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Gibberellins in shoots and developing capsules of *Populus* species

David W. Pearce[a,*], Oliver E. Hutt[b], Stewart B. Rood[a], Lewis N. Mander[b]

[a]*Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada*
[b]*Research School of Chemistry, Australian National University, Canberra, A.C.T. 0200, Australia*

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Abstract

Extracts of stems of growing shoots of *Populus deltoides* and *P. trichocarpa*, and developing capsules of *P. deltoides* were analysed for gibberellins (GAs) by gas chromatography–mass spectrometry. The following known GAs were identified by comparison of their Kovats retention indices (KRIs) and mass spectra with those of standards: GA_1, GA_8, GA_9, GA_{19}, GA_{20}, 16β,17-dihydro-17-hydroxy GA_{20}, GA_{23}, GA_{28}, GA_{29}, GA_{34}, GA_{44}, and GA_{97}. Several of these have not been previously reported from *Populus*. In addition, two new GAs were identified as 12β-hydroxy GA_{53} (GA_{127}) and 16β,17-dihydro-17-hydroxy GA_{53} and their structures were confirmed by partial synthesis. Evidence was found of 16,17-dihydro-16,17-dihydroxy GA_9, 16,17-dihydro-16,17-dihydroxy GA_{12}, 12-hydroxy GA_{14}, and GA_{34}-catabolite by comparison of mass spectra and KRIs with published data. Several putative GAs (hydroxy- and dihydroxy-GA_{12}-like) were also found. The catabolites of active GAs or of key precursors, hydroxylated at C-2 in stems and either C-2, C-12, C-17, or C-16,17 in capsules, were the major proportion of the GAs.

Keywords: *Populus deltoides*; *Populus trichocarpa*; Salicaceae; Poplar; Gibberellins

1. Introduction

The genus *Populus* is becoming increasingly important for commercial production of fibre from short-rotation stands of fast-growing hybrid trees. One focus of research on the genetic basis of many aspects related to the growth of such poplars has been a hybrid pedigree of *P. deltoides* Bartr. ex Marsh and *P. trichocarpa* Torr. & Gray (Bradshaw, 1996). The growth of these trees is likely to be regulated at least partly by gibberellins (GAs), and members of both the early 13-hydroxylation and non-early 3- or 13-hydroxylation pathways of GA biosynthesis have been described from *P. deltoides* hybrids and other *Populus* species (Bate et al., 1988; Rood et al., 1988; Zanewich and Rood, 1994; Eriksson et al., 2000). As the first part of a study of the hormonal regulation of growth in the hybrid pedigree we sought to identify and measure key GAs and their metabolites in shoots of the parents. We have also measured abscisic acid (ABA), a potential antagonist of growth, and identified several of its metabolites. In addition, to confirm the identity of compounds indicated in low quantities in the shoots, we have analysed developing capsules of *P. deltoides* as a potentially rich source of GAs. Together the shoots and capsules yielded a number of GAs not previously reported from poplars, and these suggest possible regulatory steps which may be important for future research.

2. Results and discussion

The GAs identified from extracts of stems of growing shoot tips of *P. deltoides* and *P. trichocarpa* provided evidence of one major biosynthetic pathway. Consistent with results from other studies of *Populus*—*P. deltoides* hybrids (Bate et al., 1988; Rood et al., 1988), *P. tremuloides* (Zanewich and Rood, 1994), and *P. tremula* L.×*P. tremuloides* (Eriksson et al., 2000)—GAs of the early 13-hydroxylation pathway (GA_1, GA_{19}, GA_{20} and GA_{44}) predominated together with their inactive metabolites GA_8, GA_{29} and GA_{97} (Table 1). The GAs were indicated by GC–MS and identities confirmed by GC-selected ion monitoring (SIM), based on the relative intensities of at least 6 ions, and KRI (for GA_{97}) or retention times in relation to those of the deuterated

* Corresponding author. Tel.: +1-403-329-2172; fax: +1-403-329-2242.
E-mail address: pearce@uleth.ca (D.W. Pearce).

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internal standards. We could not determine if GA_{12} and GA_{53} were present, as the internal standards were not recovered. Here, quantities of individual GAs were typically less than about 10 ng per g dry wt with the exception of that of GA_8, which exceeded GA_1 about 10-fold. We did not detect GA_4, GA_9 or other members of the non-early 3- or 13-hydroxylation pathway in shoots found in *P. tremula L.×P. tremuloides* (Eriksson et al., 2000). Evidence of 12-hydroxy GA_{14} was found in shoots, based on its mass spectrum (Table 1), in a similar quantity as the other GAs. No standard was available to confirm the identity of this GA, but comparison of its KRI with those of the other GAs found in poplar and with published data (Gaskin and MacMillan, 1991) suggests that it might be 12β-hydroxy GA_{14}.

Developing capsules of *P. deltoides* also contained GAs of the early 13-hydroxylation pathway (GA_1, GA_{19}, GA_{20} and GA_{44}; Table 1). Within this group GA_{20} predominated. In addition there were large pools of inactive metabolites of several members of this pathway. These included GA_8; one compound, 16,17-dihydro-17-hydroxy GA_{20}-like, which was later identified as the 16β,17-dihydro-epimer; and several compounds that we recognized as hydroxy-GA_{53}-like. The structures of two GAs from the latter group were subsequently elucidated as outlined below by a semi-synthetic procedure using as starting material compound **1**, with others **2**–**12** being generated as intermediates/final products, respectively.

The first GA showed a mass spectrum similar to that of 12α-hydroxy GA_{53} (GA_{123}), recently identified from immature strawberry fruits (Blake et al., 2000). The KRI differed, however, (Table 1) and so the 12β-epimer was synthesised for comparison. 12β-Hydroxy-GA_{53} dimethyl ester (**9**) was prepared from GA_{53} (Mander et al., 1996) according to well-established procedures (Chu and Mander, 1988; Bhaskar and Mander, 1996) as outlined in Scheme 1. Thus, after methylation and acetylation, hydroxylation at C-15 was effected with selenium dioxide and oxygen translocated to C-17 by subsequent oxidation with pyridinium chlorochromate. The resulting mixture of epoxy and unsaturated aldehydes was reduced with chromous chloride to give the 16β-aldehyde **5**, the stereochemistry of which was apparent from the high field shift of C-12 in the ^{13}C NMR spectrum at δ 30.2 due to the γ-gauche interaction with the endo formyl substituent. Bromination with pyrrolidone hydrotribromide then gave a mixture of bromo-aldehydes epimeric at C-16. The major 16β-aldehyde **6** was next reduced to the 17-carbinol which was used to effect transannular oxidation at C-12 to afford ether **7**. Reductive elimination with Zn–HCl followed by removal of the 13-acetate function finally gave the target diol **9**. The TMSi derivative of this product was used in GC–MS to confirm the identity of the compound from poplar as 12β-hydroxy GA_{53} (Table 1). No evidence was found in the capsules of other 12,13-dihydroxy GAs that might be derived from this, nor of 12β-hydroxy GA_{12} (GA_{112}) that might serve as a precursor for this compound. 12β-hydroxy GA_{53} has also been identified in strawberry fruits (Blake and Mander, unpublished) and has been assigned as GA_{127} (MacMillan and Takahashi, 1968).

The mass spectrum of the second compound was similar to that of 16,17-dihydro-17-hydroxy GA_{53}, tentatively identified previously from shoots of *Zea mays* L. based on its mass spectrum (Fujioka et al., 1988; Gaskin and MacMillan, 1991). In particular, there was a characteristic loss of *m/z* 131 from the Me-TMSi derivative, corresponding to loss of the oxygenated D-ring bridge. In the present study, the two C-16 epimers of 16,17-dihydro-17-hydroxy-GA_{53} dimethyl ester were prepared in equal amounts by hydroboration of GA_{53} dimethyl ester (**10**) (Scheme 2). The stereochemistry of the products was readily established from an examination of their respective ^{13}C NMR spectra. In the *endo* isomer **12**, C-12 was observed at 31.5 ppm as a result of a γ-gauche interaction with C-17, while for the C-16β,17-dihydro-epimer, i.e. *exo* isomer **11**, the chemical shift of 40.8 ppm for C-12 is similar to that of the parent GA. In analysis by GC–MS as the TMS derivatives the latter epimer proved to be the same as the compound from poplar (Table 1). It now seems likely, based on a comparison of KRIs, that the prior description of 16,17-dihydro-17-hydroxy GA_{53} from *Zea mays* L. is also that of the C-16β,17-dihydro-epimer (*exo* isomer).

A third hydroxy-GA_{53} -like compound remains unidentified. Its mass spectrum showed prominent ions at *m/z* 207/208 (Table 1, HPLC fraction 17–19), indicating a C-13-hydroxylated GA with the second hydroxyl outside of rings C/D. This and other putative GAs ranked amongst the more abundant of the GAs in the capsules. One of the others was hydroxy-GA_{12}-like; the rest were dihydroxy-GA_{12}-like.

The identity of 16β,17-dihydro-17-hydroxy GA_{20} was established by comparison in GC–MS with the TMSi derivatives of 16α- and 16β,17-dihydro-17-hydroxy GA_{20} methyl esters (Table 1). These were prepared in analogous fashion to the GA_{53} dimethyl ester analogues (**11**, **12**); details will be published elsewhere. This GA has been identified previously from strawberry fruits (Blake and Mander, unpublished).

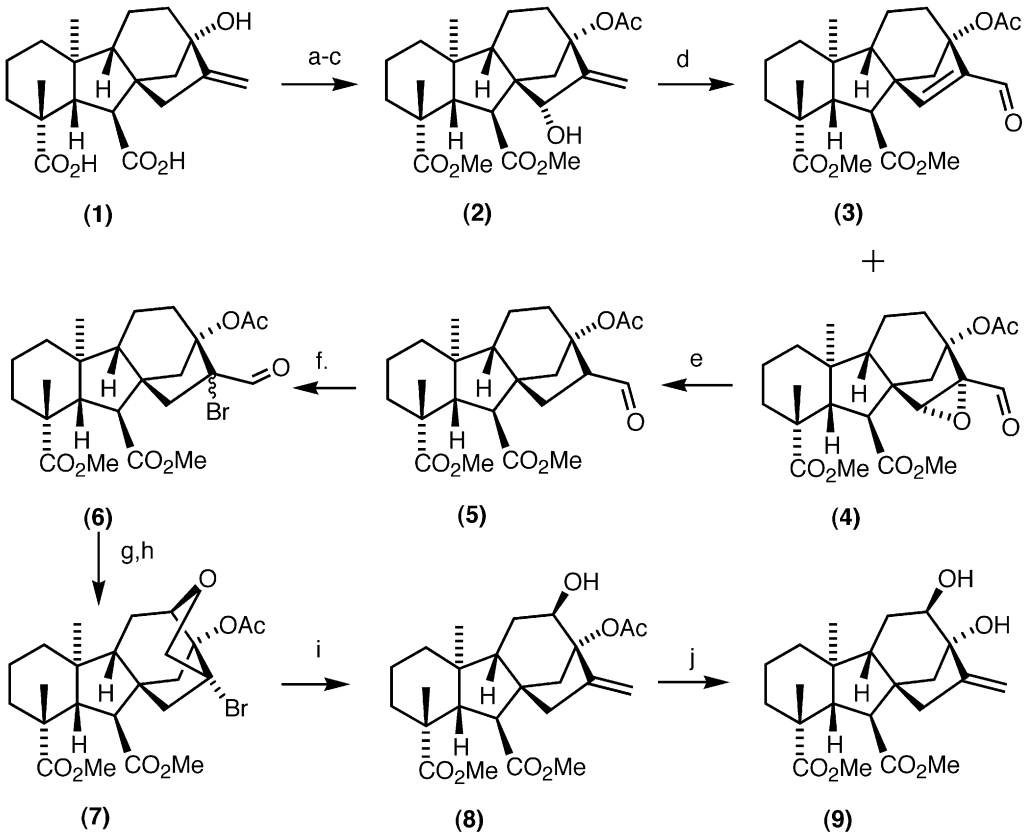
Capsules also contained a small quantity of one representative of the non-hydroxylation pathway—GA_9—and there was evidence of greater quantities of several inactive metabolites of members of this pathway. These included 16,17-dihydro-16,17-dihydroxy GA_{12} (possibly the C-16α epimer, based on its KRI) and 16,17-dihydro-16,17-dihydroxy GA_9 (possibly the C-16β epimer), and GA_{34} and GA_{34} catabolite (Table 1). The collective quantity of these compounds was less than that of the 13-hydroxylated GAs. Small quantities of GA_{23} and its inactive metabolite GA_{28} were also detected.

Table 1
Gibberellins from stems of *Populus trichocarpa* and stems and capsules of *P. deltoides*

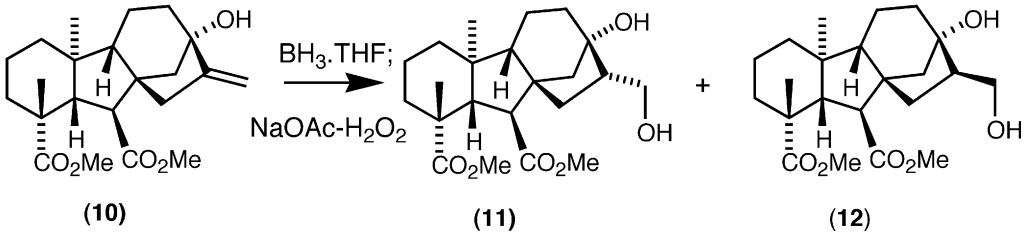
P. tri.	*P. deltoides*		Identity	HPLC	KRI[a]				Ions (m/z) and relative intensities, in *Populus* samples[b]										
Stems	Stems	Capsules		fr.	DB-5ms		HP-1												
					Smpl.	Std.	Smpl.	Std.											
+	+	++++[c]	GA_8	7–9	2785	2783	–	2822	594	579	535	448	379	375	311	281	238	207	
									100	5	9	33	25	28	19	15	31	32	
+[d]	+		GA_{29}	12–14	2672	2672			506	491	477	447	389	375	303	235	207		
									100	13	9	10	18	22	57	19	55		
		++	diOH GA_{12}-like	17–19	2658		2680		536	521	387	327	320	268	238	235	208	207	
									13	4	4	7	15	31	10	25	61	100	
		+	GA_{28}	17–19	2710	2710	2723	–	580	565	548	520	490	430	371	208	207	129	
									12	4	4	7	4	6	21	96	100	41	
		++	GA_{23}	17–19	2741	2741	2747	–	550	522	490	462	432	400	373	346	313	239	208
									7	53	9	18	19	21	38	27	38	100	36
+	+[d]		GA_{97}	19–21	2680	2680	2702	2701	536	521	504	477	446	387	386	371	327	239	207
									25	7	6	9	4	18	10	11	21	47	100
+	+[d]		*12-OH GA_{14}*[e]	19–21	2689		2709		536	504	476	414	386	297	296	237	207	129	
									7	53	65	57	82	100	61	61	90	78	
+	+	+	GA_1	20–22	2665	2664	–	2676	506	491	448	416	377	376	375	313	207		
									100	16	19	11	21	30	19	16	37		
		++	16β,17-H_2-17-OH GA_{20}	23–25	2737	2736	2741	2741	508	493	465	418	405	377	359	297	241	147	
									9	4	2	16	5	100	3	8	4	5	
			16α,17-H_2-17-OH GA_{20}[f]			2722		2721											
		++	*16β,17-H_2(OH)$_2$ GA_9*[e]	23–25	2753		2768		508	493	477	405	345	301	255	227	183		
									<1	1	<1	100	4	4	4	3	3		
			16α,17-H_2(OH)$_2$ GA_9[f]			2780		2790											
+		++++	GA_{20}	26–29	2508	2510	2496	2494	418	403	375	359	301	235	207				
									100	15	53	16	12	13	14				
		+++	*GA_{34} catabolite*[e]	26–29	2572		2556		446	387	371	327	311	297	258	237	199		
									69	41	62	100	39	39	48	31	75		
		++++	diOH GA_{12}-like	26–29	2631		2651		536	521	446	414	386	356	324	297	296	260	228
									5	67	13	26	32	22	17	71	59	100	88
		++	12β-OH GA_{53} (GA_{127})	26–29	2643	2643	2662	2663	536	504	477	433	420	301	295	251	193	181	147
									38	13	16	58	32	17	33	20	88	100	62
			12α-OH GA_{53} (GA_{123})[f]			2617		2637											
		+++	H_2-(OH)$_2$ GA_{12}-like	26–29	2829		2837		523	416	373	329	299	297	274	267	260	228	
									24	4	6	8	40	15	10	9	74	100	
		+++++	OH GA_{12}-like	30–32	2584		2588		448	433	358	326	298	283	239	223	195		
									3	3	41	24	100	19	30	15	18		
+		+++	GA_{19}	30–32	2618	2620	2606	2605	462	434	402	375	374	359	345	285	239	208	
									10	100	41	45	51	22	29	29	39	19	
		++	GA_{34}	30–32	2654	2655	2673	2672	506	459	416	372	313	288	229	223	201	147	
									100	7	11	15	22	22	30	33	26	50	
		+++++	16β,17-H_2-17-OH GA_{53}	30–32	2748	2747	2762	2763	538	448	407	389	375	347	297	207	181	147	
									4	9	39	7	100	19	17	10	10	9	
			16α,17-H_2-17-OH GA_{53}[f]			2720		2723											
		+	*16,17-H_2(OH)$_2$ GA_{12}*[e]	30–32	2782		2794		538	523	448	435	403	389	375	315	299	285	
									<1	<1	1	100	4	5	51	12	11	20	
	+	+	GA_{44}	30–32	2821	2826	2796	2795	432	417	373	251	238	207	180				
									58	8	17	18	37	100	17				
		+	GA_9	35–36	2362	2363			330	298	286	270	243	227	226	217	183	159	
									13	92	22	100	55	46	47	77	28	52	

[a] Kovats Retention Index, obtained on a DB-5ms column, and also for some samples on an HP-1 column.
[b] MeTMSi derivatives, from full scan GC–MS, or GC–SIM after identity was indicated by GC–MS.
[c] For capsules only, approx. relative quantities based on TIC in GC–MS. Each additional '+' represents a 2–3 fold increment in quantity.
[d] The source of the GC–MS data, which otherwise is from the capsules.
[e] Identities in italics are tentative, based on comparisons of MS and KRI with those published in Gaskin and MacMillan (1991).
[f] Standard, epimer of the GA from *Populus*, not found in this study. The KRI is included here for comparison with that of the natural epimer.

Taken together these data offer evidence of two main biosynthetic pathways functioning during the development of poplar capsules—principally the early 13-hydroxylation pathway, and to a lesser extent the non-hydroxylation pathway. We conclude then that GA-induced growth in both stems and capsules of *P. deltoides* and stems of *P. trichocarpa* is probably primarily due to the action of GA_1, the most abundant biologically-active GA.



Scheme 1. Partial synthesis of 12β-hydroxy GA_{53} dimethyl ester. Reagents: a. CH_2N_2; b. Ac_2O, Et_3N, DMAP; c. SeO_2/ ^tBuOOH/sonication; d. PCC/sonication; e. $CrCl_2$; f. $C_4H_9NO.HBr_3$; g. $NaBH_4$/DME; h. $PhI(OAc)_2/I_2$,hv; i. Zn/DMF/HCl; j. K_2CO_3/MeOH.



Scheme 2. Partial synthesis of 16β,17-dihydro-17-hydroxy GA_{53} dimethyl ester.

Conclusions about biosynthesis from analyses such as these are necessarily speculative. With this caveat in mind, the presence of large quantities of inactive end-products and inactive precursors suggests that pools of the active GAs (GA_1 and GA_4) have been depleted by catabolism either directly or at other key steps in their biosynthesis. First, directly by C-2- hydroxylation (to form GA_8 or GA_{34}). Second, by hydroxylation of GA_{20} at C-2 or C-17 (to form GA_{29} or 16β,17-dihydro-17-hydroxy GA_{20}), or of GA_9 at C-16 and C-17. Third, by hydroxylation of GA_{12} and GA_{53} at C-2, C-12, C-17 or C-16 and C-17 (to form GA_{97}, 12β-hydroxy GA_{53}, 16β,17-dihydro-17-hydroxy GA_{53}, or 16,17-dihydro-16,17-dihydroxy-GA_{12}, respectively). This latter group may also include some of the putative GAs as other inactive metabolites of GA_{12} and GA_{53}. In the capsules especially, the collective quantities of members of this third group of compounds points to the possible importance of GA 20-oxidase activity in regulating the metabolism of GA_{12} and GA_{53} at some stage during development of these structures. We did not separate the seeds from the remainder of the capsules and so do not know if any of the GAs predominated in either one or the other of these sources.

The relative importance of the different inactivation steps apparently differs between stems and capsules. For

example, the presence of GA_{97}, GA_{29} and GA_8 in shoots is consistent with inactivation of members of the early 13-hydroxylation pathway primarily by 2β-hydroxylation (of GA_{53}, GA_{20} and GA_1 respectively). In contrast, while GA_8 was also found in the capsules, the inactive identified GAs were primarily C-17-hydroxylated or C-16,17-diols. Their biosynthetic origin here is not known, but Hedden et al. (1993) have described the 16,17-diols as being "probably formed by non-specific oxidation of the 16,17-double bond". Such C-17-hydroxy or C-16,17-dihydroxy compounds are sometimes produced from GAs applied to plants (in the absence of their endogenous counterparts; D. Pearce, G. Abdala, R. Bottini, M. Takagi, R. Pharis and L. Mander, unpublished) and they may arise here naturally in a similar direct fashion from their non-16,17-hydroxylated antecedents. They have been identified from diverse sources, including fern croziers (Yamane et al., 1988), maize shoots (Fujioka et al., 1988) and apple seeds (Hedden et al., 1993).

ABA was of interest as a potential antagonist of growth in the shoots. Large pools of ABA were found in the growing stems of *P. deltoides* and *P. trichocarpa*, and there was evidence as well of its *trans*-isomer and metabolites 8′-hydroxy-ABA, phaseic acid (PA) and dihydrophaseic acid (data not shown). The quantities of all of these compounds exceeded that of the GAs many-fold, with ABA, for example, of the order of one μg per g dry wt. Given the known antagonism of GA and ABA in germination it seems possible that ABA (and perhaps also its catabolites 8′-hydroxy-ABA and PA; Cutler and Trochko, 1999) may exert a strong inhibiting effect on GA-induced growth here. The same compounds were also identified from *P. deltoides* capsules.

3. Experimental

3.1. Plant material

Populations of *P. deltoides* Bartr. ex Marsh and *P. trichocarpa* Torr. & Gray planted at Boardman, Oregon were sampled on 30/31 July 1996. Shoot tip cuttings were taken from branches on each of six trees representing each of the parents (*P. deltoides* clone ILL–129 and *P. trichocarpa* clone 93–968) of the hybrid pedigree. The cuttings were immediately frozen on dry ice and later lyophilized and dissected. The two rapidly-elongating uppermost internodes that subtended the first of the leaves that was at least two cm long were pooled from about 50 cuttings, for some samples. For others, used for quantitative analysis, three replicates each of 12 cuttings were used.

Developing capsules of *P. deltoides* were analysed to aid the process of identifying GAs from the shoots. These were collected from an individual growing in the Oldman River valley at Lethbridge, Alberta on 15 May 1998—about 2 weeks after pollination and 2 weeks before seeds were released. The capsules were frozen and lyophilized. One hundred grams dry wt (about 3600 capsules) was used for hormone analysis.

3.2. Extraction, purification and analysis

The smaller samples of stems from the shoot tips of *P. deltoides* and *P. trichocarpa* were extracted and purified as described below; similar procedures were used for the larger samples. The samples were ground and extracted for about 16–20 h at 4 °C in MeOH:H_2O (80:20) with internal standards added—to the larger samples to provide preliminary estimates of the quantities of endogenous GAs, and to the smaller samples for quantitative analyses to be described in detail elsewhere. The standards included some or all of [$^2H_2$]-GA_1,-GA_3,-GA_4,-GA_8,-GA_9,-GA_{12},-GA_{19},-GA_{20},-GA_{29},-GA_{44}, and-GA_{53}, and [$^2H_6$]-ABA (the latter from R. Pharis, University of Calgary; originally from L. Rivier). The extract was centrifuged, re-extracted twice with MeOH, reduced to aq., adjusted to pH 7, and loaded on to a column of C_{18} (2 g, 3 ml vol). Hormones of interest were eluted with MeOH:H_2O (80:20, 35 ml). The eluate was reduced to aq., adjusted to pH 8, and passed through a column of PVPP (sieved to remove fine particles; 0.5 g; 4 ml volume) which was then washed with H_2O (8 ml, pH 8). The combined eluate was passed though a 2.5 ml column of QAE Sephadex A-25, which was then washed with H_2O (10 ml, pH 8). The acidic compounds were eluted with HCO_2H (0.2 M, 12.5 ml), collected on a C_{18} Sep-Pak (360 mg) connected to the Q-25 column, and subsequently removed with 5 ml of MeOH:H_2O (80:20). The sample was dried and loaded onto a column of silica gel (2 g, 5 ml vol) which was then eluted with 25 ml of EtOAc:HOAc (99:1). The eluate was dried, internal standards of [^{3}H]-GA_1 and [^{3}H]-GA_4 (Amersham; 0.5 kBq; ca. 0.6 TBq mmol^{-1}) added, and hormones of interest separated by C_{18} HPLC in a solvent program of MeOH:H_2O:HOAc (Zanewich and Rood, 1993). Groups of fractions were methylated and silylated for analysis by full scan GC–MS or GC–SIM on a DB-5ms column (15 m×0.25 mm, J&W Scientific) installed in a Hewlett-Packard 5890 GC and connected to a Hewlett-Packard 5970B mass selective detector (Zanewich and Rood, 1993). Some samples were also analysed on an HP-1 column (12 m×0.22 mm, Hewlett-Packard), to facilitate comparison of KRIs with those published by Gaskin and MacMillan (1991). The temperature programme was the same in each case—from 60 °C (0.5 min) to 200 °C at 20 °C per min, then to 300 °C at 5 °C per min.

Capsules of *P. deltoides* were extracted as described above, without the addition of internal standards. The extract was reduced to aq., K-Pi buffer added to 0.01 M, adjusted to pH 8.5, and partitioned 3× against equal volumes of Et_2O. The aq. phase was then slurried with

PVPP and filtered. The filtrate was adjusted to pH 3 and partitioned three times against equal volumes of H_2O-satd. EtOAc. Internal standards of 3-*epi*-[^{3}H]GA_1 (prepared from [^{3}H]-GA_1) and [^{3}H]-GA_4 were added to the acidic EtOAc fraction, as markers for more polar- and less polar GAs, respectively, in chromatography on silica gel. The sample was dried and applied to a 20 g column of silica gel, eluted with 3×50 ml volumes of each of hexanes, hexanes:EtOAc:HOAc (2.5:97.5:1, 5:95:1, 15:85:1 and 50:50:1), and EtOAc:HOAc (100:1), respectively. Fractions expected to contain GAs ranging in polarity from GA_{12} to GA_8 were combined in two groups, each of which was further purified on Q-25 (5 ml volume)/C_{18} Sep-Pak as described above. The MeOH:H_2O eluate from the Sep-Pak was combined from the two groups before C_{18} HPLC. Groups of fractions were then prepared for GC–MS.

3.3. Synthesis of 12β-hydroxy GA_{53} dimethyl ester

Infrared spectra (υ_{max} cm^{-1}) were recorded on a Perkin-Elmer 1800 Fourier transform infrared. NMR spectra were recorded on a Varian Gemini 300 MHz Spectrometer. EI mass spectra (EIMS, 70 eV) were recorded on a VG Fisons Autospec M mass spectrometer. High resolution mass spectra (HRMS, exact masses) were by peak matching. Petroleum spirit had a boiling range of 40–60 °C. Chromatography was carried out on Merck Kieselgel 60.

3.3.1. Dimethyl ent-13-acetoxy-15β-hydroxy-gibberell-16-ene-7,19-dioate (**2**)

GA_{53} (**1**) (250 mg) was converted into its dimethyl ester with diazomethane and then treated overnight with a mixture of acetic anhydride (0.60 ml), Et_3N (0.9 ml), CH_2Cl_2 (6 ml) and 4-(*N,N*-dimethylamino)pyridine (10 mg). After an aqueous work up and extraction into ethyl acetate, the crude GA_{53} dimethyl ester 13-acetate (230 mg, 0.55 mmol) was dissolved in CH_2Cl_2 (11 ml) to which SeO_2 (182 mg, 1.65 mmol) and ^{t}BuOOH (few drops, 70% aqueous solution) were added. The reaction mixture was sonicated for 4 h at room temp, at which point TLC analysis indicated that the reaction was complete. H_2O (10 ml) was added and reaction mixture stirred for 10 min and then extracted with CH_2Cl_2 (x3, 10 ml). The organic layers were washed with brine, dried over $MgSO_4$, and filtered. Removal of the solvent under reduced pressure yielded the desired 15α-hydroxy product (**2**) (210 mg, 87%) as a colourless oil, R_f=0.53 (EtOAc/petroleum spirit 1:1). IR 3500, 1730 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.72 (3H, *s*, H20); 1.09 (3H, *s*, H18); 1.89 (1H, *d*, $J_{5,6}$=12 Hz, H5); 2.03 (3H, *s*–O_2CCH_3); 3.29 (1H, *d*, $J_{6,5}$=12 Hz, H6); 3.68 (6H, *s*, 2x –CO_2Me); 4.14 (1H, *s*, H15); 5.11 (1H, *s*, H17); 5.42 (1H, *s*, H′17). LRMS (*m*/*z*) 434 ([M]$^+$, 5%), 417 (40), 402 (26), 360 (100), 342 (64), 314 (86), 256 (67). HRMS: [M]$^+$ found 434.2305 ($C_{24}H_{34}O_7$ requires 434.2305).

3.3.2. Dimethyl ent-13-acetoxy-17-oxo-gibberell-15-ene-7,19-dioate (**3**) and dimethyl ent-13-acetoxy-15β,16β-epoxy-17-oxo-gibberellane-7,19-dioate (**4**)

The oily carbinol (**2**) (210 mg, 0.5 mmol), as prepared above, was dissolved in dry CH_2Cl_2 (11 ml) to which was added a finely ground 1:1 mixture of pyridinium chlorochromate (1.5 mmol, 360 mg): silica gel (360 mg). The resulting suspension was sonicated for 5 h, at which point TLC analysis indicated the reaction to be complete. The solvent was removed under reduced pressure and the residue subjected to flash chromatography on silica gel (EtOAc/petroleum spirit 1:4) to yield a 3:2 mixture of (**3**) and (**4**) as a clear oil (124 mg, 58%) (R_f=0.58 EtOAc/petroleum spirit 1:1). IR 1725, 1700 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67 (3H, *s*, H20); 0.70 (3H, *s*, H′20); 1.12 (3H, *s*, H18); 2.03 (3H, *s*, –O_2CCH_3); 3.21 (1H, *d*, J=12.8 Hz); 3.37 (1H, *d*, J=12.6 Hz, H′6); 3.69, 3.70, 3.73, 3.73 (12H, *s*, 4×–CO_2CH_3); 7.09 (1H, *s*, H15); 9.24 (1H, *s*, H17); 9.63 (1H, *s*, H'17). LRMS (*m*/*z*) 448 ([M]$^+$, 38%), 432 (M, 5), 372 (40), 358 (59), 328 (99), 312 (91), 299 (52), 269 (56). HRMS: [M]$^+$ found 448.2092 ($C_{24}H_{32}O_8$ requires 448.2097), [M]$^+$ found 432.2142 ($C_{24}H_{32}O_7$ requires 432.2148).

3.3.3. Dimethyl ent-13-acetoxy-17-oxo-16α-gibberellane-7,19-dioate (**5**)

$CrCl_2$ (10 ml) was freshly prepared by shaking a mixture of zinc dust (7.7 g) with $HgCl_2$ (780 mg) in H_2O (4.2 ml) and HCl (10 M, 170 μl) for 10 min to form an amalgam. A green solution of $CrCl_3$ (1.9 g) in H_2O (5.8 ml) and HCl (10 M, 1.2 ml) was then added to the amalgam and N_2 gas was bubbled through the mixture. When the supernatant turned a vibrant blue it was ready for use.

The blue supernatant of the $CrCl_2$ solution (10 ml) was added to a solution of aldehydes **3** and **4** (120 mg, 0.27 mmol) in acetone (5.5 ml) and the mixture left to stir overnight. The acetone was removed under reduced pressure and the residue partitioned between EtOAc (10 ml) and H_2O (10 ml). The aqueous phase was extracted with EtOAc (10 ml, ×3) and the combined organic layers washed with saturated $NaHCO_3$ solution, brine and dried over $MgSO_4$. After filtration the solvent was removed under reduced pressure to afford the desired aldehyde **5** (109 mg, 93%) as a clear oil (R_f=0.6 EtOAc/petroleum spirit (1:1). IR 1730 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.67 (3H, *s*, H20); 1.09 (3H, *s*, H18); 1.91 (1H, *d*, $J_{5,6}$=12.8 Hz, H5); 2.07 (3H, *s*, $-O_2CCH_3$); 3.34 (1H, *d*, $J_{6,5}$=12.5 Hz, H6); 3.67, 3.72 (6H, *s*, 2× $-CO_2CH_3$); 10.03 (1H, *s*, H17). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6 (*s*, C18); 17.4 (*d*, C11); 19.5 (*d*, C2); 28.8 (*s*, C20); 30.2 (*d*, C12); 35.8 (*d*, C1); 37.6 (*d*, C15); 39.5

(*d*, C3); 43.9, 44.3 (*q*, C4 and C10); 45. 0 (*d*, C14); 48.5 (*q*, C8); 51.6 (*t*, C6); 51.5, 51.7 (*s*, 2x–$CO_2\underline{CH_3}$); 56.9 (*t*, C9); 57.7 (*t*, C5); 85.8 (*q*, C13); 170.5, 175.2 (*q*, C7 and C19); 202.5 (*t*, C17). LRMS (*m*/*z*) 434 ([M]$^+$, 4%), 402 (42), 374 (31), 360 (15), 342 (100), 314 (79), 286 (93), 255 (28), 215 (38). HRMS: [M]$^+$ found 434.2301 ($C_{24}H_{34}O_7$ requires 434.2305).

3.3.4. Dimethyl ent-16β-bromo-13-acetoxy-17-oxo-gibberellane-7,19-dioate (6)

A solution of aldehyde **5** (27 mg, 0.06 mmol) in dry THF (1.2 ml) was treated with pyrrolidone hydrotribromide (90 mg, 0.18 mmol) and stirred overnight, by which time the reaction was complete. EtOAc (5 ml) was added and the mixture washed thoroughly with a saturated solution of sodium thiosulphate (5 ml), followed by a saturated solution of $NaHCO_3$ (5 ml) and brine (5 ml). The organic phase was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. Chromatography on silica gel yielded the desired bromo-aldehyde **6** as a yellowish oil (16 mg, 52%) (R_f = 0.74 EtOAc/petroleum spirit 1:1). IR 1740, 1730 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.66 (3H, *s*, H20); 1.12 (3H, *s*, H18); 1.84 (1H, *d*, $J_{5,6}$ = 12.9 Hz, H5); 2.06 (3H, *s*,-O_2CCH_3); 3.37 (1H, *d*, $J_{6,5}$ = 12.9 Hz, H6); 3.68, 3.75 (6H, *s*, 2×–CO_2Me); 9.54 (1H, *s*, H17). LRMS (*m*/*z*) 512 ([M]$^+$, 1%), 433 (50), 373 (40), 341 (44), 313 (100), 303 (48). HRMS: [M]$^+$ found 512.1404 ($C_{24}H_{33}O_7Br$ requires 512.1410).

3.3.5. Dimethyl ent-16β-bromo-13-acetoxy-12α, 17-epoxy-gibberellane-7,19-dioate (7)

Bromo-aldehyde **6** (15 mg, 0.03 mmol) was taken up in 1,2-dimethoxyethane (1 ml) and the solution cooled to 0 °C (H_2O/ice bath). Sodium borohydride (2 mg, 0.05 mmol) was added and solution stirred for 30 min. The reaction mixture was diluted with EtOAc (5 ml), and the remaining hydride quenched by the addition of water. The organic layer was washed with H_2O (5 ml, ×3), brine (5 ml), and dried over $MgSO_4$. After filtration the solvent was removed to afford the bromohydrin as a white solid (14 mg) (R_f = 0.5 EtOAc/petroleum spirit 1:1).

The bromohydrin was immediately taken up in CH_2Cl_2 (250 μl) and benzene (750 μl) followed by the addition of $PhI(OAc)_2$ (30 mg, 0.09 mmol) and I_2 (23 mg, 0.09 mmol), then subjected to photolysis (250 W tungsten lamp) for 45 min. The reaction mixture was diluted with EtOAc (5 ml) and washed with a saturated solution of sodium thiosulphate (5 ml). The aqueous phase was re-extracted with EtOAc (5 ml, ×3) and the combined organic layers washed with H_2O, brine, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the residue applied to a silica gel column eluted with EtOAc/petroleum spirt 1:4 to yield the desired cyclic bromo-ether **7** (6 mg, 40%) as a clear oil (R_f = 0.61 EtOAc/petroleum spirit 1:1). IR 1730 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): 0.68 (3H, *s*, H20); 1.05 (3H, *s*, H18); 1.82 (1H, *d*, $J_{5,6}$ = 12.8 Hz, H5); 2.08 (3H, *s*,–O_2CCH_3); 3.35 (1H, *d*, $J_{6,5}$ = 12.9 Hz, H6); 3.64, 3.72 (6H, *s*, 2x–CO_2CH_3); 3.84 (2H, *s*, H17); 4.36 (1H, *t*, J = 2.7 Hz, H12). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.8 (*s*, C18); 19.5 (*d*, C2); 21.4 (*s*, $-O_2C\underline{CH_3}$); 24.7 (*d*, C11); 29.0 (*s*, C20); 37.4, 39.5, 43.2 (*d*, C1, C3, C14); 44.3, 46.7 (*q*, C4, C10); 50.5 (*t*, C16); 51.5, 51.6 (*s*, 2×–$CO_2\underline{CH_3}$); 52.2 (*t*, C6); 54.9 (*t*, C9); 55.6 (*d*, C15); 56.7 (*t*, C5); 65.3 (*q*, C8); 75.7 (*d*, C17); 83.3 (*t*, C12); 88.3 (*q*, C13); 170.5 (*q*,–$O_2\underline{C}CH_3$); 175.0, 177.5 (*q*, C7, C19). LRMS (*m*/*z*) 512 ([M]$^+$, 1%), 482 (2), 454 (36), 433 (18), 373 (100), 313 (48), 253 (34), 181 (22).

3.3.6. Dimethyl ent-13-acetoxy-12α-hydroxy-gibberell-16-ene-7,19-dioate (8)

A stirred suspension of activated zinc (66 mg, 1 mmol) in a solution of the cyclic bromo-ether **7** (20 mg, 0.04 mmol) in dimethyl formamide (400 μl) was cooled to 0 °C and treated dropwise with HCl (5 M aqueous, 700 μl) and stirring continued for 2 h. The reaction mixture was diluted with EtOAc (10 ml) and washed with H_2O (10 ml). The aqueous phase was re-extracted with EtOAc (10 ml, ×2) and the combined organic layers washed with H_2O (10 ml) and brine (10 ml). The organic phase was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The residue was subjected to silica gel chromatography (EtOAc/petroleum spirit 1:1) to yield, in order of elution, the cyclic bromo-ether **7** (6 mg, 35%) and the desired 12β-carbinol **8** (6 mg, 35%) as a clear oil (R_f = 0.44 EtOAc/petroleum spirit 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.69 (3H, *s*, H20); 1.05 (3H, *s*, H18); 1.89 (1H, *d*, $J_{5,6}$ = 11.8 Hz, H5); 2.05 (3H, *s*,–O_2CCH_3); 3.36 (1H, *d*, $J_{6,5}$ = 12.1 Hz, H6); 3.66, 3.70 (6H, *s*, 2x–CO_2CH_3); 4.22 (1H, *t*, J = 6.5 Hz, H12); 5.10 (1H, *s*, H17); 5.15 (1H, *s*, H'17). IR 3500, 1730 cm^{-1}. ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6 (*s*, C18); 19.5 (*d*, C2); 22.0 (*s*, –$O_2C\underline{CH_3}$); 28.1 (*s*, C20); 29.7 (*d*, C11); 37.6, 39.1 (*d*, C1, C3); 43.6, 43.8 (*d*, C14, C15); 44.3, 48.9 (*q*, C4, C10); 50.0 (*t*, C6); 51.4, 51.7 (*s*, 2x–$CO_2\underline{CH_3}$); 56.0 (*t*, C9); 58.2 (*t*, C5); 72.7 (*t*, C12); 87.6 (*q*, C13); 109.9 (*d*, C17); 145.6 (*q*, C16); 175.5 (*q*, C7, C19). LRMS (*m*/*z*) ([M]$^+$, absent), 462 ([M $-COCH_3$]$^+$, 3%), 434 ([M $-$TMSi]$^+$, 5), 407 (24), 375 (100), 343 (43), 207 (24), 73 (38). HRMS [M]$^+$ found 434.2310 ($C_{24}H_{34}O_7$ requires 434.2305).

3.3.7. Dimethyl ent-12α, 13-dihydroxy-gibberell-16-ene-7,19-dioate (9)

Acetate **8** (6 mg, 0.01 mmol) was dissolved in MeOH (250 μl) and treated with K_2CO_3 (18 mg, 0.1 mmol) and stirred at room temperature under nitrogen. After 12 h TLC analysis indicated that the starting material had been consumed. The reaction mixture was diluted with EtOAc/10% butan-2-ol (5 ml) and washed with H_2O (5 ml, ×2), brine, and dried over $MgSO_4$. The solvent was

filtered and then removed under reduced pressure. Chromatography on silica gel (EtOAc/petroleum spirit 2:1) yielded the desired diol **9** (3 mg, 55%) as a white solid (R_f = 0.26 EtOAc/petroleum spirit 2:1). IR 3500, 1730 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.71 (3H, *s*, H20); 1.03 (3H, *s*, H18); 1.91 (1H, *d*, $J_{5,6}$ = 11.7 Hz, H5); 3.39 (1H, *d*, $J_{6,5}$ = 11.8 Hz, H6); 3.66, 3.69 (6H, *s*, 2×–CO_2CH_3); 3.78 (1H, *s*, H12); 5.11 (2H, *d*, H17). LRMS (*m*/*z*) 536 ($[M]^+$, 74%), 504 (19), 477 (27), 433 (100), 420 (60), 301 (37), 295 (42), 251 (51), 193 (100), 181 (86), 147 (41), 73 (100). HRMS $[M]^+$ found 392.2196 ($C_{22}H_{32}O_6$ requires 392.2199).

3.4. Synthesis of 16β,17-dihydro-17-hydroxy GA_{53} dimethyl ester

3.4.1. Dimethyl ent 13,17-dihydroxy-16-epi-gibberellane-7,19-dioate (*11*) and dimethyl ent-13,17-dihydroxygibberellane-7,19-dioate (*12*)

GA_{53} dimethyl ester **10** (65 mg, 0.18 mmol) was dissolved in dry THF (1.7 ml), cooled to 0 °C and flushed with nitrogen. BH_3.THF (180 μl, 0.36 mmol) was then added in a slow dropwise fashion and the reaction allowed to stir for 2 h at room temp. At this point the reaction was cooled to 0 °C, 2M NaOAc (540 μl) and H_2O_2 (370 μl) added and the mixture allowed to stir at room temp for 16 h. Water (5 ml) was then added and after extraction with EtOAc (5 ml, ×3), the combined organic layers were washed with brine (5 ml) and dried over $MgSO_4$. The organic layer was then filtered and the solvent removed under reduced pressure to yield a oil which upon chromatography on silica gel (CH_2Cl_2/2% MeOH) yielded in order of elution**: dimethyl *ent*-13,17-dihydroxy-16-*epi*-gibberellane-7,19-dioate (11)** (26 mg, 37%) (R_f = 0.17, CH_2Cl_2/2% MeOH). IR 3500, 1730 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.66 (3H, *s*, H20); 1.05 (3H, *s*, H18); 1.86 (1H, *d*, $J_{5,6}$ = 12.1 Hz, H5); 3.32 (1H, *d*, $J_{6,5}$ = 12 Hz, H6); 3.65, 3.68 (6H, *s*, 2x–$CO_2\underline{CH_3}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5 (s, C18); 18.1 (*d*, C2); 19.6 (*d*, C11); 28.3 (*s*, C20); 37.7 (*d*, C1); 39.4 (*d*, C15); 40.2 (*d*, C3); 40.8 (*d*, C12); 44.1, 44.3 (*q*, C4 and C10); 44.5 (*t*, C16); 47.4 (*d*, C14); 49.4 (*q*, C8); 50.6 (*s*, C6); 51.4, 51.5 (*t*, 2x–$CO_2\underline{CH_3}$); 55.9 (*t*, C9); 57.9 (*s*, C5); 64.7 (*d*, C17); 79.1 (*q*, C13); 175.7, 177.3 (*q*, C7 and C9). Me-TMSi: LRMS (*m*/*z*) 538 ($[M]^+$, 7%), 448 (13), 375 (100), 347 (21), 297 (18), 181 (18), 147 (15), 73 (31); and **dimethyl *ent*-13,17-dihydroxy-gibberellane-7,19-dioate (12)** (26 mg, 37%) (R_f = 0.11 CH_2Cl_2/2% MeOH). IR 3500, 1730 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 0.64 (3H, *s*, H20); 1.06 (3H, *s*, H18); 1.83 (1H, *d*, $J_{5,5}$ = 12.5 Hz, H5); 3.23 (1H, *d*, $J_{6.5}$ = 12.4 Hz); 3.65, 3.70 (6H, *s*, 2x$CO_2\underline{CH_3}$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.3 (*s*, C18); 17.5 (*d*, C2); 19.5 (*d*, C11); 28.7 (*s*, C20); 31.5 (*d*, C12); 37.6 (*d*, C1); 39.3 (*d*, C3); 39.6 (*d*, C15); 43.8, 44.3 (*q*, C4 and C10); 47.3 (*t*, C16); 47.5 (*d*, C14); 49.0 (*q*, C8); 51.4, 51.4 (*s*, 2x–$CO_2\underline{CH_3}$); 51.9 (*s*, C6); 57.3 (*s*, C15); 59.6 (*s*, C9); 63.7 (*d*, C17); 78.9 (*q*, C13); 175.8, 177.6 (*q*, C7 and C19). Me-TMSi: LRMS (*m*/*z*) 538 ($[M]^+$, 6%), 448 (8), 375 (100), 347 (22), 297 (16), 181 (12), 147 (12), 73 (18). HRMS $[M]^+$ found 394.2361 ($C_{22}H_{34}O_6$ requires 394.2355).

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