) are consistent with the use of the DB-5MS capillary column.

Bleeding sap (100 ml) from cut mature inflorescences of oil palm, *Elaeis guineesis*, containing 0.02 M sodium diethyl dithiocarbamate as anti-oxidant, was 'spiked' with *ca* 800 Bq each of high specific activity tritiated GA_1, GA_4, GA_9 and GA_{20} and the soln adjusted to pH 3.0 (2 M HCl). The EtOAc-soluble acids obtained from the aq. phase were purified by QAE-Sephadex anion exchange, Sep-Pak C18 cartridge chromatography and RP-HPLC [16]. HPLC frs 31–32 were methylated (CH_2N_2), dried and partitioned between H_2O (0.5 ml) and EtOAc (3 × 0.5 ml). The organic phases were passed through a Bond Elut Aminopropyl (100 mg) column, evapd to dryness, trimethylsilylated and analysed by GC-MS. GA_{110} (Me-

TMSi): EI-MS *m/z* (rel. int.) 448 $[M]^+$ (4), 433 (4), 416 (20), 388 (50), 373 (4), 358 (2), 326 (5), 316 (10), 298 (100), 283 (65), 272 (39), 258 (22), 257 (22), 239 (89), 223 (33), 197 (14), 145 (28); R_I 2552; R_I from archived data: 2537 [4]; R_I of synthetic sample: 2535. The discrepancies in the R_I values arose from running the oil palm sample on a 0.25 micron film thickness OV1 GC column rather than a 0.1 micron column in the other cases.

Methyl ent-2α,13-dihydroxy-19-oxo-19,20-cyclogibberell-16-en-7-oate (**5**). Dowex 50W-X2 resin (290 mg of wet resin) was added to a soln of the cyclopentanone (**4**) (49 mg, 0.11 mmol) in MeOH (17.1 ml) and H_2O (2.83 ml) and the mixt. then heated under reflux for 48 hr. The reaction mixt. was cooled, diluted with EtOAc (50 ml) and filtered through a pad of celite. The filtrate was then washed with brine (10 ml), dried over Na_2SO_4 and the solvent removed *in vacuo*. Chromatography on silica gel, eluting with hexane–EtOAc (2:1–1:2) afforded the desired diol (**5**) (31.5 mg 80%) as a slightly off-white solid. ν_{max} cm^{-1}: 1740. ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-18), 0.80–2.30 (17H, *m*), 2.40 (1H, *d*, J = 12.0 Hz, H-5), 2.52 (1H, *d*, J = 12.0 Hz, H-6), 3.70 (3H, *s*, -CO_2Me), 3.86 (1H, 7 line *multiplet*, J = *ca* 5.5 Hz, H-2), 4.94 (1H, *br s*, H-17), 5.25 (1H, *br s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (C-18), 19.9 (C-11), 38.5 (C-12), 43.9, 44.0, 44.1 (C-20, C-14, C-1), 45.4 (C-15), 47.0 (C-3), 49.4, 49.6 (C-10, C-4), 51.2 (C-6), 51.9 (-CO_2Me), 52.9 (C-8), 53.7 (C-9), 59.2 (C-5), 65.8 (C-2), 78.4 (C-13), 106.9 (C-17), 157.6 (C-16), 173.2 (C-7), 218.5 (C-19). EI-MS *m/z* (rel. int.): 360 $[M]^+$ (100), 328 (53), 301 (69), 241 (36), 157 (22), 135 (68), 121 (31), 105 (38), 91 (55), 69 (42), 55 (55). HRMS: calcd for $C_{21}H_{28}O_5$: 360.1937; found 360.1936.

Methyl ent-2α-acetoxy-13-hydroxy-19-oxo-19,20-cyclogibberell-16-en-7-oate (**6**). Dry triethylamine (0.15 ml, 1.10 mmol, 10 eq) followed by Ac_2O (0.104 ml, 1.10 mmol, 10 eq) was added to a soln of the diol **5** (50 mg, 0.11 mmol) in dry CH_2Cl_2 (7 ml), and the reaction mixt. was left stirring. After 24 hr, ice was added to quench the reaction. After stirring for 10 min, the reaction mixt. was diluted with EtOAc (50 ml) and acidified with Na_2HPO_4 soln (20%, 25 ml), then the layers were sepd and the aq. phase extracted with EtOAc (2 × 10 ml). The combined organic phases were washed with brine (3 × 10 ml) to pH 4, dried over Na_2SO_4 and the solvent removed *in vacuo*, to yield the monoacetate (**6**) (47 mg, 85% crude). The monoacetate was used without further purification. A small portion was purified for characterization by chromatography on silica gel, eluting with hexane–EtOAc (1:2). $\nu^{CHCl_3}_{max}$ cm^{-1}: 1735. ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-18), 1.20–2.40 (16H, *m*), 2.00 (3H, *s* -COMe), 2.40 (1H, *d*, J = 12.0 Hz, H-5), 2.54 (1H, *d*, J = 12.0 Hz, H-6), 3.70 (3H, *s*, -CO_2Me), 4.88 (1H, *m*, H-2), 4.93 (1H, *br s*, H-17), 5.25 (1H, *br s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (C-18), 19.9 (C-11), 21.0 (COMe), 38.5 (C-12), 41.7 (CH_2), 42.6 (CH_2), 43.3 (CH_2), 44.0 (CH_2), 44.4 (CH_2), 49.2, 49.5 (C-10, C-4), 51.2 (C-6), 51.9 (-CO_2Me), 52.9 (C-8), 53.8 (C-9), 59.0 (C-5), 68.2 (C-2), 78.3 (C-13), 106.8 (C-17), 157.6 (C-16), 170.2 (-COMe), 173.0 (C-7), 217.4 (C-19). EI MS *m/z* (rel. int.): 402 $[M]^+$ (5), 371 (13), 356 (6), 342 (100), 310 (90), 300 (98), 282 (52), 253 (25), 241 (39), 157 (18), 135 (45), 121 (24), 105 (34), 91 (35), 78 (21), 55 (27). HREI MS *m/z* calcd for $[M]^+$, $C_{23}H_{30}O_6$: 402.2042; found 402.2041.

Methyl ent-2α-acetoxy-13-methyloxalyloxy-19-oxo-19,20-cyclogibberell-16-en-7-oate (**7**). Diisopropylethylamine (68 μl, 0.39 mmol, 6 eq), plus a catalytic amount of 4-dimethylaminopyridine, followed by methyloxalyl chloride (35 μl, 0.39 mmol, 6 eq) were added to a soln of the monoacetate **6** (26 mg, 0.065 mmol) in dry CH_2Cl_2 (7 ml) and the reaction mixt. was left stirring for 24 hr. The reaction mixt. was diluted with EtOAc (50 ml) and washed with satd $NaHCO_3$ soln (15 ml) and brine (15 ml). The combined aq. phases were back-extracted with EtOAc (2 × 10 ml), then the combined organic phases were dried over Na_2SO_4, and the solvent removed *in vacuo*. Chromatography on silica gel, eluting with hexane–EtOAc (2:1) afforded **7** (24.6 mg, 80%) as a colourless oil. $\nu^{CHCl_3}_{max}$ cm^{-1}: 1770, 1745, 1740. ^1H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-18), 1.20–2.50 (15 H, *m*), 2.00 (3H, *s*, -COMe), 2.41 (1H, *d*, J = 11.9 Hz, H-5), 2.55 (1H, *d*, J = 11.9 Hz, H-6), 3.69 (3H, *s*, -CO_2Me), 3.88 (3H, *s*, (OCOCO_2Me), 4.88 (1H, 7-line *multiplet*, H-2), 5.06 (1H, *br s*, H-17), 5.26 (1H, *br s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (C-18), 19.7 (C-11), 21.1 (-COMe), 36.2 (C-12), 39.3 (C-20), 41.7 (CH_2), 42.6 (CH_2), 43.2 (CH_2), 43.3 (CH_2), 49.1 (C-10), 50.4 (C-4), 50.8 (C-9), 52.0 (-CO_2Me), 52.8 (C-8), 53.4 (C-6), 53.5 (OCOCO_2Me), 58.9 (C-5), 68.1 (C-2), 87.5 (C-13), 109.1 (C-17), 152.1 (C-16), 156.3, 158.3 (OCOCO_2Me), 170.2 (-COMe), 172.7 (C-7), 217.0 (C-19). EI MS *m/z* (rel. int.): 488 $[M]^+$ (2), 457 (11), 428 (43), 396 (68), 368 (10), 324 (100), 282 (33), 265 (30), 237 (16), 223 (35), 181 (15), 129 (15), 94 (24). HREI MS *m/z*: calcd for $[M]^+$ $C_{26}H_{32}O_9$ 488.2046; found 488.2046.

Methyl ent-2α-acetoxy-19-oxo-19,20-cyclogibberell-16-en-7-oate (**8**). Methyloxalyl ester **7** (50 mg, 0.10 mmol) was dissolved in dry C_6H_6 (5 ml) under a N_2 atmosphere. Tributyltin hydride (75 μl, 0.3 mmol, 3 eq) was added and the reaction mixt. was heated under reflux while catalytic amounts of AIBN were added at 30 min intervals. After 2 hr, the solvent was removed *in vacuo* and after repeated chromatography on silica gel, eluting with hexane–EtOAc (5:1), ketone **5** (21.7 mg, 55%) was obtained as a colourless oil. $\nu^{CHCl_3}_{max}$ cm^{-1}: 1735. ^1H NMR (300 MHz, $CDCl_3$): δ 0.94 (3H, *s*, H-18), 1.10–2.40 (15H, *m*), 2.00 (3H, *s*, -COMe), 2.39 (1H, *d*, J = 11.9 Hz, H-5), 2.51 (1H, *d*, J = 11.9 Hz, H-6), 2.62 (1H, *m*, H-13), 3.68 (3H, *s*, -CO_2Me), 4.84 (1H, *s*, H-17), 4.89 (1H, *m*, H-2), 4.96 (1H, *s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 16.8 (C-18), 19.9 (C-11), 21.1 (-COMe), 31.7 (C-12), 36.3 (C-20), 38.9 (C-13), 41.8 (C-14), 42.8 (C-1), 43.5 (C-15), 45.4 (C-3), 49.5 (C-10), 51.4 (C-6), 51.7 (C-4 + -CO_2Me), 52.7

(C-8), 54.1 (C-9), 58.6 (C-5), 68.3 (C-2), 107.0 (C-17), 157.1 (C-16), 170.2 (COMe), 173.4 (C-7), 217.8 (C-19). EI MS *m/z* (rel. int.): 386 $[M]^+$ (1), 355 (25), 326 (100), 295 (98), 284 (100), 266 (50), 239 (12), 225 (37), 183 (14), 155 (12), 129 (12), 105 (19), 79 (11). HREI MS *m/z*: calcd for $[M]^+$, $C_{23}H_{30}O_5$: 386.2093; found 386.2092.

ent-20-Hydroxy-2-oxo-gibberell-16-ene-7,19-dioic acid 7-methyl ester 19,20-lactone (**10**). An excess of dry (oil free) potassium hydride (approximately 64 mg, 1.6 mmol) was added to a soln of the cyclopentanone **8** (32 mg, 0.083 mmol) in a mixt. of dry THF (5 ml) and dry DMF (5 ml) at 0° with stirring under an atmosphere of N_2. The reaction mixt. was left stirring for 2 hr, after which time the reaction flask was thoroughly flushed with N_2 before a steady stream of dry O_2 gas was passed through the soln. After 20 min, the reaction was thoroughly flushed with N_2 then carefully quenched with MeOH (Safety Screen!) and the solvent removed *in vacuo*. The DMF was removed under high vacuum with gentle heating. The solid residue was dissolved in H_2O (20 ml) and EtOAc (50 ml), the layers were sepd and the aq. phase was extracted with EtOAc (2 × 20 ml). The combined organic phases were washed with brine (10 ml), dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The residue was dissolved in MeOH (10 ml) and treated with a small excess of diazomethane (persistent yellow colour), then the solvent removed under a gentle stream of N_2. Purification on silica gel, eluting with hexane–EtOAc, 3:1, afforded lactone **10** (22 mg, 74%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (3H, *s*, H-18), 0.80–2.30 (9H, *m*), 2.39 (1H, *d*, J = 15.4 Hz, H-3), 2.49 (2H, *s*, H-1), 2.66 (1H, *m*, H-13), 2.74 (1H, *d*, J = 15.4 Hz, H-3), 2.80 (1H, *d*, J = 12.6 Hz, H-5), 2.85 (1H, *d*, J = 12.6 Hz, H-6), 3.72 (3H, *s*, CO_2Me), 4.11 (1H, *d*, J = 12.1 Hz, H-20), 4.38 (1H, *d*, J = 12.1 Hz, H-20), 4.83 (1H, *br s*, H-17), 4.95 (1H, *br s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.8 (C-11), 22.3 (C-18), 31.1 (C-12), 36.3 (C-14), 39.1 (C-13), 43.5 (C-15), 44.6 (C-1), 46.2 (C-4), 50.7 (C-3), 51.1 (C-6), 52.1 (C-5), 52.2 (CO_2Me), 52.7 (C-8), 53.3 (C-10), 55.1 (C-9), 74.2 (C-20), 107.3 (C-17), 155.8 (C-16), 173.0, 173.8 (C-7, C-19), 205.0 (C-2). EI MS *m/z* (rel. int.): 358 $[M]^+$ (70), 326 (37), 298 (66), 253 (100), 211 (22), 143 (23), 129 (28), 121 (23), 105 (28), 91 (49), 77 (27), 69 (33), 55 (34). HREI MS *m/z*: calcd for $[M]^+$ $C_{21}H_{26}O_5$: 358.1780; found 358.1780.

Methyl ent-2α-benzoyloxy-13-hydroxy-19-oxo-19,20-cyclogibberell-16-en-7-oate. Dry triethylamine (0.26 ml, 1.76 mmol, 10 eq) followed by benzoyl chloride (0.205 ml, 1.75 mmol, 10 eq) was added to a soln of diol **5** (64 mg, 0.18 mmol) in dry CH_2Cl_2 (10 ml), and the reaction mixt. was left stirring for 24 hr. Satd $NaHCO_3$ was then added to quench the reaction. After stirring for 10 min, the reaction mixt. was diluted with EtOAc (50 ml), the layers were sepd and the aq. phase was extracted with EtOAc (2 × 10 ml). The combined organic phases were washed with brine (10 ml), dried over Na_2SO_4 and the solvent removed *in vacuo*. Chromatography on silica gel (hexane–EtOAc, 1:1) afforded the desired monobenzoate (48.8 mg, 60% crude) as a colourless oil. $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735, 1715. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, *s*, H-18), 1.20–2.50 (16H, *m*), 2.43 (1H, *d*, J = 11.7 Hz, H-5), 2.62 (1H, *d*, J = 11.7 Hz, H-6), 3.71 (3H, *s*, -CO_2Me), 4.94 (1H, *br s*, H-17), 5.15 (1H, 7-line *multiplet*, H-2), 5.25 (1H, *br s*, H′-17), 7.40–8.00 (5H, *m*, $C_6H_5CO_2$-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.8 (C-18), 19.8 (C-11), 38.5 (C-12), 41.8 (CH_2), 42.7 (CH_2), 43.3 (CH_2), 44.0 (CH_2), 44.3 (CH_2), 49.2, 49.5 (C-10, C-4), 51.1 (C-6), 51.9 (CO_2Me), 52.9 (C-8), 53.8 (C-9), 59.0 (C-5), 68.8 (C-2), 78.2 (C-13), 106.9 (C-17), 128.3, 129.4, 129.9, 133.0 ($C_6H_5CO_2$), 157.6 (C-16), 165.6 ($C_6H_5CO_2$), 173.8 (C-7), 217.4 (C-19). EI MS *m/z* (rel. int.): 464 $[M]^+$ (4), 433 (15), 356 (12), 342 (100), 310 (64), 282 (41), 241 (29), 135 (29), 105 (86), 77 (48), 55 (20). HREI MS *m/z*: calcd for $C_{28}H_{32}O_6$: 464.2199; found 464.2198.

Methyl ent-2α-benzoyloxy-13-methyloxalyloxy-19-oxo-19,20-cyclogibberell-16-en-7-oate. This compound was prepd as described for acetate **7**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (3H, *s*, H-18), 1.20–2.50 (15H, *m*), 2.45 (1H, *d*, J = 11.9 Hz, H-5), 2.64 (1H, *d*, J = 11.9 Hz, H-6), 3.71 (3H, *s*, -CO_2Me), 3.89 (3H, *s*, $OCOCO_2$Me), 5.06 (1H, *br s*, H-17), 5.15 (1H, 7-line *multiplet*, H-2), 5.27 (1H, *br s*, H′-17), 7.40–8.00 (5H, *m*, $C_6H_6CO_2$-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 18.8 (C-18), 19.7 (C-11), 36.2 (C-20), 39.3 (C-20), 41.8 (CH_2), 42.7 (CH_2), 43.2 (CH_2), 43.4 (CH_2), 49.2, 50.4 (C-10, C-4), 50.8 (C-9), 52.0 (CO_2Me), 52.9 (C-8), 53.4 (C-6 + $OCOCO_2$Me), 58.9 (C-5), 68.7 (C-2), 87.5 (C-13), 109.1 (C-17), 128.3, 129.5, 129.9, 133.0 ($C_6H_6CO_2$-), 152.1 (C-16), 156.3, 158.2 ($OCOCO_2$Me), 165.6 ($C_6H_6CO_2$), 172.7 (C-7), 216.9 (C-19). EI MS *m/z* (rel. int.): 550 $[M]^+$ (1), 519 (7), 428 (58), 396 (62), 386 (52), 324 (84), 292 (28), 282 (38), 265 (24), 223 (30), 105 (100), 91 (22), 77 (34), 59 (20). HREI MS *m/z*: calcd for $[M\text{-}OMe]^+$ $C_{30}H_{31}O_8$: 519.2019; found 519.2020.

Methyl ent-2α-benzoyloxy-19-oxo-19,20-cyclogibberell-16-en-7-oate. This intermediate was prepd as described for acetate **8**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, *s*, H-18), 1.10–2.60 (15H, *m*), 2.42 (1H, *d*, J = 11.9 Hz, H-5), 2.59 (1H, *d*, J = 11.9 Hz, H-6), 2.62 (1H, *m*, H-13), 3.69 (3H, *s*, -CO_2Me), 4.84 (1H, *br s*, H-17), 4.96 (1H, *br s*, H′-17), 5.15 (1H, 7-line *multiplet*, H-2), 7.30–8.00 (5H, *m*, C_6H_6CO-). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.9 (C-18), 19.0 (C-11), 31.7 (C-12), 36.3 (C-20), 38.9 (C-13), 41.9 (CH_2), 42.8 (CH_2), 43.5 (CH_2), 45.4 (C-3), 49.5 (C-10), 51.5 (C-6), 51.7 (-CO_2Me), 51.8 (C-4), 52.8 (C-8), 54.1 (C-9), 58.6 (C-5), 68.9 (C-2), 107.0 (C-17), 128.3, 129.5, 130.0, 133.0 (C_6H_6CO-), 157.1 (C-16), 165.7 (C_6H_6CO-), 173.4 (C-7), 217.8 (C-19). EI MS *m/z* (rel. int.): 448 $[M]^+$ (1), 417 (7), 326 (74), 294 (88), 284 (100), 266 (66), 225 (42), 183 (16), 155 (14), 105 (100), 91 (18), 77 (44). HREI MS *m/z*: calcd for $[M\text{-}OMe]^+$ $C_{27}H_{29}O_4$: 417.2066; found 417.2067.

Oxidative cleavage of methyl ent-2α-benzoyloxy-19-oxo-19,20-cyclogibberell-16-en-7-oate. This procedure

was carried out on 23 mg of material as described for acetate **8**. Purification on silica gel (hexane–EtOAc, 3:1) afforded lactone **10** (14 mg, 70%) as a colourless oil, identical to the lactone prepd from acetate **8**.

Reduction of ent-20-hydroxy-2-oxo-gibberell-16-ene-7,19-dioic acid 7-methyl ester 19,20-*lactone* (**10**). Sodium borohydride (1.3 mg, 0.03 mmol) was added to a soln of the keto-lactone **10** (12 mg, 0.03 mmol) in MeOH (3 ml) at 0°. After 15 min TLC analysis showed that the reaction was complete. The soln was diluted with EtOAc (30 ml) and acidified with Na_2HPO_4 soln (20%, 5 ml). The layers were sepd and the aq. phase was extracted with EtOAc (2 × 10 ml). The combined organic phases were washed with brine (2 × 5 ml), dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. Chromatography on silica gel, eluting with hexane–EtOAc (2:1) afforded compounds **11** and **12**.

Methyl ent 19,20-*epoxy-19ζ-hydroxy-2-oxo-gibberell-16-en-7-oate* (**12**). (4.3 mg, 36%, 2 isomers, approx. 3:1 ratio, only major isomer assigned below). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (3H, *s*, H-18), 0.80–2.20 (9H, *m*), 2.17 (1H, *d*, J = 15.7 Hz, H-3), 2.21 (2H, *s*, H-1), 2.50–2.80 (4H, *m*, H-5, 1 × H3, H-13), 3.68 (1H, *d*, J = 11.6 Hz, H-6), 3.67 (1H, *d*, J = 11,4 Hz, H-20), 3.73 (3H, *s*, -CO_2Me), 4.06 (1H, *d*, J = 11.5 Hz, H-20), 4.74 (1H, *d*, J = 4.4 Hz, H-19), 4.82 (1H, *br s*, H-17), 4.94 (1H, *br s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.9 (C-11), 22.5 (C-18), 31.9 (C-12), 36.2 (C-14), 38.8 (C-4), 39.2 (C-13), 45.6 (C-15), 46.0 (C-1), 50.2 (C-6), 50.7 (C-3), 51.9 (C-5), 52.9 (CO_2Me), 53.3 (C-8), 53.7 (C-10), 54.8 (C-9), 62.3 (C-20), 98.7 (C-19), 106.8 (C-17), 157.1 (C-16), 174.8 (C-7), 208.0 (C-2). EI MS *m/z* (rel. int.): 360 $[M]^+$ (18), 342 (100), 310 (35), 284 (82), 239 (52), 223 (89), 171 (48), 143 (48), 129 (58), 105 (67), 91 (82), 71 (65), 55 (63). HREI MS *m/z*: calcd for $C_{21}H_{28}O_5$: 360.1937; found 360.1936.

ent-2β,20-dihydroxy-gibberell-16-en-7,19-dioic acid 7-methyl ester 19,20-*lactone* (**11**). (3.7 mg, 31%). $\nu^{CHCl_3}_{max}$ cm^{-1}: 1730. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.16 (3H, *s*, H-18), 0.80–2.30 (14H, *m*), 2.11 (1H, *d*, J = 12.6 Hz, H-5), 2.65 (1H, *m*, H-13), 2.84 (1H, *d*, J = 12.6 Hz, H-6), 3.68 (3H, *s*, CO_2Me), 4.24 (1H, *br s*, H-2), 4.33 (1H, *d*, J = 11.3 Hz, H-20), 4.37 (1H, *d*, J = 11.3 Hz, H-20), 4.81 (1H, *br s*, H-17), 4.94 (1H, *br s*, H′-17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.9 (C-11), 24.0 (C-18), 31.4 (C-12), 36.6 (C-14), 39.2 (C-1), 39.3 (C-13), 40.5 (C-3), 44.3 (C-15), 46.2 (C-10), 48.0 (C-4), 49.9 (C-8), 51.4 (C-6), 51.8 (CO_2Me), 52.0 (C-5), 56.9 (C-9), 66.1 (C-2), 73.4 (C-20), 106.7 (C-17), 156.6 (C-16), 173.7, 176.2 (C-7, C-19). EI MS *m/z* (rel. int.): 360 $[M]^+$ (65), 329 (27), 310 (60), 282 (79), 255 (46), 237 (82), 195 (45), 143 (43), 129 (65), 91 (72), 73 (80), 57 (100). HREI MS *m/z*: calcd for $C_{21}H_{28}O_5$: 360.1937; found 360.1936.

Dimethyl ent-2β-methoxymethoxy-20-oxogibberell-16-ene-7,19-dioate (**13**). To a soln of acetate **8** (106 mg) in MeOH (5 ml) was added K_2CO_3 (75 mg) and the mixt. stirred for 16 hr. H_2O was added and the product extracted into EtOAc (2 × 20 ml). After drying and removal of solvent, the crude product was dissolved in CH_2Cl_2 (6 ml), treated with diisopropylethylamine (0.15 ml) and chloromethyl ether (47 mg), then stirred for 2 days at room temp. The mixt. was reduced to dryness, the residue extracted into EtOAc and this soln washed with diluted HCl, $NaHCO_3$ soln, then dried (Na_2SO_4). Chromatography on silica gel (6:1 hexane–EtOAc) afforded the 2β-methoxymethyl ether (86 mg, 80%) as a colourless foam. $\nu^{CHCl_3}_{max}$ cm^{-1}: 1740. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (3H, *s*, H-18), 0.80–2.30 (17H, *m*), 2.40 (1H, *d*, J = 12.0 Hz, H-5), 2.52 (1H, *d*, J = 12.0 Hz, H-6), 2.62 (1H, *m*, H-13), 3.32 (3H, *s*, OCH_2OMe), 3.67 (3H, *s*, -CO_2Me), 3.86 (1H, *m*, H-2), 4.56, 4.72 (2 × 1H, *ABd*, J = 7.1 Hz, OCH_2OMe), 4.84 (1H, *s*, H-17), 4.96 (1H, *s*, H′-17). A portion of this material (33 mg) was subjected to oxidative cleavage as described for acetate **8**. Purification on silica gel, eluting with hexane–EtOAc (5:1) afforded aldehyde **13** as a colourless oil (27.8 mg, 76%). $\nu^{CHCl_3}_{max}$ cm^{-1}: 1725. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.99 (1H, *t*, J = 11.3 Hz, H-1β), 1.17 (3H, *s*, H-18), 1.22 (1H, *m*, H-3β), 1.40–1.90 (5H, *m*), 1.99 (1H, *dt*, J = 15.5 Hz, J = 2.5 Hz, H-15), 2.24 (1H, *d*, J = 15.5 Hz, H′-15), 2.30 (1H, *d*, J = 12.8 Hz, H-5), 2.45 (1H, *dd*, J = 13.2 Hz, J = 1.4 Hz, H-3α), 2.62 (1H, *m*, H-13), 2.68 (1H, *dd*, J = 12.1 Hz, J = 1.3 Hz, H-1α), 3.37 (3H, *s*, -OCH_2OMe), 3.64, 3.73 (2 × 3H, *s*, -CO_2Me), 3.84 (1H, *d*, J = 12.8 Hz, H-6), 3.91 (1H, *m*, H-2), 4.69 (2H, *s*, OCH_2OMe), 4.84 (1H, *br s*, H-17), 4.92 (1H, *br s*, H′-17), 9.63 (1H, *s*, H-20). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.6 (C-11), 27.8 (C-18), 31.6 (C-12), 37.8, 39.0 (C-1, C-14), 39.2 (C-13), 43.8 (C-3), 44.3 (C-4), 45.8 C-15), 49.6 (C-6), 50.5 (C-8), 51.6, 51.7 (2 × CO_2Me), 55.3 (C-9 + OCH_2OMe), 56.0 (C-5), 60.7 (C-10), 70.9 (C-2), 95.5, (OCH_2OCH_3), 106.6 (C-17), 155.5 (C-16), 174.5, 175.8 (C-7, C-19), 204. 3 (C-20). EI MS *m/z* (rel. int.): 434 $[M]^+$ (1), 402 (12), 372 (100), 344 (35), 342 (32), 312 (50), 284 (93), 225 (65), 223 (43).

Dimethyl ent-2α-hydroxy-20-oxogibberell-16-ene-7,19-dioate (**14**) *and dimethyl ent-2α-hydroxy-20-oxogibberell-15-ene-7,19-dioate* (**15**). A stirred soln of methoxymethyl ether **13** (10 mg) in CH_2Cl_2 (5 ml) at −78° was treated with dimethylbromomethane (0.10 ml). After 4 min the reaction was quenched by the addition of satd $NaHCO_3$ soln (5 ml). Further CH_2Cl_2 was added, the organic layer washed with Na_2HPO_4 soln, dried and evapd to dryness. ^{1}H NMR and GC-MS revealed a 1:3 mixt. of 2β-hydroxy-GA_{24} (**14**) with its 15-ene isomer **15**. Sepn was effected by HPLC on a Waters Prep NovaPak HR C18 6 μm column (7.8 × 300 mm)—isocratic elution with MeOH–H_2O (3:2 plus 0.05% HOAc) afforded the 15-ene (**15**) followed by the 16-ene (**14**). EI MS *m/z* (rel. int.): (Me-TMSi): 462 $[M]^+$ (2), 447 (7), 430 (69), 402 (23), 387 (7), 374 (19), 372 (20), 358 (9), 344 (48), 312 (51), 298 (14), 284 (100), 269 (22), 253 (57), 241 (31), 225 (85), 223 (50), 216 (44) (R_I 2613). ^{1}H NMR data are provided in Table 1.

Dimethyl ent-2α-methoxymethoxygibberell-16-ene-

7,19-*dioate* (**16**). Anhydrous hydrazine (0.25 ml) was added to a soln of the aldehyde **13** (30 mg) in ethylene glycol (2 ml) and the reaction mixt. was heated at 100° for 30 min. Half a pellet of NaOH (approximately 200 mg) was added and the temp. was raised to 116° for 1 hr. Finally, the temp. was raised to 178° and the reaction continued overnight. After cooling, the mixt. was diluted with EtOAc–2-butanol (4:1, 50 ml) and was acidified with phosphoric acid (10%, 10 ml). The layers were sepd and the aq. phase was extracted with the EtOAc–2-BuOH mixt. (2 × 20 ml). The combined organic phases were washed with brine to pH 4. The organic phase was dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The residue was dissolved in MeOH (10 ml) and treated with an excess of ethereal CH_2N_2, the solvent was removed under a gentle stream of N_2 and finally purification on silica gel, eluting with hexane–EtOAc (3:1) afforded **16** as a colourless oil (4.2 mg). $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730.^{1}H NMR (300 MHz, $CDCl_3$): δ 0.70 (3H, *s*, H-20), 1.16 (3H, *s*, H-18), 0.80–2.40 (*m*, H-12), 1.97 (1H, *d*, J = 12.5 Hz, H-5), 2.19 (1H, *d*, J = 15.8 Hz, H-15), 2.52 (1H, *ddd*, J = 13.1 Hz, J = 3.2 Hz, J = 1.4 Hz, H-3α), 2.58 (1H, *m*, H-13), 3.38 (1H, *d*, J = 12.5 Hz, H-6), 3.38 (3H, *s*, -$OCH_2O\underline{Me}$), 3.68, 3.72 (2 × 3H, *s*, CO_2Me), 4.01 (1H, *m*, H-2), 4.56, 4.76 (2 × 1H, *ABd*, J = 6.8 Hz, $OC\underline{H_2}$ OMe), 4.83 (1H, *br s*, H-17), 4.90 (1H, *br s*, H′-17).

Dimethyl ent-2α-hydroxygibberell-16-ene-7,19-dioate (**17**) *and dimethyl ent-2α-hydroxygibberell-15-ene-7,19-dioate* (**18**). A stirred soln of methoxymethyl ether **16** (4.2 mg) in CH_2Cl_2 (1 ml) at −78° was treated with dimethylbromoborane (0.10 ml). After 4 min the reaction was quenched by the addition of satd $NaHCO_3$ soln (5 ml). Further CH_2Cl_2 was added, the organic layer washed with Na_2HPO_4 soln, dried and evapd to dryness. ^{1}H NMR and GC-MS revealed a 1:2 mixt. of 2β-hydroxy-GA_{12} (**17**) with its 15-ene isomer (**18**) which was resolved by HPLC on a Waters Prep NovaPak HR C18 6 μm column (7.8 × 300 mm)—isocratic elution with MeOH–H_2O (13:7 plus 0.05% HOAc); the 15-ene eluted first, followed by the 16-ene (**17**). EI MS *m/z* (rel. int.) (Me-TMSi): 448 $[M]^+$ (7), 433 (13), 416 (31), 388 (62), 373 (5), 358 (4), 326 (8), 316 (7), 298(100), 283 (74), 272 (36), 258 (23), 257 (28), 239 (92), 223 (29), 197 (17), 145 (25) (R_I 2535). ^{1}H NMR data are provided in Table 1.

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