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SYNTHESIS AND CONFIRMATION OF STRUCTURE OF THREE 13,15β-DIHYDROXY C-20 GIBBERELLINS, GA_{100}, GA_{101} AND GA_{102}, ISOLATED FROM THE SEEDS OF *HELIANTHUS ANNUUS* L.

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Key Word Index—*Helianthus annuus*; Compositae; sunflower seeds; 13,15β-dihydroxylated C-20 gibberellins; GA_{100}, GA_{101} and GA_{102}; synthesis; selenium dioxide oxidation; Wolff–Kishner reduction.

Abstract—The structures of three 13,15β-dihydroxylated C-20 gibberellins isolated from the seeds of *Helianthus annuus* have been confirmed by partial synthesis of authentic samples from gibberellic acid and GC-mass spectral comparison with the endogenous samples.

INTRODUCTION

Thirteen 15β-hydroxy gibberellins have been isolated from the seeds of the sunflower (*Helianthus annuus* L.) and their structures established [1, 2]. Three of these gibberellins GA_{64} (**1**), GA_{65} (**2**) and GA_{66} (**3**), were identified as C-20 derivatives and their structures established by metabolic transformation of 15β-hydroxykaur-16-en-19-oic acid with cultures of *Gibberella fujikuroi* (B1-41 a mutant). The putative structures, 15β-hydroxy GA_{53} (**4**), 15β-hydroxy GA_{44} (**5**), 15β-hydroxy-GA_{19} (**6**), and 15β-hydroxy GA_{17} (**7**), were assigned to four further C-20 gibberellins, but attempts to confirm these structures by similar metabolism of 13,15β - dihydroxykaur - 16 - en - 19 - oic acid were unsuccessful [1]. We have therefore undertaken the syntheses of compounds **4–7** from the readily available fungal gibberellin, gibberellic acid, in order to confirm the tentative structural assignments. The successful completion of the syntheses is described in this paper.

RESULTS AND DISCUSSION

The GA_{19} derivative **8** is available in eight steps from gibberellic acid [3, 4] and serves as a common intermediate for the preparation of all four targets, following the well-established protocol for the introduction of the 15β-hydroxyl [2, 5, 6], although with some useful improvements to the original procedures. Thus, allylic oxidation of **8** with selenium dioxide and *t*-butylhydroperoxide under sonication readily yielded the 15α-hydroxy derivative. Because of the ease with which the 15α-hydroxy group is prone to react with the 7-methyl ester function to form the 7,15α-lactone, the material was used directly without purification and oxidized to the enone **9** by the Dess–Martin procedure [7], which was found to be more reliable than the Swern method [8] used previously. In this way, the enone **9** was isolated cleanly in an overall yield of 80% from the two steps. Characteristic ^{1}H NMR resonances for the C-17 olefinic protons at δ 5.45 and 6.08, combined with the appearance of the carbonyl resonance at δ 205.1 in the ^{13}C NMR spectrum, were consistent with the enone structure. For the 1,2-reduction of gibberellin 16-en-15-ones to allylic alcohols, the use of zinc and acetic acid [5, 6] has been shown to be superior to the hydride–lanthanide reagents more commonly employed for this transformation [9], although the yield deteriorates when applied to some C-19 GAs containing 13-oxygen substituents, especially with polyhydroxylated GAs [2]. It was therefore a pleasant surprise when the enone **9** was reduced to the desired 15β-hydroxy compound **10** in 91% yield.

Treatment of aldehyde **10** with Dowex resin in methanol under reflux [10] efficiently removed the methoxymethyl protecting group in 80% yield, thereby providing the first of the target compounds, 15β-hydroxy GA_{19} dimethyl ester (**11**) (57% overall yield from the starting aldehyde **8**). Reduction of the aldehyde **10** with sodium borohydride in methanol at 0°C cleanly provided the expected 15β-hydroxy GA_{44} derivative **12** in 67% yield, and then removal of the methoxymethyl protecting group by treatment with

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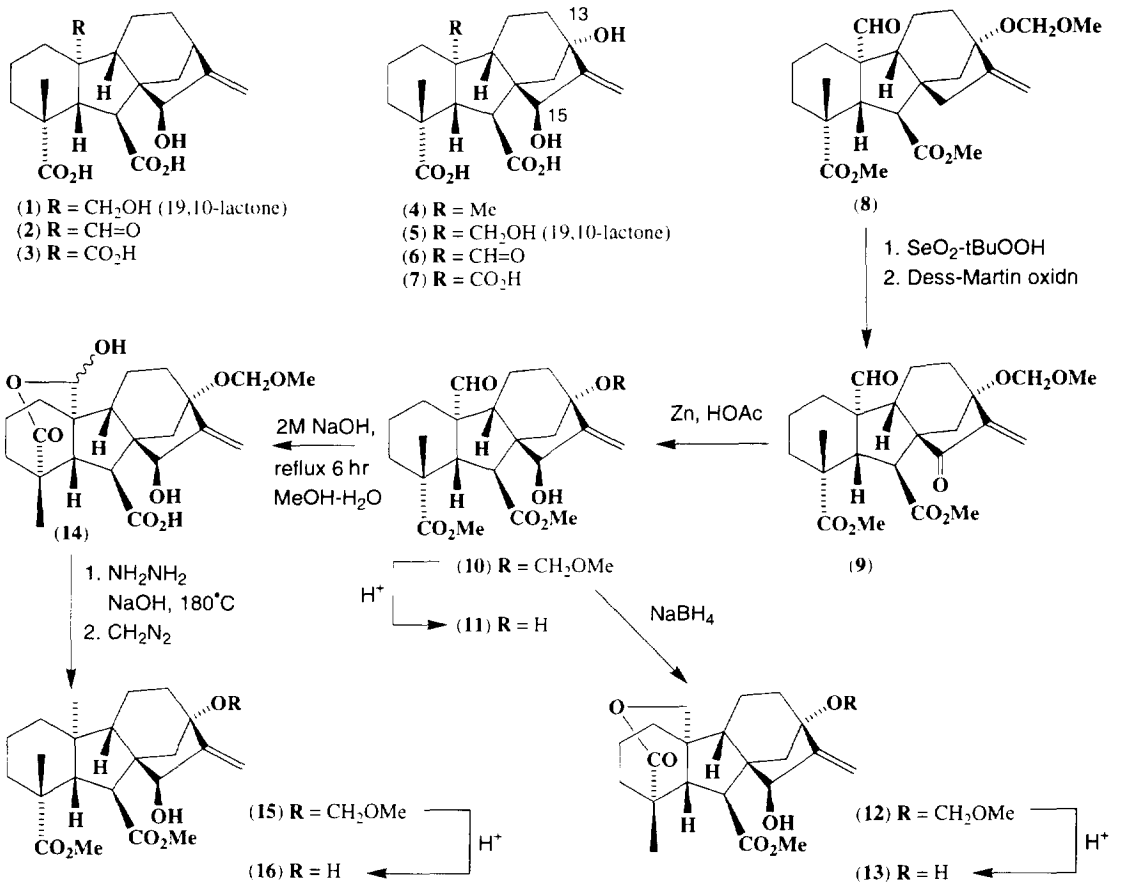

Scheme 1. Preparation of 13,15β-dihydroxy C-20 gibberellins.

Dowex resin in methanol under reflux yielded 15β-hydroxy GA_{44} methyl ester (**13**), in 77% yield.

The synthesis of the dimethyl ester of 15β-hydroxy GA_{53} (**6**) was based on the conditions optimized for the synthesis of the parent GA_{53} [11]. Thus, hydrolysis of the dimethyl ester **10** to **14**, followed by Wolff–Kishner reduction at the carefully regulated temperature of 180° and treatment of the crude product with diazomethane, yielded the 15β-hydroxy GA_{53} derivative **15** in 35% yield. The identity of the product was apparent from the ^{1}H NMR spectrum which showed characteristic resonances for the 15α-proton at δ 4.02, and the 10α-methyl group at δ 0.69. Finally, treatment of compound **15** with Dowex resin in methanol under reflux yielded the desired 15β-hydroxy GA_{53} dimethyl ester (**16**) in 67% yield.

While the synthesis of the trimethyl ester of 15β-hydroxy-GA_{17} (**7**) was not attempted, GC-mass spectral analysis of the bis(trimethylsilyl) derivative of 15β-hydroxy GA_{19} dimethyl ester **11** revealed a substantial amount of an impurity characteristic of the (trimethylsilyl) trimethyl ester derivative of 15β-hydroxy GA_{17} (**7**), indicating that some of the aldehyde **11** had undergone auto-oxidation. GC-mass spectral analysis of trimethylsilyl methyl ester derivatives of all four compounds matched well with those obtained from the natural source (Table 1) and GA-numbers are therefore allocated as follows: GA_{100} to 15β-hydroxy GA_{53} (**4**); GA_{101} to 15β-hydroxy GA_{44} (**5**); and GA_{102} to 15β-hydroxy GA_{19} (**6**) [12]. (Gibberellin GA_{99} has been assigned to 2β-hydroxy GA_{19}, the identity of which has been confirmed by synthesis from gibberellic acid [13].)

The ^{1}H and ^{13}C NMR data for the new gibberellins are shown in Tables 2 and 3, respectively. Although the identification of 15β-hydroxy GA_{17} in seeds of *H. annuus* is not in doubt, allocation of a GA-number of this GA must await its full chemical characterizations.

EXPERIMENTAL

General. IR spectra were recorded on $CDCl_3$ solns on a Perkin-Elmer 683 infrared spectrophotometer. NMR spectra were recorded on Varian Gemini 300 and VXR 300 spectrometers. Chemical shifts (δ ppm) are measured relative to the residual peak of $CHCl_3$ (7.26 ppm) for ^{1}H spectra or the central peak of $CDCl_3$ (77.0 ppm) for ^{13}C spectra. Distortionless enhancement by polarization transfer (DEPT) and the attached proton test (APT) were used in the assignment of ^{13}C spectra. Low-resolution EI-mass spectra (70 eV) were recorded on a VG Micromass 7070F double focusing mass

Table 1. Comparison of Kovats retention indices and relative intensities of characteristic ions for methyl TMSi derivatives of GA_{100}, GA_{101} and GA_{102}

Identified compound	Kovats retention index*	Diagnostic ions (m/z) with % abundance in reference and sample																			
15β-OH GA_{53} Me TMSi		Ion	229	244	296	324	348	386	414	446	476	504	521	536							
(GA_{100}-Me-TMSi)	2567	Reference	100	70	60	12	16	32	18	27	29	8	18	28							
Helianthus annuus	2576	Sample	96	100	83	21	18	49	37	31	43	12	16	37							
15β-OH GA_{44} Me TMSi		Ion	147	229	244	269	281	295	371	415	430	461	489	492	505	520					
(GA_{101}-Me-TMSi)	2856	Reference	36	100	35	15	14	82	22	12	75	11	9	7	35	95					
Helianthus annuus	2865	Sample	22	100	30	16	15	93	21	8	59	8	3	4	23	54					
15β-OH GA_{19} Me TMSi		Ion	181	223	229	244	282	296	313	372	400	428	432	461	490	493	522	535	550		
(GA_{102}-Me-TMSi)	2669	Reference	23	36	89	43	34	28	33	84	45	21	63	18	32	26	100	31	22		
Helianthus annuus	2680	Sample	19	31	92	39	43	67	35	100	51	24	63	21	31	25	88	27	15		
15β-OH GA_{17} Me TMSi		Ion	147	221	229	244	281	295	311	339	370	371	399	430	458	460	490	520	521	548	565	580
(impurity in 15β-OH GA_{19})	2632	Reference	33	16	74	41	23	100	22	17	38	46	28	51	21	23	16	25	26	14	16	44
Helianthus annuus	2640	Sample	12	10	62	30	16	100	11	13	32	25	21	42	15	22	8	16	19	10	10	22

*The KRIs are slightly different as a consequence of carrying out the experiments on different columns (the column used for the original isolations had been discarded prior to the completion of the syntheses).

Table 2. ^{1}H NMR spectral data for methyl esters of GA_{100} (**16**), GA_{101} (**13**) and GA_{102} (**11**)

H	GA_{100} (**16**)	GA_{101} (**13**)	GA_{102} (**11**)
5	1.81 *d* (12.6)	2.13 *d* (12.7)	2.12 *d* (13.0)
6	3.39 *d* (12.6)	2.82 *d* (12.7)	3.94 *d* (13.0)
15	4.08 *m*	4.05 *br s*	4.14 *m*
17	5.25 *d* (2.6)	5.24 *d* (2.6)	5.26 *d* (2.5)
	5.35 *d* (3.0)	5.37 *d* (3.0)	5.34 *d* (3.0)
18	1.05 *s*	1.10 *s*	1.09 *s*
20	0.68 *s*	4.15 *d* (13.2)	9.64 *s*
		4.37 *dd* (13.2, 2.2)	
CO_2CH_3	3.67, 3.76 *s*	3.76 *s*	3.63, 3.79 *s*

Table 3. ^{13}C NMR spectral data for methyl esters of GA_{100} (**16**), GA_{101} (**13**) and GA_{102} (**11**)

C	GA_{100} (**16**)	GA_{101} (**13**)	GA_{102} (**11**)
1	37.5	37.65	32.4
2	19.5	20.6	20.7
3	39.1	39.1	37.5
4	43.4	42.6	45.2
5	59.3	52.7	58.2
6	44.9	43.8	44.8
7	176.9	176.3	176.3
8	53.3	53.0	53.2
9	51.5	55.0	50.0
10	44.6	41.1	59.3
11	17.6	15.9	18.1
12	39.3	39.8	39.2
13	75.7	76.7	76.3
14	43.9	41.2	42.2
15	76.7	76.6	76.4
16	157.6	158.2	157.5
17	108.3	109.1	109.0
18	28.5	22.9	27.8
19	177.8	174.9	177.8
20	15.2	75.1	205.6
OCH_3	50.8	53.0	51.7
	52.3		52.6

spectrometer. Flash chromatography was carried out on Merck Kieselgel 60.

Dimethyl ent-13-methoxymethoxy-15,20-dioxogiberell-16-ene-7,19-dioate (**9**). SeO_2 (50 mg, 0.450 mmol, 3.3 eq.), followed by one drop of *t*-BuOOH soln were added to a soln of aldehyde **8** (59 mg, 0.145 mmol) in dry CH_2Cl_2 (5 ml). The mixture was sonicated for 3 hr, then diluted with EtOAc (30 ml) and washed with dilute HCl (10 ml) and H_2O (10 ml). The combined aq. phases were extracted with EtOAc (2 × 10 ml). The combined organic phases were washed with aq. $NaHCO_3$ (10 ml), dried over Na_2SO_4, filtered, and the solvent was removed *in vacuo*. The 15α-hydroxy compound (60 mg, 0.136 mmol) was dissolved in dry CH_2Cl_2 (15 ml) and Dess–Martin reagent (116 mg, 0.273 mmol, approx. 2 eq.) was added. After 10 min the reaction appeared as a cloudy white soln. The reaction mixt. was diluted with CH_2Cl_2 (20 ml) and satd $NaHCO_3$ soln containing 7% sodium thiosulphate (20 ml) added. The mixt. was stirred for 20 min, the layers were sepd and the organic phase was washed with $NaHCO_3$ soln (2 × 10 ml), followed by brine (15 ml), then dried over Na_2SO_4 and the solvent removed *in vacuo*. Purification by silica gel CC (hexane–EtOAc, 3:1) yielded the desired enone **9** (49 mg, 80% from **8**) as a white foam. IR cm^{-1}: 1750, 1725, 1715. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.32 (3H, *s*, H-18), 2.43 (1H, *d*, J = 12.3 Hz, H-5), 3.41 (3H, *s*, OCH_2OCH_3), 3.63 (2 × 3H, *s*, CO_2CH_3), 3.93 (1H, *d*, J = 12.8 Hz, H-6), 4.68, 4.80 (2 × 1H, *ABd*, J = 7.3 Hz, OCH_2OCH_3), 5.45 (1H, *s*, H-17), 6.08 (1H, *s*, H-'17), 9.71 (1H, *s*, H-20). ^{13}C NMR (75 MHz, $CDCl_3$): δ 19.0 (C-11), 20.4 (C-2), 29.0 (C-18), 32.5 (C-3), 35.9 (C-1), 37.7 (C-12), 38.9 (C-14), 45.6 (C-4), 48.4 (C-6), 51.5, 51.6 (7-CO_2CH_3, 19-CO_2CH_3), 52.7 (C-9), 54.8 (CH_2OCH_3), 55.6 (C-5), 59.6 (C-8), 61.1 (C-10), 80.3 (C-13), 91.5 (OCH_2OCH_3), 117.0 (C-17), 149.9 (C-16), 172.1, 176.8 (C-7, C-19), 203.5 (C-15), 205.1 (C-20). EI-MS m/z (rel. int.): 420 $[M - CO]^+$

(24), 388 (52), 360 (56), 344 (38), 326 (46), 315 (60), 299 (94), 285 (52), 255 (100), 239 (62), 227 (54), 145 (50), 111 (70), 91 (66), 55 (100). HREI-MS *m/z* calcd for $[M-CO]^+$ $C_{23}H_{32}O_7$; 420.2148; found 420.2150.

Dimethyl ent-15α-hydroxy-13-methoxymethoxy-20-oxogibberell-16-ene-7,19-dioate (**10**). To a soln of enone **9** (49 mg, 0.109 mmol) in benzene (10 ml) was added AcOH (5 ml) and freshly activated Zn (100 mg). The reaction mixt. was then sonicated for 1 hr, after which time TLC analysis indicated that the reaction was complete. The mixt. was filtered through a small pad of celite and washed thoroughly with Et_2O (30 ml). The organic phase was washed with H_2O (10 ml), $NaHCO_3$ soln (10 ml) and brine (10 ml). The organic phase was dried over Na_2SO_4, and the solvent was removed *in vacuo*. Purification by silica gel CC (hexane–EtOAc, 2:1) yielded the desired 15β-hydroxy compound **10** (45 mg, 91%) as a foam, plus a small amount of the 1,4 reduction product that could not be removed by flash chromatography. IR cm^{-1}: 1715. ^1H NMR (300 MHz, $CDCl_3$): δ 1.08 (3H, *s*, H-18), 2.10 (1H, *d*, *J* = 12.8 Hz, H-5), 3.35 (3H, *s*, $-OCH_2OCH_3$), 3.63, 3.79 (2 × 3H, *s*, $-CO_2CH_3$), 3.92 (1H, *d*, *J* = 12.8 Hz, H-6), 4.07 (1H, *m*, H-15), 4.53, 4.76 (2 × 1H, *ABd*, *J* = 7.3 Hz, $-OCH_2OCH_3$), 5.23 (1H, *s*, H-17), 5.31 (1H, *s*, H-'17), 9.65 (1H, *s*, H-20). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8 (C-11), 20.7 (C-2), 27.6 (C-18), 32.4 (C-3), 37.6 (C-1), 38.6 (C-12), 38.8 (C-14), (C-4 not observed), 45.1 (C-6), 50.1 (C-9), 51.7, 52.7 (CO_2CH_3), 52.6 (C-8), 55.4 (OCH_2OCH_3), 58.4 (C-5), 59.3 (C-10), 76.9 (C-15), 81.5 (C-13), 91.9 (OCH_2OCH_3), 109.9 (C-17), 153.7 (C-16), 176.2, 178.0 (C-7, C-19), 205.7 (C-20). EI-MS (rel. int.): 418 $[M-CH_3OH]^+$ (16), 373 (15), 356 (26), 328 (28), 300 (30), 285 (34), 267 (28), 239 (26), 227 (100), 195 (30), 165 (32), 145 (28). 91 (32), 55 (30). HREI-MS *m/z* calcd for $[M-CH_3OH]^+$, $C_{23}H_{30}O_7$: 418.1992; found 418.1990.

Dimethyl ent-15α,13-dihydroxy-20-oxogibberell-16-ene-7,19-dioate (**11**) (*GA_{102} methyl ester*). Dowex 50W-X2 resin (80 mg of wet resin) was added to a soln of the aldehyde **10** (10 mg, 0.022 mmol) in MeOH (3 ml) and H_2O (0.5 ml). The reaction mixt. was then heated under reflux for 3 hr. After cooling the reaction mixture was 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*. CC on silica gel (hexane–EtOAc, 1:1) yielded the desired dihydroxy aldehyde **11** (7.2 mg, 80%) as a foam, plus some starting material **10** (1.2 mg, 13%). IR cm^{-1}: 1710. EI-MS *m/z* (rel. int.): 374 $[M-CH_3OH]^+$ (17), 346 (28), 328 (66), 300 (100), 286 (36), 268 (48), 241 (70), 183 (67), 159 (38), 145 (43), 129 (36), 105 (41), 91 (51), 55 (35). HRMS (EI) *m/z* calcd for $[M-CH_3OH]^+$, $C_{21}H_{26}O_6$: 374.1729; found 374.1729.

ent 15α,20-Dihydroxy-13-methoxymethoxygibberell-16-ene-7,19-dioic acid 7-methyl ester 19,20-lactone (**12**). $NaBH_4$ (4 mg, 0.10 mmol) was added to a soln of the aldehyde **10** (9 mg, 0.02 mmol) in methanol (5 ml) at 0°C. After 1 hr, TLC showed that the reaction was complete. The soln was diluted with EtOAc (50 ml) and acidified with NaH_2PO_4 soln (20%, 20 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 × 20 ml), dried over Na_2SO_4 and the solvent removed *in vacuo*. CC on silica gel (hexane–EtOAc, 2:1) yielded lactone **12** (5.7 mg, 67%) as an oil. R_f: 0.28 (hexane–EtOAc, 2:1), IR cm^{-1}: 1723. ^1H NMR (300 MHz, $CDCl_3$): δ 1.09 (3H, *s*, H-18), 2.11 (1H, *d*, *J* = 12.8 Hz, H-5), 2.81 (1H, *d*, *J* = 12.8 Hz, H-6), 3.36 (3H, *s*, OCH_2OCH_3), 3.76 (3H, *s*, CO_2CH_3), 3.97 (1H, *m*, H-15), 4.15 (1H, *d*, J_{gem} = 11.2 Hz, 20-pro-*S*-H), 4.37 (1H, *dd*, J_{gem} = 11.2 Hz, $J_{20R,1\beta}$ = 2.6 Hz, 20-pro-*R*-H), 4.53, 4.74 (2 × 1H, *ABd*, *J* = 7.3 Hz, OCH_2OCH_3), 5.26 (1H, *d*, *J* = 3 Hz, H-17), 5.30 (1H, *d*, *J* = 2.7 Hz, H-'17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.6 (C-11), 20.6 (C-2), 22.8 (C-18), 37.3, 37.6 (C-3, C-1), 38.7 (C-12), 39.8 (C-14), 41.1 (C-10), 42.6 (C-4), 44.1 (C-6), 51.8 (CO_2CH_3), 52.4 (C-8), 52.8 (C-5), 55.3 (C-9), 55.5 (OCH_2OCH_3), 75.1 (C-20), 77.2 (C-15), 81.9 (C-13), 92.0 (OCH_2OCH_3), 110.0 (C-17), 154.3 (C-16), 174.9, 176.5 (C-7, C-19). EI-MS *m/z* (rel. int.): 420 $[M]^+$ (8), 391 (13), 375 (17), 359 (23), 343 (62), 328 (18), 298 (20), 269 (29), 227 (100), 195 (31), 145 (23), 105 (21), 91 (26), 55 (29). HREI-MS *m/z* calcd for M^+, $C_{23}H_{32}O_7$: 420.2148; found 420.2150.

ent-13β,15α,20-Trihydroxygibberell-16-ene-7,19-dioic acid 7-methyl ester 19,20-lactone (**13**) (*GA_{101} methyl ester*). Dowex 50W-X2 resin (80 mg wet resin) was added to a soln of lactone **12** (5.5 mg, 0.013 mmol) in MeOH (3.3 ml) and H_2O (0.66 ml). The reaction mixt. was then heated under reflux for 3 hr, after which time TLC indicated that the reaction was complete. The reaction mixt. was 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 was removed *in vacuo*. CC on silica gel (hexane–EtOAc, 2:1 to 1:2) yielded the desired dihydroxy lactone **13** (3.8 mg, 77%) as an off-white foam. EI-MS *m/z* (rel. int.) 376 $[M]^+$ (20), 344 (83), 316 (23), 298 (49), 260 (35), 241 (32), 183 (100), 159 (55), 145 (42), 105 (42), 91 (51), 79 (32), 55 (43). HREI-MS *m/z* calcd for $[M]^+$, $C_{21}H_{28}O_6$: 376.1886; found 376.1887.

ent-15α,20,20-Trihydroxy-13-methoxymethoxygibberell-16-ene-7,19-dioic acid 19,20-lactone (**14**). The aldehyde **10** (25 mg, 0.055 mmol) was dissolved in MeOH (0.5 ml) and 2 M NaOH soln (3 ml). The reaction mixture was heated at reflux for 6 hr. After cooling the mixture was diluted with EtOAc–20% 2-BuOH (50 ml) and was acidified with H_3PO_4 (10%, 10 ml). The layers were sepd and the aq. phase was extracted with the EtOAc–2-BuOH mixture (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*. Purification on silica gel (hexane–EtOAc: acetic acid, 2:1:0.1) provided carboxylic acid **14** (23 mg, 100%) as

a gum. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.20 (3H, *s*, H-18), 2.15 (1H, *d*, $J = 13.4$ Hz, H-5), 3.38 (4H, *s*, OCH_2OCH_3 and H-6 overlapped), 4.20 (1H, *br s*, H-15), 4.55, 4.79 (2 × 1H, *ABd*, $J = 7.3$ Hz, OCH_2OCH_3), 5.25 (1H, *br s*, H-17), 5.29 (1H, *br s*, H-'17), 5.80 (1H, *br s*, H-20). EI-MS *m/z* (rel. int.): 404 $[M - H_2O]^+$ (25), 376 (12), 359 (28), 331 (28), 314 (38), 285 (50), 257 (59), 239 (61), 213 (62), 149 (43), 91 (58), 71 (65), 57 (100). HREI-MS *m/z* calcd for $[M - H_2O]^+$, $C_{22}H_{28}O_7$: 404.1835; found 404.1834.

Dimethyl ent-15α-hydroxy-13-methoxymethoxygibberell-16-ene-7,19-dioate (**15**). Anhydrous NH_2NH_2 (0.25 ml) was added to a soln of carboxylic acid **14** (23 mg, 0.055 mmol) in $(CH_2OH)_2$ (2 ml) and the reaction mixture was heated at 100°C for 30 min. Half a pellet of NaOH (approximately 200 mg) was added and the temperature was raised to 116°C for 1 hr. Finally, the temperature was raised to 180° and the reaction left overnight. After cooling the mixt. was diluted with EtOAc–20% 2-BuOH (50 ml) and was acidified with H_3PO_4 (10%, 10 ml). The layers were sepd and the aq. phase was extracted with the EtOAc–2-BuOH mixture (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 was removed *in vacuo*. The residue was dissolved in MeOH (10 ml) and treated with an excess of CH_2N_2. The solvent was removed under a gentle stream of N_2 and finally, purification on silica gel (hexane–EtOAc, 3:1) yielded **15** (8.5 mg, 35%) as an oil. IR cm^{-1}: 1720. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.69 (3H, *s*, H-20), 1.04 (3H, *s*, H-18), 1.78 (1H, *d*, $J = 12.5$ Hz, H-5), 3.36 (3H, *s*, OCH_2OCH_3), 3.39 (1H, *d*, $J = 12.5$ Hz, H-6), 3.67, 3.75 (2 × 3H, *s*, CO_2CH_3), 4.02 (1H, *m*, H-15), 4.56, 4.77 (2 × 1H, *ABd*, $J = 7.3$ Hz, OCH_2OCH_3), 5.21 (1H, *d*, $J = 3.1$ Hz, H-17), 5.31 (1H, *d*, $J = 2.5$ Hz, H-'17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.1 (C-20), 17.3 (C-11), 19.5 (C-2), 28.3 (C-18), 37.5 (C-1), 38.6, 39.0 (C-3, C-12), 40.5 (C-14), 43.4, 44.3 (C-4, C-8), 45.2 (C-6), 50.8 (C-9), 51.4, 52.3 (CO_2CH_3), 52.9 (C-10), 55.4 (OCH_2OCH_3), 59.5 (C-5), 77.1 (C-15), 81.8 (C-13), 91.8 (–OCH_2OCH_3), 108.9 (C-17), 153.7 (C-16), 177.2, 178.8 (C-7, C-19). EI-MS *m/z* (rel. int.): 436 $[M]^+$ (8), 404 (49), 376 (34), 359 (100), 342 (52), 299 (52), 271 (45), 255 (38), 227 (33), 197 (31), 149 (39), 91 (37). HREI-MS *m/z* calcd for $[M]^+$, $C_{24}H_{36}O_7$: 436.2461; found 436.2463.

Dimethyl ent-13,15α-dihydroxygibberell-16-ene-17,19-dioate (**16**) (*GA_{100} methyl ester*). Dowex 50W-X2 resin (80 mg of wet resin) was added to a soln of diester **15** (11.5 mg, 0.026 mmol) in MeOH (3.3 ml) and H_2O (0.66 ml). The reaction mixt. was then heated under reflux for 4 hr. The soln was 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*. CC on silica gel (hexane–EtOAc, 1:1) yielded **16** (7.3 mg, 77%) as a white foam. EI-MS *m/z* (rel. int.): 392 $[M]^+$ (2), 374 (3), 360 (100), 342 (20), 332 (66), 314 (39), 300 (30), 285 (18), 272 (31), 255 (39), 173 (30), 159 (30), 105 (30), 91 (30), 69 (22), 55 (26). HREI-MS *m/z* calcd for $[M - CH_3OH]^+$, $C_{21}H_{28}O_5$: 360.1937; found 360.1936.

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