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GIBBERELLINS A_{82} AND A_{83} IN SEED OF *LUPINUS ALBUS*

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Key Word Index—*Lupinus albus*; Leguminosae; seeds; gibberellins; new gibberellins A_{82} and A_{83}; GC-MS; partial synthesis; bioassay.

Abstract—Extracts from seeds of *Lupinus albus* at 14, 22, 35 and 52 days after anthesis were separated into free gibberellins, ester conjugates and ether conjugates. Capillary GC-MS of the methylated and trimethylsilylated free gibberellin fractions showed the presence of the known gibberellins A_1, A_3, A_4, A_{17}, A_{18}, A_{23} and A_{43}. In addition six new gibberellin-like compounds were detected that corresponded to the addition of the elements of water to gibberellins A_3, A_4, A_7, A_{14}, A_{18} and A_{34}. Two of these components were identified by chemical syntheses as *ent*-3α,10β,17-trihydroxy-20-nor-16αHgibberellane-7,19-dioic acid 19,10-lactone and *ent*-3α,17-dihydroxy-16αHgibberellane-7,19-dioic acid, which are accorded the gibberellin numbers A_{82} and A_{83}, respectively. *ent*-3α,10β,16β,17-Tetrahydroxy-20-nor-16βHgibberellane-7,19-dioic acid 19,10-lactone was also identified by synthesis of the methyl ester. Similar analyses of the hydrolysed ether conjugate fractions showed the presence of the known gibberellins A_1, A_3, A_{13}, A_{18} and A_{43}, the new gibberellin A_{82} and the 'hydrated' gibberellins A_{18} and A_{34}; the 15-ene isomers of gibberellins A_{13} and A_{43} and the 16ξ17-epoxide of gibberellin A_{18} were also identified as probable artefacts. In the hydrolysed ester conjugate fractions the new gibberellin A_{82} and the 'hydrated' gibberellin A_{34} were detected. Gibberellin A_{18} was by far the most abundant GA but quantitation of the GAs was not carried out.

INTRODUCTION

Seeds of *Lupinus* species are a rich source of gibberellin-like substances [1, 2] and gibberellin A_{18} (GA_{18}) (**9**), GA_{19} (**12**), GA_{23} (**13**) and GA_{28} (**11**) have been identified [3–6] in seeds of *L. luteus* (yellow lupin). This paper describes the detection and identification of GAs in developing seeds of *L. albus* (white lupin) cv Vladimir. Two new GAs, GA_{82} (**34**) and GA_{83} (**37**), were characterized by chemical syntheses of their methyl esters from GA_4 (**3**) and GA_{14} (**8**), respectively.

RESULTS

Seeds of *L. albus*, cv Vladimir, were extracted by a modification of the method of Hiraga *et al.* [7] to provide: (i) the neutral compounds soluble in ethyl acetate (NE), (ii) the compounds extracted with ethyl acetate at pH 3.0 (AE) and (iii) the compounds extracted with *n*-butanol at pH 3.0 (AB). The AE fraction was separated into free GAs and presumed GA glycosyl esters by chromatography using DEAE cellulose (DE-52, hydroxyl form) [8]. The presumed glycosyl esters were hydrolysed to the free GAs by a pectinolytic enzyme preparation. The AB fraction was similarly hydrolysed to free GAs. The free GAs from the AE and AB fractions were subjected to reverse-phase HPLC. The HPLC fractions were combined on the basis of bioassay results using the lettuce hypocotyl assay [9] (data not presented) and all fractions from the column, whether biologically active or inactive, were methylated and trimethylsilylated, then analysed by capillary GC-mass spectrometry. The GAs and GA derivatives that were identified by full scan GC-mass spectrometry are discussed below under the separate headings of known GAs, new GAs and putative GAs. The distribution of these GAs in seeds of different ages is shown in Table 1. Although no internal standard was available for quantitation by GC-mass spectrometry, it was shown from the strength of the intensity of its response that GA_{18} was present in the seeds in far greater amounts than any of the other GAs.

Known gibberellins

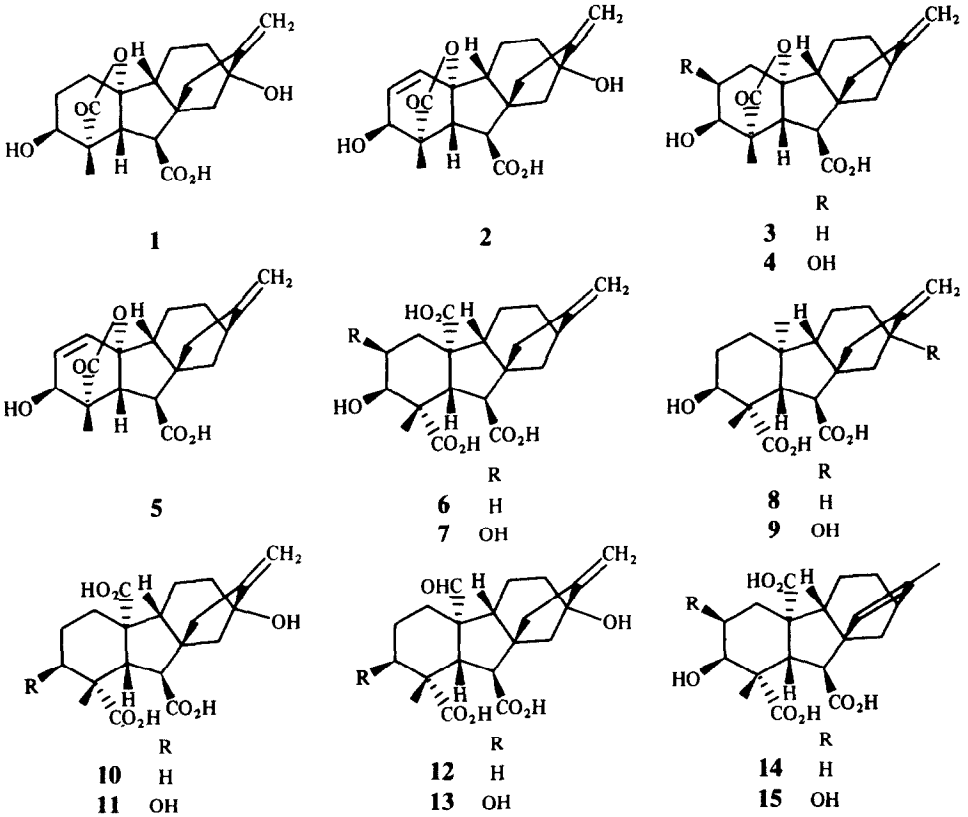
The following GAs were identified in the AE fractions: GA_1 (**1**), GA_3 (**2**), GA_4 (**3**), GA_{17} (**10**), GA_{18} (**9**), GA_{23} (**13**) and GA_{43} (**7**). In the enzyme-hydrolysed AB fractions GA_1 (**1**), GA_3 (**2**), GA_{13} (**6**) and GA_{18} (**9**) were identified together with GA_{13}-15-ene (**14**) and GA_{43}-15-ene-(**15**).

New gibberellins

Six new GAs were detected as free GAs and after enzyme hydrolysis of the conjugate fractions. They corresponded to the addition of the elements of water to **2**, **3**, GA_7 (**5**), **8**, **9** and GA_{34} (**4**). However they were not the analogues of GA_2 (**16**) and GA_{10} (**17**) since the mass spectra of their MeTMSi derivatives did not contain an *m/z* 130 ion, characteristic [10] of the MeTMSi derivatives of GA_2 and GA_{10}. Possible structures for these new GAs were the 16α- or 16β,17 dihydro-17-ols, for example, in the case of the 'hydrated'-GA_4, the structures **18** or **20**, or the 3-epimers **19** or **21**. The partial synthesis of the

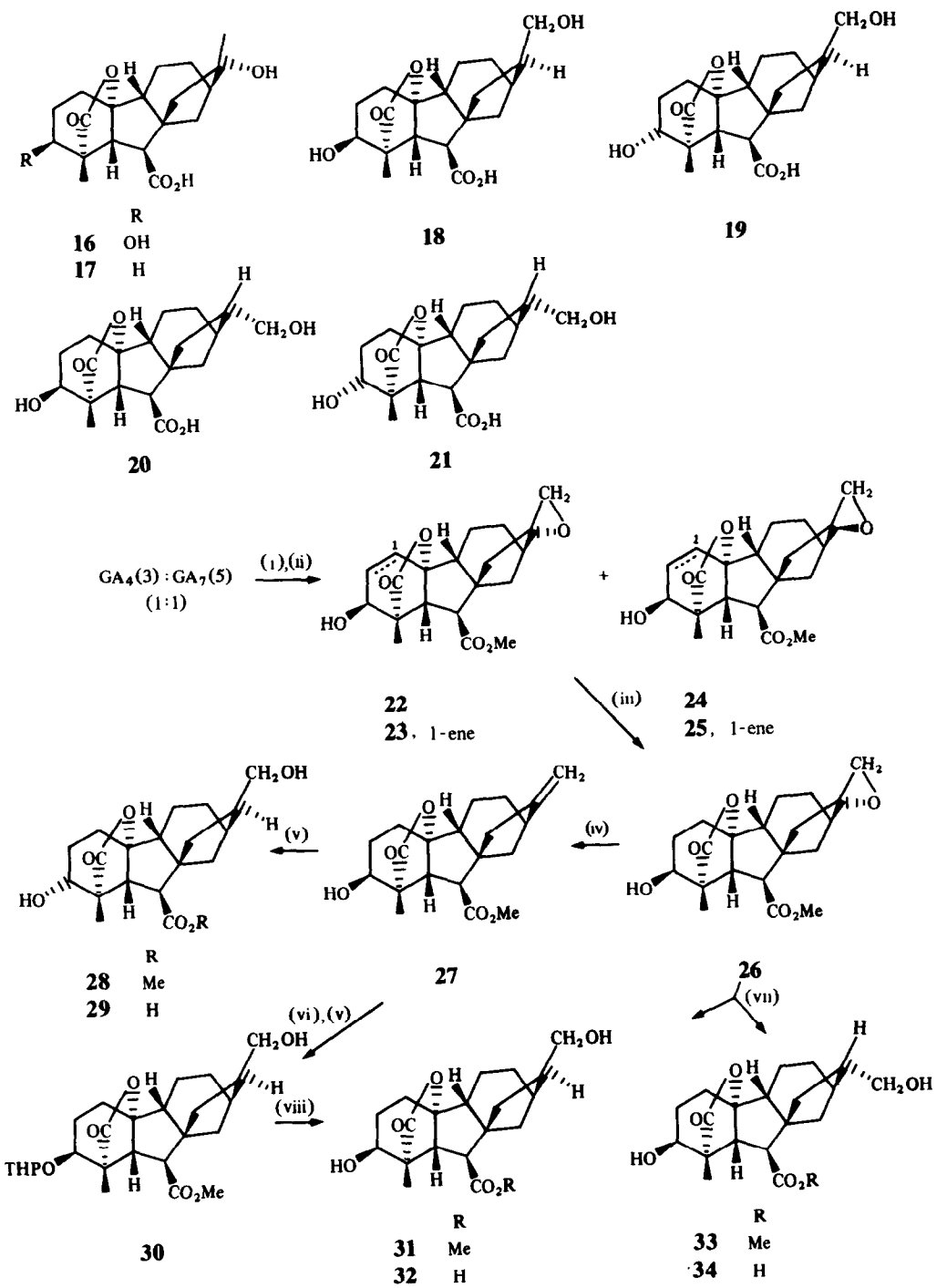
Table 1. Gibberellins and gibberellin-like compounds in developing seeds of *L. albus*

(A) 14 Days after anthesis	
Free GAs	GA_3 (2), GA_{18} (9), GA_{23} (11), GA_{82} (34), 'Hydrated' GA_7
(B) 22 Days after anthesis	
Free GAs	GA_1 (1), GA_3 (2), GA_{18} (9), GA_{23} (13), GA_{43} (7), GA_{82} (34), GA_{83} (37), 'Hydrated' GA_7, 'Hydrated' GA_{18}, putative GA_{18} 16,17-epoxide (42)
Hydrolysed glycosyl ester fraction	GA_{82} (34)
Hydrolysed glycosyl ether fraction	GA_1 (1), GA_3 (2), GA_{13} (6), GA_{18} (9), GA_{43} (7), GA_{82} (34), 'Hydrated' GA_{18}, 'Hydrated' GA_{34}, putative GA_{18} 16,17-epoxide (42)
(C) 35 Days after anthesis	
Free GAs	GA_4 (3), GA_{18} (9), GA_{23} (11), GA_{82} (34), 'Hydrated' GA_{18}, putative GA_{18} 16,17-epoxide (42)
Hydrolysed glycosyl ester fraction	Hydrated GA_{34}
Hydrolysed glycosyl ether fraction	GA_3 (2), GA_{13} (6), GA_{13}-15-ene (14), GA_{43}-15-ene (15), 'Hydrated' GA_{34}
(D) 52 Days after anthesis	
Free GAs	GA_{17} (10), GA_{18} (9), GA_{23} (13), GA_{82} (34), 'Hydrated' GA_{18}, 'Hydrated' GA_{34}



methyl esters of three of these possible structures from **3** was achieved as shown in Scheme 1.

The starting material, GA_4 methyl ester (**27**, Scheme 1), was prepared from a commercial mixture (1:1) of **3** and **5**. This mixture was methylated then treated with 3-chloroperbenzoic acid to give a mixture (10:1) of 16α,17-epoxides (**22** and **23**) and the 16β,17-epoxides (**24** and **25**). The pair of 16α-epoxides (**22** and **23**) were separated from the pair of 16β-epoxides (**24** and **25**) by flash chromatography and hydrogenated over palladium on charcoal to give GA_4 methyl ester 16α,17-epoxide (**26**). Deoxygenation [11] of the epoxide **26** gave pure GA_4 methyl ester (**27**) in 40% overall yield from the starting mixture of **3** and **5**. The tetrahydropyranyl ether **30** was prepared prior to hydroboronation of **27** to avoid possible epimerization [12] of the 3-alcohol. Deprotection of the hydroboronation product **30** with toluene-4-sulphonic acid gave 16α,17-dihydro-17-hydroxyGA_4 methyl ester (**31**). The hydroboronation product **30** was also hydrolysed with aqueous sodium hydroxide before removal of the protecting tetrahydropyranyl ether to provide the free acid (**32**) for bioassay (see later). The stereochemistry of the hydroboronation products was assigned by the precedents of exo-attack at the 16-ene in GAs and was



Scheme 1. Partial synthesis of epimers of 16,17-dihydro GA_4-17-ol. Reagents: (i) CH_2N_2; (ii) 3-$ClC_6H_4CO_3H$; (iii) H_2, $CaCO_3$; (iv) NaI, NaOAc, HOAc, Zn; (v) $BH_3 \cdot THF$, H_2O_2, NaOH; (vi) Dihydropyran, 4-$MeC_6H_4SO_3H$; (vii) Na (CN) $BH_3 \cdot Et_2O$; (viii) 4-$MeC_6H_4SO_3H$.

supported by ^{13}C NMR data discussed later. The full scan GC-mass spectrum of the TMSi derivatives of the synthetic 16α,17 dihydro-17-diol (**32**) and the *Lupinus* 'hydrated' GA_4 were very similar but the KRI values were slightly different (2813 for the natural GA and 2815 for the synthetic compound). Thus the *Lupinus* GA was not the 16α,17-dihydro-17-hydroxyGA_4 (**32**). Nor was it the 3α-epimer (**29**), the methyl ester of which was prepared by direct hydroboronation of **27** without protection of the 3-hydroxyl from epimerization. The synthesis of the alternative structure, 16β,17 dihydro-17-hydroxyGA_4 (**34**), was, therefore, undertaken (see Scheme 1).

Anti-Markownikoff hydration of the 16-ene in GAs is not a trivial task. As discussed earlier, hydroboronation of **27** occurs from the exo (least hindered)-face, and

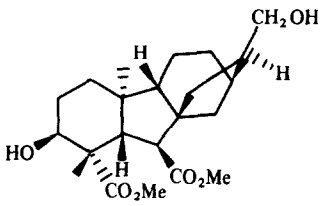
epoxidation gives predominantly the 16α,17-epoxide (**26**). In principle, hydride reduction of **26** at the more substituted 16-position provides a route to the required 16β,17-dihydro-17-ol (**34**). Several methods for the anti-Markownikoff reduction of epoxides have been described. Of these the AlH_3–$AlCl_3$ reagent [13] was rejected because of its poor regio- and stereo-selectivity. Attempted hydrogenolysis of **26** with either Pd-C or PtO_2 [14] and attempted reduction with $NaBH_4$–B_2H_6 [13] gave no reaction. Reaction of **26** with $Na(CN)BH_3$–$BF_3.Et_2O$ [15] gave a product (57%) that appeared to be homogeneous by capillary GC. However a detailed analysis of the ^1H and ^{13}C NMR spectra indicated a mixture (2:1) of two compounds. The major component showed all the signals of the previously prepared **31** and the minor component was assigned the 16β,17-dihydro-17-ol structure **33** on the basis of the following NMR and GC-mass spectral data. In the ^{13}C NMR spectrum of the mixture (**31**, **33**) the chemical shift of C-17 in the endo isomer (**31**) occurred at lower field (δ63.88) than that (δ67.47) of the minor isomer (**33**). This chemical shift difference is consistent with the assigned sterochemistry at C-16 and appears to be characteristic for 16-epimeric 16,17-dihydroGAs (see also later). Although the TMSi derivatives of the mixture of epimers (**31**, **33**) were not separated by capillary GC, they were just resolved by capillary GC-mass spectrometry by scanning over a limited mass range of 750 to 200 amu in order to decrease the cycle time to 1 sec. By monitoring the ions at *m/z* 508 $[M]^+$, 493 $[M-15]^+$ and 490 $[M-18]^+$, it was found that the KRI (2813) of the TMSi of the minor component (**33**) was identical to the MeTMSi of the natural GA which was therefore identified as 16β,17-dihydro-17-hydroxyGA_4 (**34**).

The mixture of methyl esters (**31** and **33**) was protected from epimerization of the 3-hydroxyl by formation of the tetrahydropyranyl ethers. The mixture of ethers was directly hydrolysed using aqueous alkali then deprotected using acid to give a mixture (3:7) of **34** and its 16-epimer (**32**) used for bioassay (see later).

In a similar manner the structure of the 'hydrated' GA_{14} in the *Lupinus* seeds was shown to be 16β,17-dihydro-17-hydroxyGA_4 (**37**). The dimethyl ester (**35**) of the 16αH-epimer was prepared in 60% yield by hydroboronation of dimethyl ester of GA_{14} (**8**); no epimerization of the 3-hydroxyl occurred. The dimethyl ester (**36**) of the 16βH-epimer was prepared by epoxidation of the dimethyl ester of GA_{14} (**8**), then reduction of the resultant epoxide (**38**) with $Na(CN)BH_3$–$BF_3.Et_2O$. As in the case of GA_4 dimethyl ester 16α,17-epoxide (**26**, Scheme 1), the dimethyl esters **35** and **36** of the 16α,17-dihydro-17-diol and the 16β,17-dihydro-17-diol were obtained as a 2:1 mixture that was resolved by fast scanning capillary GC-mass spectrometry of the TMSi derivatives. The KRI (2776) of the TMSi derivatives of the minor component and the MeTMSi of the natural GA were the same and different to that (2775) of the TMSi of **35**. In the ^{13}C NMR spectrum of the mixture the minor component (**36**) showed the 17-carbon signal at δ67.41, compared to δ64.45 for the major component, and is therefore assigned the exo-17 stereochemistry. The structure of the 'hydrated' GA_{14}, detected in the lupin seeds, is therefore concluded to be 16β,17-dihydro-17-hydroxyGA_{14} (**37**).

Since 16β,17-dihydro-17-hydroxyGA_{14} (**34**) and **37** have been identified as naturally occurring GAs, they are allocated [16] the numbers GA_{82} and GA_{83}, respectively. The other 'hydrated' GAs detected in the lupin seeds may be the 16β,17-dihydro-17-hydroxy derivatives of **2**, **4**, **5** and **9** but authentic samples were not available for direct comparison.

16α,17-Dihydro-16α,17-dihydroxyGA_4 (**39**) was also identified in extracts of the lupin seeds by direct comparison of the full scan GC-mass spectrum and KRI of the MeTMSi derivative with an authentic sample. The authentic diol **39** was prepared as the methyl ester by treatment of **27** (see Scheme 1) with osmium tetraoxide in the presence of *N*-methylmorpholine *N*-oxide [17, 18]. An attempt to prepare the diol **39** by treatment of the tetrahydropyranyl ether of the epoxide (**26**, see Scheme 1) with aqueous alkali, followed by acidic work-up, gave the



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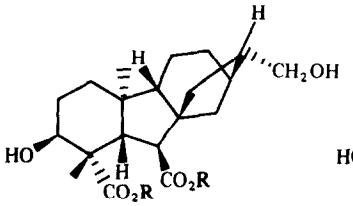
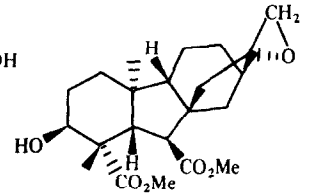


36 R = Me
37 R = H



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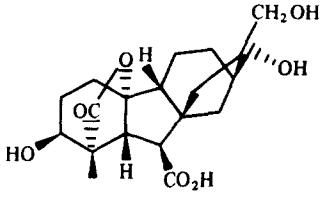

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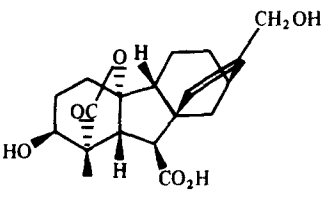

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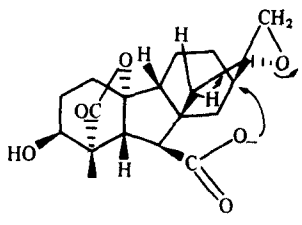

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15-ene **40**, characterized by ^{1}H NMR and mass spectrometry. A possible mechanism for this unexpected result is shown in **41**.

Putative gibberellins

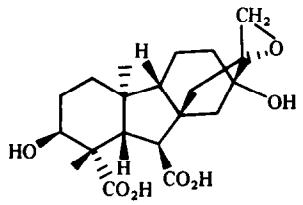
Five putative GAs were detected as their MeTMSi derivatives by capillary GC-mass spectrometry. One may be GA_{18}-16,17-epoxide (**42**) but no authentic sample was available for direct comparison. The other four were unidentified isomers of a monohydroxyGA_{18}.

Bioassay of GA_{82} (34)

This mixture of GA_{82} (**34**) and its 16-epimer (**32**) and the pure 16-epimer (**32**), prepared as described earlier, were bio-assayed using the Tan-ginbozu rice assay [19]. The results (Table 2) showed that **34** and its 16-epimer (**32**) were inactive. In contrast, **16** is reported [20] to be no less active than **3**.

DISCUSSION

Ten GAs were identified by full scan GC-mass spectrometry and KRI in developing seeds of *L. albus* cv Vladimir. Gibberellin A_{18} was by far the most abundant but quantitation of it and other GAs was not undertaken. The detected GAs comprise an equal number of 13-hydroxy- and 13-non-hydroxy GAs. The new GAs, GA_{82} (**34**) and GA_{83} (**37**), may be the first examples of a family of natural 16β,17-dihydro-17-hydroxyGAs since a further four putative GAs were detected that may be such derivatives of GA_3 (**2**), GA_7 (**5**), GA_{18} (**9**) and GA_{34} (**4**).



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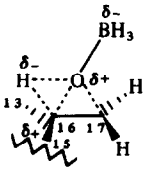

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Also putative 16,17-dihydro-17-hydroxy derivatives of GA_{53}, GA_{44} and GA_{19} have been detected in extracts from shoots of maize [21, and unpublished results]. However, **34** is biologically inactive in the dwarf rice assay and may be a deactivation product of **3**. The other putative 16,17-dihydro-17-hydroxy GAs may also be deactivation products. In the present work, 16α,17-dihydro-16α,17-dihydroxyGA_4 (**39**) was also identified but a GA number has not been allocated to it since it may have been formed as an artefact via GA_4 16,17-epoxide. It is unlikely to be formed from **3** via **34** since all precedents [22, 23] indicate that enzymatic hydroxylation of GA_{82} (**34**) at C-16 would occur with retention of configuration.

There are two points of chemical interest. Firstly, the lower chemical shift of the endo C-17 in the epimeric pairs **31**, **33** and **35**, **36** may provide a useful method of distinguishing 16-epimers of 16,17-dihydroGAs where both isomers are available. Secondly, the predominant retention of configuration in the cyanoborohydride reduction of the epoxides **26** and **38** is notable. A possible explanation [24, 25] is that hydride nucleophile forms a loose ion-pair with the complexed oxygen as shown in **43** and is thereby directed to attack C-16 from the same side as the oxygen with resultant retention of configuration.

EXPERIMENTAL

General experimental details have been described previously [26].

Plant material. Nodulated plants of *Lupinus albus* L. cv Vladimir were grown in a naturally lit glasshouse from April to September in 4 inch 'Long Tom' pots containing John Innes No. 1 compost. Plants received regular applications of 'Vitafeed 101' and 'Instant Bio' culture solns. Plants were tagged at anthesis. Seeds were collected at 14, 22, 35 and 52 days after anthesis.

Extraction procedure. Seeds (10 g dry wt) were ground, extracted with MeOH–H_2O (4:1, 50 ml × 4) and MeOH removed from the filtrate under red. pres. The residue was washed with 10 ml H_2O, the two aq. frs combined, adjusted to pH 8 with NaOH and slurried with PVP for 2 hr. After filtering, the filtrate was extracted with petrol (50 ml × 3), the petrol phases discarded and the aq. phase extracted with EtOAc (50 ml × 5). The pooled EtOAc phases (designated NE) were taken to dryness under red. pres. The aq. phase was then adjusted to pH 3 with HCl and partitioned against EtOAc (50 ml × 5). The pooled EtOAc

Table 2. Bioassay data for GA_4 (**3**), GA_{82} (**34**) and 16α,17-dihydro-17-hydroxy GA_4 (**32**) in the Tan-ginbozu dwarf rice assay

(i) Total length of seedling (mm)				
		μg Gibberellin per vial		
Gibberellin	10^{-2}	10^{-1}	10^{0}	10^{1}
GA_4 (**3**)	24.8±1.4	28.0±1.7	39.0±4.0	68.7±6.9
16α,17-Dihydro-17-hydroxy GA_4 (**32**)	25.2±1.7	24.9±1.9	26.0±1.6	27.4±3.1
GA_{82} (**34**): 16-epimer (**32**) (3:7)	24.4±1.5	24.6±2.4	25.6±1.6	26.3±1.6
Control	22.7±1.3			
(ii) Length of second leaf sheath (mm)				
		μg Gibberellin per vial		
Gibberellin	10^{-2}	10^{-1}	10^{0}	10^{1}
GA_4 (**3**)	15.1±1.1	16.9±1.8	25.4±3.2	53.3±6.7
16α,17-Dihydro-17-hydroxy GA_4 (**32**)	15.3±1.2	15.1±1.4	15.8±1.9	17.8±2.6
GA_{82} (**34**): 16-epimer (**32**) (3:7)	15.0±1.1	14.9±1.7	14.9±1.8	19.3±1.6
Control	13.7±0.9			

phases (designated AE) were taken to dryness under red. pres. The aq. phase was extracted with *n*-BuOH (50 ml × 5) and the aq. phase discarded. The pooled *n*-BuOH phases were divided into two equal parts and evapd to dryness under red pres. One part (designated AB) was retained, the other dissolved in H_2O (50 ml) and hydrolysed (see below).

The presumed glycosyl esters in fraction NE were sepd from free GAs using a silicic acid column [27]. Acid washed silicic acid was slurried in hexane and packed into a 20 × 2.0 cm i.d. glass column. The extract was added to 1.0 g silicic acid in EtOAc, dried and added to the column in a hexane slurry. The column was eluted stepwise with 9 increasing concs of EtOAc in hexane (0–90%) and finally EtOAc. Ten frs (20 ml) were collected and taken to dryness under N_2.

A Whatman DEAE cellulose column was used to separate the presumed glycosyl esters from free GAs in the acidic EtOAc fr. (AE). The method was a modification of that of ref. [8]. A 20 × 2.0 cm i.d. column of DEAE cellulose (DE52, hydroxylated form) was pre-washed with 0.1 M H_2SO_4 and then 0.1 N NaOH and rinsed until the eluate reached pH 7.0. The fr. (AE) was dissolved in dist. H_2O (5 ml) and added to the column which was then washed with 200 ml dist. H_2O to elute the presumed glycosyl esters and 200 ml 0.5 M Na_2SO_4, pH 7.0, to elute the free GAs. All frs from CC were chromatographed on TLC plates in ETOAc–$CHCl_3$–HOAc (15:5 1) and then bioassayed [9] to monitor the elution of biologically active GAs.

The presumed glycosyl esters were hydrolysed to the free GAs using a pectinolytic enzyme preparation (Boots Co.). The enzyme was released from the Kieselguhr support by slurrying with 100 ml 0.1 M KPi–citrate buffer (pH 4.0) for 1 hr and then filtering. The filtrate was added to the aqueous plant extract (100 ml) which was incubated for 48 hr at 37° in darkness. After hydrolysis the aq. extract was slurried with PVP, adjusted to pH 3.0 and partitioned against EtOAc.

The free GAs from the AE and AB fractions were subjected to reverse phase HPLC. A semi-prep. column (15 × 1.0 cm i.d.) was packed with Apex ODS (5 μm totally porous microspherical silica with a C18 non-polar bonded phase, Jones Chromatography). Samples were taken up in 20% aq. MeOH, Millipore filtered (0.5 μm), loaded onto the column and eluted with a linear gradient of 20% MeOH in H_2O to 100% MeOH containing 100 $\mu l\,l^{-1}$ HOAc (30 min, 3 ml min^{-1}). Frs (3 ml) were taken to dryness under N_2. Aliquots of each fr. were bioassayed [9] and the remainder of the frs derivatized (Me esters and TMSi ethers) prior to GC-MS.

Derivatization of seed extracts and GC-MS. Samples were methylated with excess CH_2N_2, taken up in dry pyridine and trimethylsilated by the addition of trimethylchlorosilane and hexamethyldisilazane and, after mixing, heating at 120° for 5 min. GC-MS was done using WCOT fused silica column (25 m × 0.2 mm i.d.) coated with OV1. The He pressure was 2 bars, and the inj. temp. 250°. Injections were made in the Grob splitless mode, with CH_2Cl_2 as solvent. The injector purge gas was activated after 30 sec and the column temp. rapidly increased to 150° and then at 3° min^{-1} to 300°. The column effluent led directly into the ion source via a heated interface maintained at 250°. The source pressure was 3×10^{-6} mbar. Data were acquired and processed using a VG7035 computerized GC-MS.

Preparation of epoxides **22**, **23** *and* **24**, **25**. A mixt. of the Me esters of GA_4 (**3**) and GA_7 (**5**), prepd by methylation (CH_2N_2) of a mixt. (1:1, 4.5 g) of GA_4 (**3**) and GA_7 (**5**) was dissolved in $CHCl_3$ (150 ml) and treated with 3-chloroperoxybenzoic acid (2.64 g) at 0° for 24 hr. The mixt. was dil. with $CHCl_3$, washed sequentially with aq. $NaHSO_3$, aq. $NaHCO_3$ and finally H_2O. Removal of solvent and fractionation of the residue by flash CC gave, on elution with 40% EtOAc in petrol, unchanged starting material (0.59 g). Elution with 60% EtOAc in petrol gave the previously unisolated 16β,17-epoxides of GA_4 and GA_7 Me esters (**24**, **25**) as an inseparable mixt. (231 mg). (Found: $[M]^+$ 362.1694 and $[M]^+$ 360.1565; $C_{20}H_{26}O_6$ requires $[M]^+$ 362.1729 and $C_{20}H_{24}O_6$ requires $[M]^+$ 360.1573.) ^{1}H NMR, ($CDCl_3$); δ (**24**) 1.14 (*s*, H_3-18), 2.67 (*d*, *J* = 11 Hz, H-6), 2.77 (*d*, *J* = 5 Hz, H-17), 2.85 (*d*, *J* = 5 Hz, H-17), 3.18 (*d*, *J* = 11 Hz, H-5), 3.71 (*s*, OMe) and 3.83 (*br s*, H-3); δ($CDCl_3$) (**25**) 1.24 (*s*, H_3-18), 2.76 (*d*, *J* = 11 Hz, H-6), 2.79 (*d*, *J* = 5 Hz, H-17), 2.85 (*d*, *J* = 5 Hz, H-17), 3.18 (*d*, *J* = 11 Hz, H-5), 3.73 (*s*, OMe), 4.14 (*br s*, H-3), 5.91 (*dd*, *J* = 9 and 3.5 Hz, H-2) and 6.36 (*d*, *J* = 9 Hz, H-1); MS *m/z* (rel. int.): 362 $[M]^+$ (30), 360 $[M]^+$ (6), 344 (8), 342 (3), 331 (7), 330 (4), 329 (9), 328 (8), 310 (15), 302 (10), 300 (51), 239 (72), 238 (100), 135 (70) and 91 (78).

Further elution with 60% EtOAc in petrol gave an inseparable mixt. (2.87 g) of the required 16α,17-epoxides (**22**, **23**) of GA_4 and GA_7 Me esters, the components being identified by NMR [26]. ^{1}H NMR ($CDCl_3$): δ (**22**), 1.15 (*s*, H_3-18), 2.74 (*d*, *J* = 11 Hz, H-6), 2.84 (*s*, H_2-17), 3.18 (*d*, *J* = 11 Hz, H-5), 3.71 (*s*, OMe) and 3.84 (*br s*, H-3); (23), 1.25 (*s*, H_3-18), 2.83 (*d*, *J* = 11 Hz, H-6), 2.84 (*s*, H_2-17), 3.18 (*d*, *J* = 11 Hz, H-5), 3.74 (*s*, OMe), 4.16 (*d*, *J* = 3.5 Hz, H-3), 5.92 (*dd*, *J* = 9 and 3.5 Hz, H-2) and 6.34 (*d*, *J* = 9 Hz, H-1); MS *m/z* (rel. int.): 362 $[M]^+$ (30), 360 $[M]^+$, (6), 344 (8), 342 (3), 331 (7), 330 (4), 329 (9), 328 (8), 302 (10), 300 (34), 297 (100) and 238 (25).

ent-3α,10β-*Dihydroxy*-16β,17-*epoxy*-20-*norgibberellane*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**26**). A mixt. (2.65 g) of the 16α,17-epoxides (**22**, **23**) of GA_4 and GA_7 Me esters in EtOAc (110 ml) was rapidly stirred with 10% Pd-$CaCO_3$ catalyst (150 mg) for 2 hr at room temp. under an atmosphere of H_2. The mixt. was dil. with EtOAc, filtered through Celite and the solvent removed under red. pres. to yield GA_4 Me ester 16α,17-epoxide (**26**) (2.60 g), mp 185–187° (lit. [28] 187–188°). ^{1}H NMR, ($CDCl_3$): δ 1.14 (*s*, H_3-18), 2.74 (*d*, *J* = 10.5 Hz, H-6), 2.84 (*s*, H_2-17), 3.19 (*d*, *J* = 10.5 Hz, H-5), 3.71 (*s*, OMe) and 3.84 (*d*, *J* = 2 Hz, H-3); MS *m/z* (rel. int.): 362$[M]^+$(100), 344 (20), 331 (26), 330 (11), 316 (24), 303 (24), 302 (32), 301 (13), 300 (42), 240 (36), 135 (33) and 91 (30).

ent-3α,10β-*Dihydroxy*-20-*norgibberell*-16-*ene*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**27**). NaI (5 g) and NaOAc (2.5 g) were dissolved in a mixt. of HOAC (80 ml) and H_2O (5 ml). Freshly activated Zn dust (*ca* 2 g) was added and the mixt. stirred at room temp. for 5 min. Gibberellin A_4 Me ester 16α,17-epoxide (**26**) (2.60 g) was added as a soln in Me_2CO (10 ml) and the stirring continued for a further 4.5 hr. The suspension was filtered and the solvent removed under red. pres. to leave a residue which was redissolved in EtOAc and washed with H_2O. Removal of solvent left a gum which was purified by flash CC. Elution with 40% EtOAc in petrol gave GA_4 Me ester (**27**) (1.83 g), mp 171–173° (lit. [29] 176°). ^{1}H NMR, ($CDCl_3$): δ1.14 (*s*, H_3-18), 2.67 (*d*, *J* = 10.5 Hz, H-6), 3.07 (*d*, *J* = 10.5 Hz, H-5), 3.71 (*s*, OMe), 3.83 (*br s*, H-3), 4.85 (*br s*, H-17) and 4.96 (*br s*, H-17); MS *m/z* (rel. int.): 346 $[M]^+$ (7), 328 (7), 314 (100), 300 (6), 286 (12), 284 (63), 268 (13) and 224 (68).

ent-10β,17-*Dihydroxy*-3α-*tetrahydropyranyloxy*-20-*nor*-16β*Hgibberellane*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**30**). A soln of GA_4 Me ester (**27**) (1 g) in CH_2Cl_2 (50 ml) was stirred with fr. dist. 2,3-dihydropyran (1 ml) in the presence of a catalytic amount of *p*-toluene sulphonic acid for 2 hr at room temp. Work-up gave the crude tetrahydropyranyl ether (1.8 g) as a mobile gum which, without further purification, was dissolved in THF (20 ml) and treated with a borane–tetrahydrofuran complex (5 ml of 1 M soln) at room temp. for 3 hr. The reaction mixt. was cooled in ice, treated with 2 M NaOH (10 ml) and 30% H_2O_2 (10 ml) and allowed to warm to room temp. The

reaction mixt. was stirred for a further hr, poured into H_2O, acidified and the product recovered into EtOAc. Flash CC gave, on elution with 55% EtOAc in petrol, 16α,17-dihydro-17-hydroxy-GA_4 Me ester 3β,tetrahydropyranyl ether (**30**) as an equimolar mixt. of diastereoisomers (683 mg). (Found: $[M-31]^+$ 417.2329. $C_{25}H_{36}O_7$-MeO requires $[M-31]^+$ 417.2277.) ^1H NMR, ($CDCl_3$): δ1.08 and 1.18 (*s*, H_3-18), 2.64 and 2.66 (*d*, *J* = 11 Hz, H-6), 3.18 and 3.19 (*d*, *J* = 11 Hz, H-5), 3.62 (*m*, H_2-17), 3.705 and 3.71 (*s*, OMe), 4.62 (*m*, H-3) and 4.74 (*t*, *J* = 2.5 Hz, H-3); MS *m/z* (rel. int.): 448 $[M]^+$ (abs), 417 (4), 364 (41), 346 (37), 332 (9), 318 (29), 304 (22), 303 (19), 243 (27) and 85 (100).

ent-3α,10β,17-*Trihydroxy*-20,16β*Hgibberellane*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**31**). The 3β-tetrahydropyranyl ether (**30**) (150 mg) in a mixture of Me_2CO (15 ml) and MeOH (3 ml) was stirred with *p*-toluene sulphonic acid (10 mg) at room temp. for 4 hr. The solvent was removed under red. pres. and the residue purified by flash CC. Elution with EtOAc gave 16α,17-dihydro-17-hydroxy-GA_4 Me ester (**31**) (112 mg), a portion of which was recrystallized from Me_2CO–petrol as fine needles, mp 182–183°. (Found: C, 65.42; H, 7.68; $[M]^+$ 364.1897. $C_{20}H_{28}O_6$ requires C, 65.91; H, 7.74%; $[M]^+$ 364.1886.) ^1H NMR, ($CDCl_3$): δ1.12 (*s*, H_3-18), 2.67 (*d*, *J* = 11 Hz, H-6), 3.15 (*d*, *J* = 11 Hz, H-5), 3.65 (*m*, H_2-17) and 3.82 (*br s*, H-3); ^{13}C NMR, $[(CD_3)_2CO]$: δ15.18, 15.99, 21.06, 28.16, 29.16, 34.36, 39.12, 40.64, 44.11, 51.76, 51.92, 53.74, 55.38, 56.77, 63.88 (C-17), 70.26 (C-3), 94.40 (C-10), 173.77 (C-7) and 178.61 (C-19); MS *m/z* (rel. int.): 364 $[M]^+$ (1), 362 (1), 346 (33), 333 (13), 332 (13), 318 (32), 314 (9), 304 (25), 302 (100), 243 (60) and 242 (51).

ent-3α,10β,17-*Trihydroxy*-20-*nor*-16β*Hgibberellane*-7,19-*dioic acid* 19,10-*lactone* (**32**). A soln of 16α,17-dihydro-17-hydroxyGA_4 Me ester 3-tetrahydropyranyl ether (**30**) (495 mg) in MeOH (10 ml) and 3 M NaOH (50 ml) was heated under reflux for 17 hr. The reaction mixt. was dil. with H_2O and extd with EtOAc. The aq. portion was brought to pH 1 with 2 M HCl and extd with EtOAc. The solvent was removed under red. pres. and the residue redissolved in MeOH (5 ml) and Me_2CO (10 ml). *p*-Toluenesulphonic acid (15 mg) was added and the soln allowed to stand at room temp. After 4 hr the solvent was removed and the residue purified by flash CC. Elution with EtOAc–HOAc (100:1) gave 16α,17-dihydro-17-hydroxy-GA_4 (**32**) (159 mg), a portion of which recrystallized from MeOH–Me_2CO–petrol as cubes, mp 195–197°. (Found: C, 65.42; H, 7.68; $[M]^+$ 364.1897. $C_{19}H_{26}O_6$ requires C, 65.12; H, 7.48%; $[M]^+$ 364.1886.) ^1H NMR, $[(CD_3)_2CO]$: δ1.10 (*s*, H_3-18), 2.55 (*d*, *J* = 11 Hz, H-6), 3.17 (*d*, *J* = 11 Hz, H-5), 3.58 (*m*, H_2-17) and 3.71 (*d*, *J* = 3 Hz, H-3); GC-MS (Me, TMSi): *m/z* (rel. int.) 508$[M]^+$ (5), 493 (13), 490 (5), 480 (7), 476 (8), 448 (7), 390 (11), 379 (14), 359 (4), 358 (9), 289 (43), 225 (51), 129 (26), 75 (100) and 73 (12).

ent-3β,10β,17-*Trihydroxy*-20-*nor*-16β*Hgibberellane*-7,19-*dioic acid* 7-*methyl ester* 19,10-*lactone* (**28**). Gibberellin A_4 Me ester (27) (100 mg) in THF (10 ml) was treated with a borane–tetrahydrofuran complex (2 ml of a 1 M soln) at room temp. After standing for 3 hr, 30% H_2O_2 (2 ml) and 2 M NaOH (4 ml) were added and the reaction mixt. stirred for 18 hr. Work-up and fractionation of the product by flash CC gave, on elution with EtOAc, 16α,17-dihydro-17-hydroxyGA_4 Me ester (**31**) (11 mg) identical to that previously synthesized. Elution with 5% Me_2CO in EtOAc gave 16α,17-dihydro-17-hydroxy-3-epiGA_4 Me ester (**28**) (44 mg) which recrystallized from Me_2CO–petrol as prisms, mp 179–180°. (Found: $[M]^+$ 364.1853 $C_{20}H_{28}O_6$ requires $[M]^+$ 364.1886.) ^1H NMR ($CDCl_3$): δ1.16 (*s*, H_3-18), 2.51 (*d*, *J* = 10.5 Hz, H-5), 2.74 (*d*, *J* = 10.5 Hz, H-5), 3.64 (*m*, H-3 and H_2-17) and 3.72 (*s*, OMe); MS *m/z* (rel. int.): 364 $[M]^+$ (3), 363 (5), 362 (2), 346 (98), 333 (27), 332 (25), 320 (11), 318 (100), 314 (25), 305 (19), 304 (74), 302 (23), 286 (37), 260 (39), 243 (41) and 91 (39).

Reduction of GA_4 *methyl ester* 16α,17-*epoxide* (**26**). A soln of GA_4 Me ester 16α,17-epoxide (**26**) (300 mg), sodium cyanoborohydride (160 mg, 3 equivalents) and 18-crown-6 (5 mg) in dry THF (30 ml) was stirred whilst BF_3-etherate (0.4 ml) was added dropwise. Stirring was continued and the reaction mixt. heated to reflux with the exclusion of moisture. After 2 hr, the mixt. was dil. with H_2O and the product recovered into EtOAc. Removal of solvent under red. pres. gave a solid which was purified by flash CC. Elution with EtOAc gave a product (171 mg) which, after derivatization, exhibited a single narrow peak on analysis by capillary GC. Analysis by NMR revealed a 2:1 mixt. of (i) 16α,17-dihydro-17-hydroxyGA_4 Me ester (**31**) (67%); ^1H NMR, $[(CD_3)_2CO]$: δ1.04 (*s*, H_3-18), 2.57 (*d*, *J* = 11 Hz, H-6), 3.18 (*d*, *J* = 11 Hz, H-5), 3.67 (*m*, H_2-17), 3.69 (*s*, OMe) and 4.47 (*t*, *J* = 4 Hz, H-3); (ii) 16β,17-dihydro-17-hydroxyGA_4 Me ester (**33**) (33%); ^1H NMR, $[(CD_3)_2CO]$: δ1.04 (*s*, H_3-18), 2.60 (*d*, *J* = 11 Hz, H-6), 3.21 (*d*, *J* = 11 Hz, H-5), 3.59 (*m*, H_2-17), 3.67 (*s*, OMe) and 4.47 (*t*, *J* = 4 Hz, H-3); ^{13}C NMR, $[(CD_3)_2CO]$: δ (signals downfield from 65 ppm only), 67.47 (C-17), 94.31 (C-3), 94.31 (C-10), 173.50 (C-7), 178.60 (C-19); MS *m/z* (rel. int.) 364 $[M]^+$, (2), 362 (2), 346 (41), 333 (15), 332 (15), 318 (35), 314 (13), 304 (34), 303 (25), 302 (100), 290 (10), 273 (11), 272 (13), 271 (23), 243 (82), 242 (56), 43 (33) and 31 (15).

Demethylation of 16ξ,17-*dihydro*-17-*hydroxy*-GA_4 *methyl ester* (**31, 33**). A mixt. of 16α,17-dihydro-17-hydroxyGA_4 Me ester (**31**) and 16β,17-dihydro-17-hydroxyGA_4 Me ester (**33**) (141 mg 2:1) in CH_2Cl_2) (15 ml) was stirred with freshly dist. 2,3-dihydropyran (0.4 ml) in the presence of a catalytic amount of *p*-toluenesulphonic acid for 2.5 hr at room temp. Removal of solvent gave a mobile oil, which without purification, was dissolved in MeOH (10 ml) and refluxed with 3 M NaOH (5 ml) for 4 hr. The residue after work-up was redissolved in MeOH (3 ml) and Me_2CO (10 ml), *p*-toluenesulphonic acid (10 mg) added and the soln allowed to stand at room temp. for 6 hr. The solvent was removed under red. pres. and the product purified by flash CC. Elution with EtOAc–HOAc (100:1) gave the following as an inseparable mixt. (8 mg): (i) *ent*-3α,10β,17-trihydroxy-20-nor-16βHgibberellane-7,19-dioic acid 19,10-lactone (**32**) (70%), spectroscopically identical to that previously obtained; (ii) *ent*-3α,10β,17-trihydroxy-20-nor-16αHgibberellane 7,19-dioic acid 19,10-lactone (**34**) (30%); ^1H NMR, $[(CD_3)_2CO]$: δ1.11 (*s*, H_3-18), 2.56 (*d*, *J* = 11 Hz, H-6), 3.18 (*d*, *J* = 11 Hz, H-5), 3.58 (*m*, H_2-17) and 3.70 (*br s*, H-3); GC-MS (Me, TMSi): *m/z* (rel. int.) 508 $[M]^+$ (5), 493 (7), 490 (7), 480 (8), 476 (15), 462 (6), 448 (12), 390 (21), 379 (20), 359 (12), 358 (11), 289 (75), 261 (39), 233 (61), 225 (60), 129 (38), 75 (100) and 73 (14).

ent-3α,17-*Dihydroxy*-16β*Hgibberellane*-7,19-*dioic acid* 7,19-*dimethyl ester* (**35**). The diMe ester of GA_{14} (**9**) (450 mg) in dry THF (20 ml) was treated with a borane–tetrahydrofuran complex (4 ml of a 1 M soln) at room temp. After 3.5 hr, the reaction mixt. was cooled in ice, 2 M NaOH (10 ml) added, followed by 30% H_2O_2 (8 ml). The ice bath was removed and the mixt. stirred rapidly for 30 min. The mixt. was poured into H_2O, acidified with 2 M HCl to pH 1 and then extd with EtOAc. Purification by flash CC gave, on elution with 70% EtOAc in petrol, 16α,17-dihydro-17-hydroxy-GA_{14} diMe ester (**35**) (284 mg) as a gum. (Found: $[M]^+$ 394.2372. $C_{22}H_{34}O_6$ requires $[M]^+$ 394.2355.) ^1H NMR ($CDCl_3$): δ0.65 (*s*, H_3-20), 1.18 (*s*, H_3-18), 2.30 (*d*, *J* = 13 Hz, H-5), 3.27 (*d*, *J* = 13 Hz, H-6), 3.65 (*m*, H_2-17), 3.67 (*s*, OMe), 3.70 (*s*, OMe), and 4.13 (*t*, *J* = 2.5 Hz, H-3); ^{13}C NMR, ($CDCl_3$): δ14.29, 16.33, 21.49, 23.95, 26.95, 33.67, 34.10, 39.61, 41.05, 42.69, 43.89, 48.43, 49.43, 49.54, 51.29, 51.35, 51.91, 60.29, 64.45 (C-17), 71.26 (C-3), 175.91 (C-7) and 177.49 (C-19); MS *m/z* (rel. int.) 394 $[M]^+$ (3), 376 (2), 363 (23), 362 (68), 344

(28), 335 (25), 334 (100), 277 (18), 257 (15) and 31 (6). GC-MS (Me, TMSi): *m/z* (rel. int.) 538 $[M]^+$ (1), 523 (11), 506 (4), 482 (7), 481 (5), 478 (7), 463 (2), 419 (4), 416 (5), 409 (6), 408 (7), 377 (97), 348 (39), 321 (19), 259 (43), 231 (100), 199 (19), 171 (16), 129 (48), 75 (21) and 73 (26).

ent-16*β*,17-*Epoxy-3α-hydroxy-gibberellane-7,19-dioic acid 7,19-dimethyl ester* (**38**). The diMe ester (186 mg) of GA_{14} (**8**) in $CHCl_3$ (20 ml) was treated with 3-chloroperoxybenzoic acid (168 mg, 2.0 equivalents) at 5° for 17 hr. The mixt. was dil. with $CHCl_3$ and washed sequentially with satd aq. $NaHCO_3$, then H_2O and dried (Na_2SO_4). Removal of solvent under red. pres. followed by flash CC gave, on elution with 30% EtOAc in petrol, GA_{14} diMe ester 16α,17-epoxide (**38**) (165 mg) as a gum. (Found: $[M]^+$ 392.2215. $C_{22}H_{32}O_6$ requires $[M]^+$ 392.2199.) ^1H NMR ($CDCl_3$): δ0.69 (*s*, H_3-20), 1.18 (*s*, H_3-20), 2.32 (*d*, *J* = 12.5 Hz, H-5), 2.80 (*d*, *J* = 4.5 Hz, H-17), 2.85 (*d*, *J* = 4.5 Hz, H-17), 3.35 (*d*, *J* = 12.5 Hz, H-6), 3.69 (*s*, × 2 OMe) and 4.14 (*br s*, H-3); MS *m/z* (rel. int.) 392 $[M]^+$ (23), 374 (4), 360 (100), 342 (19), 332 (96), 314 (24, 301 (11), 300 (20), 273 (15), 272 (18), 255 (22) and 91 (29).

Reduction of GA_{14} dimethyl ester 16α,17-*epoxide* (**38**). A soln of GA_{14} diMe ester 16α,17-epoxide (**38**) (130 mg), sodium cyanoborohydride (70 mg, 3 equivalents) and 18-crown-6 (5 mg) in dry THF (15 ml) was treated with BF_3-etherate (0.2 ml) with stirring and the resultant mixt. heated to reflux. After 1.25 hr, the reaction mixt. was poured into H_2O and extd with EtOAc. The organic ext. was dried (Na_2SO_4) and solvent removed under red. pres. Purification by flash CC gave, on elution with 35% EtOAc in petrol, unreduced starting material (39 mg). Elution with 70% EtOAc in petrol gave a gum (54 mg), a portion of which was analysed by full scan and GC-SIM and shown to contain (i) *ent*-3α,17-dihydroxy-16αHgibberellane-7,19-dioic acid 7,19-diMe ester (**35**) (66%), identical to that previously obtained, (ii) *ent*-3α,17-dihydroxy-16*β*Hgibberellane-7,19-dioic acid 7,19-diMe ester (**36**) (34%). ^1H NMR, ($CDCl_3$): δ0.67 (*s*, H_3-20), 1.17 (*s*, H_3-18), 2.32 (*d*, *J* = 12.5 Hz, H-5), 3.30 (*d*, *J* = 12.5 Hz, H-6), 3.63 (*m*, H_2-17), 3.68 (*s*, OMe), 3.69 (*s*, OMe) and 4.13 (*br s*, H-3); ^{13}C NMR, ($CDCl_3$): δ14.32, 15.62, 16.99, 17.13, 23.64, 26.48, 31.06, 33.56, 35.52, 37.81, 41.61, 43.93, 45.64, 48.38, 49.66, 50.36, 50.75, 57.05, 67.41 (C-17), 71.20 (C-3), 175.93 (C-7) and 177.50 (C-19); GC-MS (Me, TMSi): *m/z* (rel. int.) 538 $[M]^+$ (0.5), 523 (14), 506 (2), 482 (10), 481 (7), 478 (7), 463 (1), 419 (5), 416 (2), 409 (8), 408 (8), 377 (95), 348 (39), 231 (100), 75 (23) and 73 (27).

ent-3α,10*β*,16*β*,17-*Tetrahydroxy-20-nor-16βHgibberellane-7,19-dioic acid 7-methyl ester* 19,10-*lactone* (**39**). A soln of GA_4 Me ester (**27**) (105 mg) in Me_2CO (2 ml) was added to a pre-prep. soln of OsO_4 (*ca* 10 mg) and *N*-methylmorpholine-*N*-oxide (100 mg of a 60% soln in H_2O) in Me_2CO (2 ml), pyridine (2 ml) and H_2O (1 ml). The resultant mixt. was stirred in a sealed vial for 2 days. Satd aq. $Na_2S_2O_7$ was added and the mixt. stirred for a further 2 hr. The mixt. was poured into H_2O, brought to pH 4 with 2 M HCl and the product recovered in EtOAc. Removal of solvent under red. pres. yielded a black oil which was purified by flash CC. Elution with EtOAc gave 16α,17-dihydro-16α,17-dihydroxy-GA_4 Me ester (**39**) (42 mg) a portion of which was recrystallized from Me_2CO–petrol as cubes, mp 195–197°. (Found: C, 62.95; H, 7.48. $C_{20}H_{28}O_7$ requires C, 63.14; H, 7.42%.) ^1H NMR [$(CD_3)_2CO$]: δ1.05 (*s*, H_3-18), 2.60 (*d*, *J* = 11 Hz, H-6), 3.15 (*d*, *J* = 11 Hz, H-5), 3.68 (*m*, H_2-17), 3.69 (*s*, OMe) and 4.48 (*br d*, *J* = 3 Hz, H-3); ^{13}C NMR [$(CD_3)_2CO$]: δ15.13, 17.05, 22.41, 27.97, 29.11, 36.41, 44.16, 50.73, 51.76, 51.97, 52.81, 53.81, 55.35, 55.48, 67.50, (C-17), 70.27 (C-3), 83.29 (C-16), 94.43 (C-10), 173.77 (C-7) and 178.56 (C-19); GC-MS (Me, TMSi) *m/z* (rel. int.): 596 $[M]^+$ (1), 581 (2), 506 (3), 493 (100), 359 (5), 299 (13), 269 (8), 241 (5), 217 (5), 147 (6), 129 (7), 75 (39) and 73 (23).

Attempted hydrolysis of GA_4 methyl ester 16α,17-*epoxide* (**26**). A soln of GA_4 Me ester 16α,17-epoxide (**26**) (870 mg) in CH_2Cl_2 (45 ml) was stirred with fr. dist. 2,3-dihydropyran (1 ml) and *p*-toluenesulphonic acid (5 mg) for 2 hr at room temp. The solvent was removed under red. pres., the residue dissolved in a mixt. of DMSO (30 ml) and 2.5 M NaOH (10 ml) and heated under reflux for 16 hr. The reaction mixt. was poured into dist. H_2O, brought to pH 3 with 2 M HCl and the product recovered in EtOAc. The solvent was removed under red. pres. and the crude 3-tetrahydropyranyl ether redissolved in Me_2CO (30 ml) and MeOH (10 ml) and stirred for a further 12 hr at room temp. in the presence of *p*-toluenesulphonic acid (10 mg). Work-up as above, followed by flash CC gave, on elution with EtOAc–Me_2CO–HOAc (94:5:1), a product (357 mg) identified spectroscopically as *ent*-3α,10*β*,17-trihydroxy-20-norgibberell-15-ene-7,19-dioic acid 19,10-lactone (**40**), a portion of which recrystallized from Me_2CO–MeOH–petrol as prisms, mp 119–121°. (Found: C, 60.96; H, 7.69; $[M]^+$ 348.15. $C_{19}H_{24}O_6.2CH_3OH$ requires C, 61.15; H, 7.82%; $[M]^+$ 348.1573.) ^1H NMR, [$(CD_3)_2CO$]: δ1.12 (*s*, H_3-18), 2.50 (*d*, *J* = 10 Hz, H-6), 3.16 (*d*, *J* = 10 Hz, H-5), 3.72 (*d*, *J* = 2 Hz, H-3), 4.12 (*m*, H_2-17) and 5.75 (*br s*, H-15); MS *m/z* (rel. int.): 348 $[M]^+$, (10), 346 (7), 332 (6), 330 (100), 312 (7), 302 (11), 286 (13), 284 (21), 268 (17), 91 (43), 43 (24) and 28 (40); GC-MS (Me, TMSi), *m/z* (rel. int.): 506 $[M]^+$ (100), 491 (8), 474 (1), 446 (15), 416 (10), 384 (4), 372 (9), 357 (9), 356 (10), 313 (19), 287 (12), 282 (20), 223 (21), 207 (17), 156 (24), 129 (25), 103 (14), 75 (64) and 73 (70).

Bioassays. Lettuce hypocotyl assay. Assays were based on the method of ref. [9].

Tan-ginbozu dwarf rice immersion assay. Dwarf rice seeds were sterilized by immersion in a 2% aq. 'Domestos' soln for 10 min and, after rinsing with copious amounts of sterile H_2O, were covered with sterile H_2O and allowed to germinate at 26° in a controlled environment over 60 hr, during which time the H_2O was changed at intervals of 12 hr. After germination, seeds were selected for uniformity and six placed in each cylindrical sample vial (17 mm diam. × 48 mm) which contained 0.5 ml sterile H_2O and 10 μl of a soln of compound in MeOH. The concns of GAs applied were 10^1, 10^0, 10^{-1} and 10^{-2} μg per 10 μl of MeOH, each concn being tested in duplicate. Two vials containing sterile H_2O were used as controls. After a growing period of 7 days, the length of the second leaf-sheath and the total length (mm) from seed to longest leaf tip was recorded (Table 2).

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