Pergamon

PII: S0031-9422(98)00310-0

Phytochemistry Vol. 49, No. 8, pp. 2195–2206, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

EFFECTS OF D-RING MODIFIED GIBBERELLINS ON FLOWERING AND GROWTH IN *LOLIUM TEMULENTUM*

LEWIS N. MANDER,†§* MICHAEL SHERBURN,§ DAVID CAMP,§ ROD W. KING,‡§ LLOYD T. EVANS‡§ and RICHARD P. PHARIS‖

†Research School of Chemistry, Australian National University, Canberra, A.C.T. 0020, Australia, ‡CSIRO Division of Plant Industry, G.P.O. Box 1600, Canberra, A.C.T. 2601, Australia, §Cooperative Research Centre for Plant Science, G.P.O. Box 475, Canberra, A.C.T. 2601, Australia and ‖Biological Sciences Dept., University of Calgary, Alberta, Canada

(*Received in revised form* 23 *February* 1998)

Key Word Index—*Lolium temulentum*; Gramineae; darnel; bioactivity; flowering; stem elongation, growth inhibition; D-ring modifications; cyclopropyl; dichloromethanogibberellins; gibberellin A_3; gibberellin A_5.

Abstract—The modified gibberellins, 16-methyl-16,17-dihydro-GA_5, 16,17-methano-16,17-dihydro-GA_5, 16,17-methano-16,17-dihydro-GA_3 and several halogenated derivatives of 16,17-methano-16,17-dihydro-GA_3 and GA_5 were prepared and tested for bioregulating properties in *Lolium temulentum*. The effects ranged from the promotion of both stem elongation and flowering, the promotion of flowering but not elongation, and *vice versa*, the inhibition of stem elongation combined with the promotion of flowering, and the inhibition of both.

INTRODUCTION

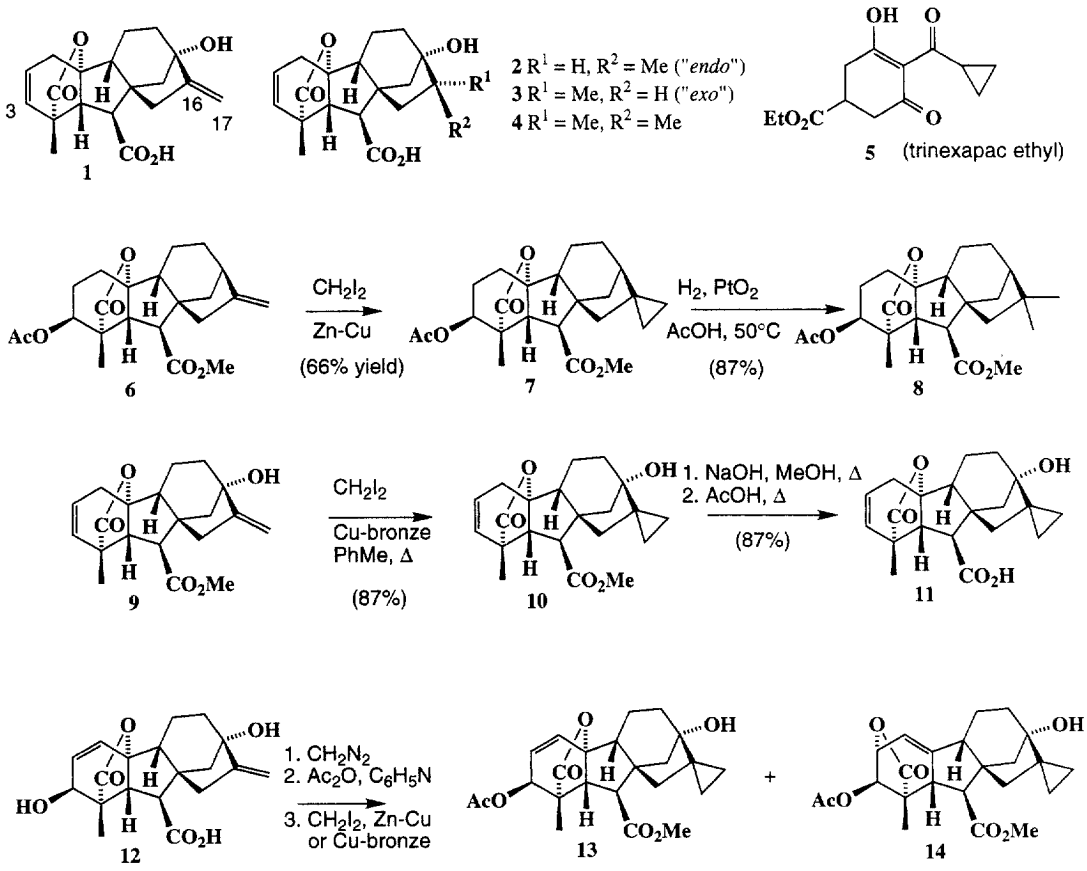
Gibberellins (GAs) are implicated in the induction of flowering in the long day (LD) plant *Lolium temulentum* and are the only chemical compounds known to induce the initiation of inflorescences of this species when held in short days (SD) [1,2]. They also induce flowering *in vitro* in shoot apices excised from *Lolium* plants held in SD [3]. Single applications of floral-promotive GAs to plants in non-inductive conditions cause an early rise in the rate of primordium initiation at the shoot apex, as does exposure to one LD [4]. There is also a rise in the endogenous level of GA-like substances in the *Lolium* shoot apex following exposure to one LD [5]. While many natural GAs also induce stem elongation, a recent study [6] on the effects of a number of 16,17-dihydro-GAs revealed that elongation was reduced, relative to that by the parent GA, even though they remained effective for floral induction. For both the 16,17-dihydro-GA_5 derivatives **2** and **3**, stem elongation was actually less than that in the untreated controls. Indeed, some GA derivatives actually inhibited stem elongation to an extent comparable with growth retardants such as trinexapac-ethyl (**5**) [7]. Following these intriguing discoveries, we have explored the impact of further changes in the D-ring area of the GA structure with a view to preparing further GA-based bioregulators with differential effects on flowering and growth [8]. The present study was initially focused on derivatives of GA_5 (**1**) and began with the question of how bioactivity might be altered by the addition of a second methyl group at C-16 in 16,17-dihydro-GA_5. The chosen method of preparation of the target GA (**4**) involved the intermediacy of a C-16 spirocyclopropyl unit, hydrogenolysis [9] of which, was expected to lead to the desired derivative. This approach was chosen for synthetic expediency, but also in the expectation that the cyclopropyl moiety might serve as a surrogate for the 16,16-gem-dimethyl group.

*Author to whom correspondence should be addressed.

RESULTS AND DISCUSSION

Preparation of GA_5 derivatives **4** *and* **11** *and their effects on growth and flowering*

The feasibility of introducing an additional methyl group at C-16 in the GA molecule was first tested on GA_4 methyl ester 3-acetate (**6**) as a model substrate, using a standard Simmons-Smith methylenation [10] followed by hydrogenolysis as



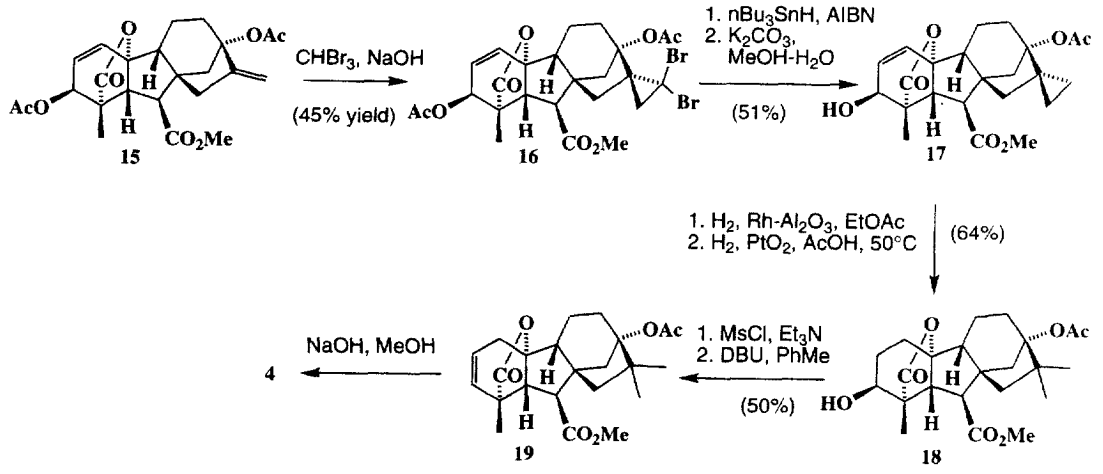
Scheme 1. Application of the Simmons–Smith methylation to the preparation of 16-cyclopropyl-GAs.

outlined in Scheme 1. The most effective conditions for the latter step were based on the use of platinum oxide as catalyst in acetic acid at 50°. Methylenation of GA_5 methyl ester (**9**) [11] was examined next and was found to proceed just as smoothly using either traditional methods based on a zinc-copper couple [10], or by using copper-bronze [12]; hydrolysis then afforded 16,17-methano-dihydro-GA_5 (**11**).

Since access to the dimethyl analogue **4** by hydrogenolysis of **11** was not practical in the presence of the double bond in the A-ring, we turned to the cyclopropanation of GA_3 (**12**). After methylation of the carboxy group and acetylation of the 3-hydroxyl, the standard Simmons-Smith conditions [10] were utilised, and although successful, the desired product (**12**) was severely contaminated by the isolactone (**14**). Rather better outcomes were achieved with copper-bronze [12], but because of difficulties in purifying **13**, we proceeded to examine the addition of dihalocarbenes to the 16-methylene group, expecting that removal of halogen from the dibromo adducts would be readily achieved. The sequence that ultimately led to **4**, was based on adduct **16** (Scheme 2), which was readily formed from the GA_3 derivative **15** under phase-transfer conditions [13]. The dibromide was reduced with tri-*n*-butylstannane [14], but this treatment also induced partial loss of the 3-acetate function, so the resulting mixture of mono- and di-acetates was hydrolysed briefly with potassium carbonate in methanol to give **17**. Hydrogenation of the A-ring double bond in **17** over rhodium-alumina (this catalyst minimises hydrogenolysis of the allylic lactone function), then hydrogenolysis of the cyclopropyl ring over platinum oxide, afforded the dimethyl analogue **18**, from which the target GA_5 analogue **4** was prepared by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) induced elimination of the derived 3-mesylate followed by hydrolysis of the 13-acetate and 7-methyl ester functions [15].

A summary of biological effects (flowering and stem elongation of *L. temulentum*) based on 25 μg doses of **4** and **11**, as well as further synthetic GAs (see below for preparation) is presented in Table 1. More detailed bioassays (1, 5, and 25 μg doses) by compound type are provided in Figs 1 and 2.

The application of 16,17-methano-dihydro-GA_5 (**11**) to *Lolium* plants promoted flowering to a degree comparable with *exo*-16,17-dihydro-GA_5 (**3**) and GA_5 (**1**) itself (Expts 2 and 5, Table 1). In two



Scheme 2. Preparation of 16-methyl-16,17-dihydro-GA_5.

experiments, stem elongation was inhibited to a similar extent to that observed for *exo*-16,17-dihydro-GA_5 (**3**) (*e.g.* Expt 2, Table 1), but (**11** was rather more inhibitory in two other experiments (*e.g.* Expt 5, Table 1). In the two experiments conducted with 16-methyl-dihydro-GA_5 (**4**), there was a moderate promotion of flowering at a dose of 25 μg per plant, but stem elongation was strongly inhibited (Fig. 1, Expt 2); moreover, lower doses were almost as effective.

Preparation of 16,17-methano-dihydro-GA_3 derivatives **22**, **24**, **26**, **28** *and* **30** *and their effects on growth and flowering*

Given the interesting bioregulating properties of **11**, and having established that GA_3 derivatives could be satisfactorily cyclopropanated with dihalocarbenes, we decided to extend the study to include the 16,17-methano-dihdro-GA_3 derivative **30**, the preparation of which is outlined in Scheme 3. The 7-methoxymethyl ester **20** was chosen as starting

Table 1. The effect of a single dose of 25 μg of either GA_3 (**12**), GA_5 (**1**), their various derivatives or, in one case, trinexapac-ethyl (**5**) on both shoot apex length (mm) and stem length (mm) at dissection three weeks after exposure of *L. temulentum* plants to one long day. Shoot apex length is an index of floral induction. Values are means ± standard errors

Expt No.	Treatment	Apex length (mm)	Stem length (mm)
1.	Control	2.73 ± .10	194 ± 5.7
	GA_3 (**12**)	2.95 ± .18	319 ± 13.6
	16,17-methano-dihydro-GA_3 (**30**)	4.65 ± .25	248 ± 5.5
	GA_5 (**1**)	3.18 ± .17	249 ± 6.6
2.	Control	1.99 ± .08	102 ± 3.1
	GA_5 (**1**)	3.22 ± .14	190 ± 19.5
	16,17-dihydro-GA_5 (*exo*) (**3**)	2.97 ± .10	66.3 ± 4.0
	16,17-methano-dihydro-GA_5 (**11**)	3.20 ± .33	77.4 ± 4.0
	16-methyl-dihydro-GA_5 (**4**)	2.66 ± .11	67.3 ± 5.1
3.	Control	1.92 ± .06	98.1 ± 3.5
	GA_3 (**12**)	3.05 ± .14	382 ± 10.3
	16,17-chloromethano-dihydro-GA_3 (**26**)	3.16 ± .11	106 ± 2.8
	16,17-dichloromethano-dihydro-GA_3 (**22**)	1.99 ± .06	94.3 ± 5.1
	16,17-dichloromethano-dihydro-GA_5 (**32**)	1.53 ± .05	48.7 ± 4.0
	GA_5 (**1**)	3.39 ± .13	209 ± 10.7
4.	Control	1.89 ± .09	119 ± 5.4
	GA_3 (**12**)	2.69 ± .09	379 ± 7.2
	16,17-chloromethano-dihydro-GA_3 (**26**)	3.31 ± .08	135 ± 3.8
	16,17-dichloromethano-dihydro-GA_3 (**22**)	1.80 ± .11	119 ± 5.6
	16,17-dichloromethano-dihydro-GA_5 (**32**)	1.48 ± .07	57.9 ± 4.4
	GA_5 (**1**)	2.71 ± .11	205 ± 10.6
5.	Control	1.89 ± .07	116 ± 4.6
	GA_3 (**12**)	3.04 ± .11	331 ± 18.6
	GA_5 (**1**)	3.01 ± .20	192 ± 10.5
	16,17-dihydro-GA_5 (*exo*) (**3**)	2.79211	93.4 ± 5.6
	16,17-methano-dihydro-GA_5 (**11**)	3.16 ± .12	83.0 ± 3.5
	16,17-chloromethano-dihydro-GA_5 (**33**)	2.03 ± .04	65.1 ± 2.9
	16,17-dichloromethano-dihydro-GA_5 (**32**)	1.81 ± .06	55.0 ± 3.6
	16,17-methano-dihydro-GA_3 (**30**)	3.05 ± .11	158 ± .8.6
	16,17-chloromethano-dihydro-GA_3 (**26**)	2.69 ± .06	129 ± 3.2
	trinexapac-ethyl (**5**)	2.34 ± .09	66.0 ± 2.5

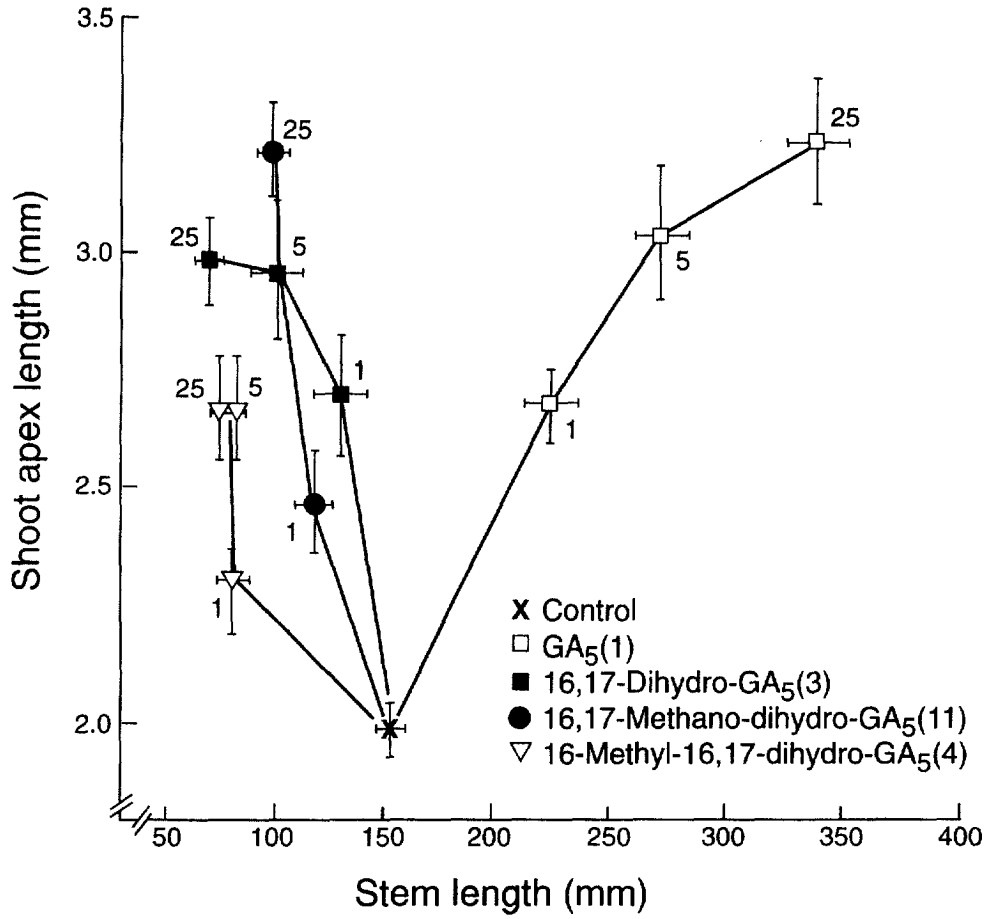


Fig. 1. Effect of single dose at the time of the long day with various GAs and their derivatives on shoot apex length (mm) and stem length (mm) in the same plants of *Lolium temulentum*. All measurements were made three weeks after treatment. At the start of treatment stem length was 15 ± 2 mm. Control plants (*x*) were treated with 95% ethanol:water and were exposed to a single long day. Values are means $\pm$ SE (n = 14). Dose rates of 1, 5 or 25 μg/plant are indicated by numbers near the symbols (data from Expt. 2, Table 1).

material so that liberation of the free GA acids would be a simple task [16]. Only moderate yields were obtained on the addition of dibromocarbene, so we initially based our approach on the intermediacy of the dichlorocarbene adduct **21**, obtained in 85% yield. Removal of one chlorine atom by treatment with tri-*n*-butylstannane proceeded smoothly to afford **25**, but further reduction to **29** was completed with difficulty. We therefore reverted to the intermediacy of the dibromo derivative **23**, which was reduced smoothly and in good yield, first to the monobromo product **27**, and then to **29**. Liberation of the target acid (**30**) was routine, and since the parent acids corresponding to the series of ester intermediates could also be obtained with equal ease, the set of halogenated methano derivatives was also screened for biological effects.

The dibromo and monobromo derivatives of GA_3, (**24** and **28**), were of limited interest, since they had only a marginal effect on either stem elongation or flowering in LD-exposed plants. While 16,17-methano-dihydro-GA_3 (**30**) promoted flowering to the same (Expt 5) or greater extent (Expt 1) as the parent GA_3 (**12**), promotion of stem elongation by **30** (Expts 1 and 5) was significantly reduced, a remarkable result, given the strong growth promotion normally associated with the presence of the 3β-hydroxyl in the GA molecule [17]. 16,17-Methano-dihydro-GA_3 (**30**) was also highly florigenic on plants maintained under non-inductive SD, more so than either GA_3 or GA_5. With a 25 μg dose of **30**, shoot apex length (and floral stage) was at least as great as those of plants exposed to one LD (SD, 1.09 ± 0.03 mm; LD, 2.22 ± 0.11 mm; 16,17-methano-dihydro-GA_3 (**30**), 2.40 ± 0.11 mm).

With the chloromethano derivative of GA_3 (**26**), the strongly promotive effect on flowering was maintained, relative to **30**, but the effect on stem elongation was further reduced to the point that little enhancement occurred in any of experiments 3,4 and 5, compared with the controls. The presence of

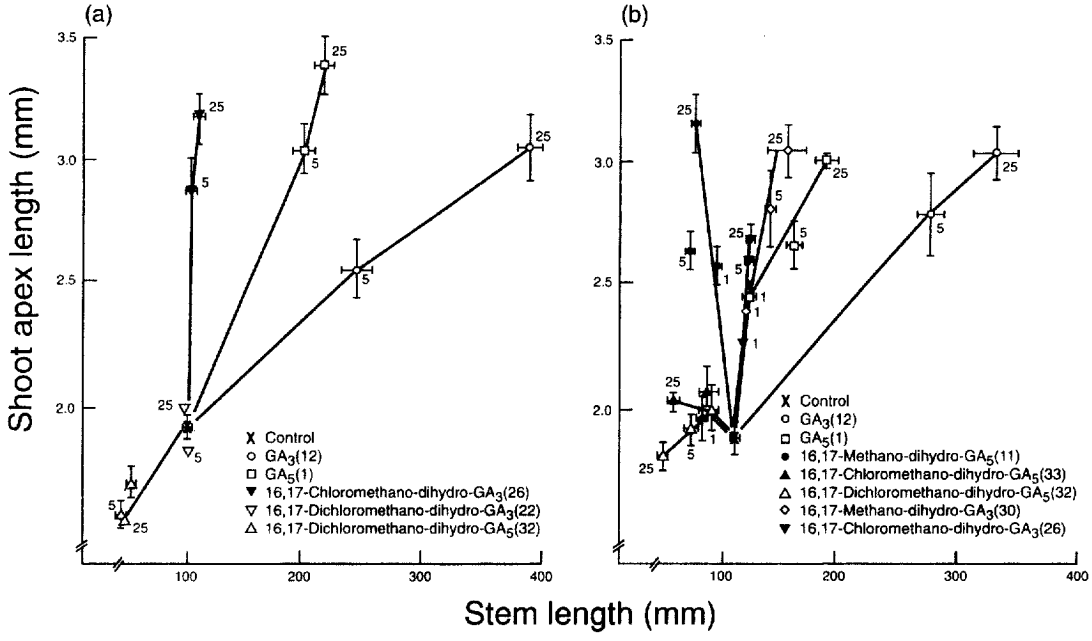
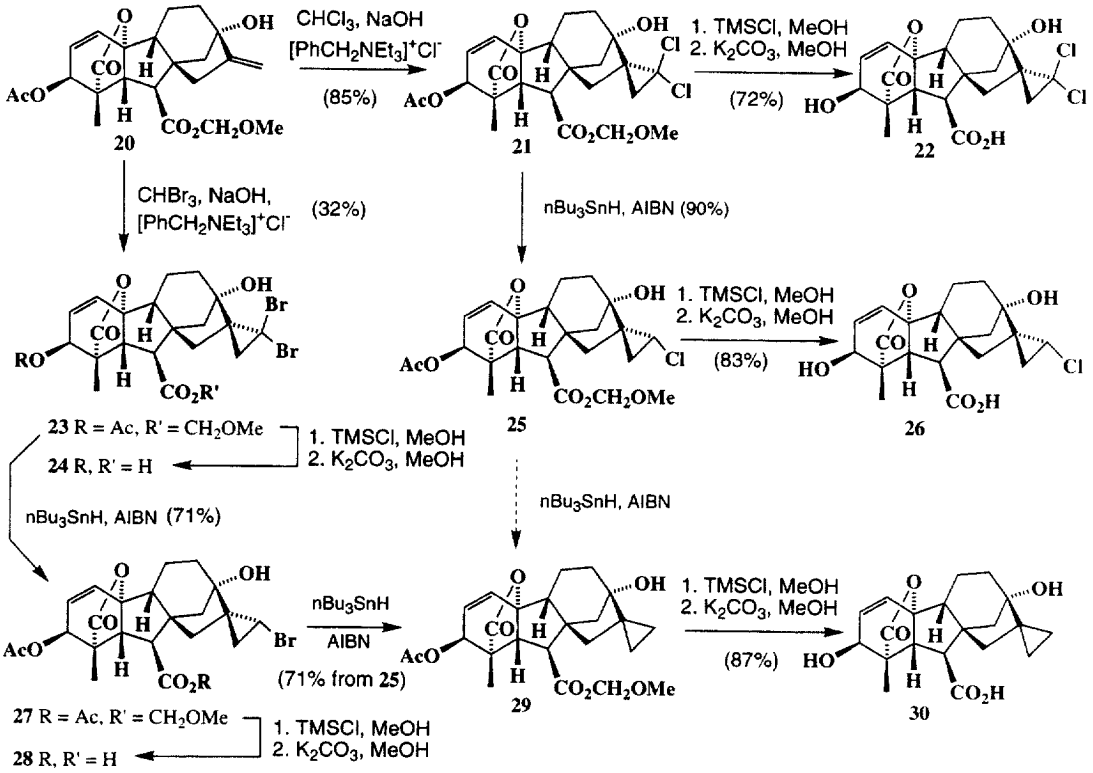
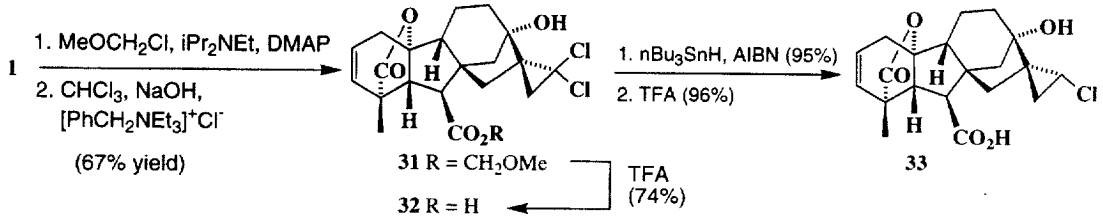


Fig. 2. Effect of various GA_3 or GA_5 derivatives [data from (a) Expt. 3, and (b) Expt. 5, Table 1] on shoot apex length (mm) and stem length (mm) after three weeks. At the start of GA treatment stem length was 23 ± 5 mm in (b). Treatments and conditions as for Fig. 1.

a second chlorine atom, as in **22**, however, effectively resulted in neglible bioactivity (Expts 3 and 4). An indication of the flowering/stem elongation profile in *Lolium* for the active GAs in this selection of compounds, relative to GA_3 and GA_5, is provided in Fig. 2.



Scheme 3. Synthesis of 16,17-methano-GA_3 derivatives.



Scheme 4. Synthesis of 16,17-methano-GA_5 derivatives.

Preparation of chloromethano-dihydro-GA_5 derivatives **32** *and* **33** *and their effects on stem elongation and flowering*

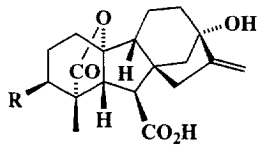
The major impact on growth promotion and flowering associated with the introduction of a halogenated cyclopropyl unit into the D-ring of GA_3 naturally prompted us to return to the GA_5 series and to examine the effects of analogues **32** and **33**. Their preparation was straightforward and is outlined in Scheme 4.

Although chloromethano-dihydro-GA_5 (**33**) had only a barely significant promotive effect on flowering (unlike its GA_3 counterpart), it had a very inhibitory effect on stem elongation (Table 1, Expt 5). This inhibition was confirmed in a further experiment (not shown). Growth inhibition was even more accentuated with the dichloromethano analogue **32** (*cf.* Table 1, Expts 3–5; Fig. 2 and two experiments not shown). This derivative was found to be highly inhibitory (up to 70%) to the stem elongation of *L. temulentum*, an effect that was greater than that seen (Expt 5) with similar doses of the growth retardant trinexapac-ethyl (**5**) [7]. Inhibition by **32** was highly significant, even with the very low dose of 1 μg per plant. Rather strikingly for a GA derivative, in two of the experiments in Table 1, at all three doses dichloromethano-dihydro-GA_5 (**32**) significantly reduced the flowering response of *Lolium* to one LD. However, in one experiment (not shown), although there was a significant inhibition of flowering with doses of 1 and 5 μg, there was a slight promotion of flowering at the highest dose (25 μg per plant).

The first objective of these experiments had been to identify further structural variants of the GA molecule which might promote flowering without additional stem elongation. Our earlier studies [6, 17] had shown that, in general, florigenicity was remarkably unaffected by changes in the D-ring region of the molecule, *inter alia*, tolerating the change in the orientation of the methyl group attached to C-16 in the dihydro-GA_5 derivatives **2** and **3** [6]. With the introduction of a second 16-methyl group as in **4**, however, the enhancement of flowering response was diminished relative to both the simple dihydro analogues. In the case of the 16,17-methano-dihydro GA_3 derivatives, however, high florigenicity is maintained by the parent (**30**) and monochloro derivative (**26**), although lost with the dichloro- (**22**) and bromo-derivatives (**24** and **28**). On the other hand, in the 16,17-methano-dihydro-GA_5 series, the monochloro-derivative (**33**) had largely lost its florigenicity, while the dichloro-derivative (**32**) was quite inhibitory to flowering, even at doses as low as 1 μg.

In contrast to the flowering response, stem elongation in *L. temulentum* has been shown to be very sensitive to modifications of the D-ring region of the molecule. We have previously found the 16,17-dihydro-GA_5 derivatives **2** and **3** to be inhibitory to stem elongation [6], and can now add 16,17-methano-dihydro-GA_5, and its chloro- and dichloro-derivatives.

The molecular basis of activity for the growth-inhibiting effects of 16,17-dihydro-GA_5 (**3**) and 16,17-dichloromethano-dihydro-GA_5 (**32**), have been examined by applying these compounds plus isotopically labelled GA_{20} to dwarf rice (*Oryza sativa* L. cv. Tan-ginbozu) [18] and to *Lolium* [19]. The GA_{20} (**34**) would normally be converted by both plants into the growth-active GA_1 (**35**), but in



34 R = H

35 R = OH

the presence of *exo*-16,17-dihydro-GA_5 (**3**), the pool of endogenous GA_{20} was increased 25-fold in *Lolium* and GA_1 was barely detectable; the effect was duplicated in *Lolium* with 16,17-dichloromethano-dihydro-GA_5 (**32**), for which a 12-fold increase in GA_{20} was observed. Furthermore, a recent study with sorghum [20] also showed that the application of **32** results in low levels of endogenous GA_1 (8–16% of controls), with GA_{20} content elevated 3 to 5-fold. It may be concluded, therefore, that these dihydro-GA_5 derivatives inhibit 3β-hydroxylation of GA_{20}, perhaps by serving as competitive substrates, as opposed to trinexapac and related cyclohexanetriones which are presumed to suppress non-haeme dioxygenases by mimicking 2-oxoglutarate [21].

The experiments reported here, highlight again the independent effects of various GAs on stem

elongation on the one hand and flowering on the other, with examples ranging from the promotion of both, the promotion of flowering but not elongation, and *vice versa*, the inhibition of stem elongation combined with the promotion of flowering, and the inhibition of both. Promotion of flowering without additional elongation, or even with reduced growth would have many uses in horticulture. For turf applications, on the other hand, the inhibition of stem or leaf elongation, with or without the inhibition of flowering, could be of considerable value. Field experiments have been conducted on turf with 16,17-dichloromethano-dihydro-GA_5 and are reported elsewhere [22].

EXPERIMENTAL

Plants

Plants of the *Ceres* strain of *Lolium temulentum* L. were grown in pots of vermiculite/perlite irrigated with nutrient soln each morning and with water each afternoon. For the first 5 weeks the plants were held in a glasshouse of the Canberra phytotron in SD of 8 hr of natural light at 25° followed by 16 hr of darkness at 20°. They were then transferred to the same temp. and daylength regime, but under light from fluorescent lamps with a photosynthetically-active irradiance of about 250 μmol m^{-2} s^{-1} PAR at plant height from fluorescent lamps. After 2–3 weeks they were then exposed to only one LD by a photoperiod extension of 16 hr of low irradiance light from incandescent lamps (11–12 μmol m^{-2} s^{-1} PAR). All plants were dissected 3 weeks after the one LD, before the beginning of the rapid stem elongation that occurs in *L. temulentum* after anther primordia have been initiated. At dissection, both shoot apex and stem length were measured and the stage of inflorescence development was scored. As in our previous work, floral score was highly correlated with shoot apex length across a wide range of GA treatments [17] and only shoot apex lengths are presented here. There were 14 replicate plants per treatment.

Gibberellin treatments

All GAs and related compounds were applied once only in a 10 μl drop of 95% aqu. EtOH to the uppermost fully-expanded leaf blade between 1400 and 1530 hr on the long day, towards the end of the daily period of high irradiance. Control plants were treated with 10 μl of 95% aqu. EtOH. The usual dose rates for all GAs were 1, 5 or 25 μg per plant.

Chemicals

Trinexapac-ethyl (**5**) was a technical grade sample supplied by Novartis Crop Protection Inc. (Kansas, U.S.A.). Gibberellin derivatives were prepared as outlined below. ^{13}C NMR data of selected compounds are provided in Table 2.

*ent-3α-Acetoxy-16,17-methano-20-norgibberellane-7,19-dioic acid 19,10-lactone 7-methyl ester (**7**)*

To freshly prepared Zn–Cu couple (137 mg, 2.1 mmol) in dry Et_2O (1.0 ml) was added CH_2I_2

Table 2. ^{13}C NMR spectral data for 16,17-methanogibberellins **10**, **21**, **23**, **27**, **29**, **31** and **33**

C↓ halogen→	**27** Br	**23** Br_2	**21** Cl_2	**31** Cl_2	**33**‡ Cl	**10**	**29**
1	129.2	129.2	129.1	34.9†	34.9†	35.0†	129.1
2	134.0	133.7	133.7	127.9	127.7	127.8	134.3
3	70.2	70.0	70.0	132.9	132.5	132.5	70.3
4	52.1	51.9	51.8	49.7	51.7	51.9	52.0
5	53.4	53.3	53.3	55.9	55.9	56.0	53.4
6	50.9	50.0	50.9	51.2	51.2	50.9	51.0
7	174.5	171.3	171.3	172.3	172.3	173.4	171.6
8	51.2	50.6	49.1	48.0	48.0	47.9	50.9
9	51.1	50.6	50.2	52.9	53.0	52.8	51.6
10	90.3	90.0	89.9	91.6	91.8	92.0	90.2
11	17.0	16.5	16.5	17.0	17.2	17.3	17.2
12	34.9	36.1	35.9	36.1†	35.0†	35.1†	35.4
13	75.6	78.5	78.3	78.7	75.6	75.2	75.3
14	45.5*	46.4	46.3*	47.8*	47.1	47.6*	46.2*
15	45.7*	49.1	47.6*	47.6*	42.6	47.0*	47.2*
16	33.8	38.5	39.7	39.0	33.4	27.3	28.9
17	19.5	33.2	30.2	30.1	18.5	10.5§	11.0
17′	27.7	35.5	66.7	66.9	36.8	11.9§	12.1
18	14.3	14.2	14.1	15.2	15.2	15.1	14.3
19	177.3	176.8	176.8	177.4	177.7	178.1	177.1
$CO_2CH_2O\mathit{Me}$	58.2	58.0	57.9	58.1	58.1	–	57.9
$CO_2CH_2O\mathit{Me}$	91.1	91.0	90.9	91.1	91.1	–	90.8
$\mathit{Me}CO_2$	20.6	20.7	20.6	–	–	–	20.8
$\mathit{Me}CO_2$	171.0	171.3	169.8	–	–	–	170.0
$CO_2\mathit{Me}$	–	–	–	–	–	51.6	–

*,†,§ assignments interchangeable; ‡ Methoxymethyl ester.

(140 ml, 1.75 mmol) and a crystal of I_2. The resulting mixture was heated under reflux with stirring under N_2 for 30 min, then GA_4 7-methyl ester 3-acetate (194 mg, 0.50 mmol) in dry Et_2O (2.0 ml) was added. Reflux and stirring was continued for 14 hr, then the reaction quenched with sat. aq. NH_4Cl. The product was extracted into Et_2O, washed with NH_4Cl (2×), brine and dried (Na_2SO_4). Chromatography on silica gel (EtOAc–hexane, 1:4) gave the desired product as a white solid (132 mg, 66%). ^1H NMR ($CDCl_3$): δ 0.43, 0.58 (4H, 2 × *m*, H_2-17, H_2-21), 1.05 (3H, *s*, H_3-18), 2.13 (3H, *s*, OAc), 2.69 (1H, *d*, J = 10.7 Hz, H-6), 3.13 (1H, *d*, J = 10.7 Hz, H-5), 3.70 (3H, *s*, OMe, 4.96 (1H, *m*, H-3). EIMS *m/z* (rel. int.): 402 $[M]^+$ (18) 384 (8), 371 (22), 370 (18), 347 (32), 342 (28), 298 (100), 282 (25), 270 (27), 244 (53), 239 (50), 238 (50), 211 (24), 209 (23), 185 (25), 183 (33), 181 (27), 169 (22), 157 (27), 155 (23).

*ent-3α-Acetoxy-16-methyl-20-norgibberellane-7,19-dioic acid 19,10-lactone 7-methyl ester (**8**)*

A soln of ester **7** (39 mg, 0.10 mmol) in acetic acid (3.0 ml) containing PtO_2 (10 mg) was stirred under H_2 at 50° for 6 hr. The mixture was filtered through Celite and the pad washed thoroughly with CH_2Cl_2. Removal of solvent and chromatography on silica gel (EtOAc-hexane, 1:3) gave the product as a white solid (34 mg, 87%). ^1H NMR ($CDCl_3$): δ 1.01 (6H, *s*, 2 × Me), 1.02 (*s*, 3H, Me), 2.13 (3H, *s*, OAc), 2.63 (1H, *d*, J = 10.9 Hz, H-6), 3.08 (1H, *d*, J = 10.9 Hz, H-5), 3.71 (3H, *s*, OMe, 4.94 (1H, *m*, H-3). EIMS *m/z* (rel. int.): 404 $[M]^+$ (10) 386 (14), 373 (13), 344 (22), 316 (17), 300 (100), 285 (27), 243 (48), 241 (38), 240 (45), 185 (20), 183 (21), 169 (11), 157 (17), 155 (13).

*ent-13-Hydroxy-16,17-methano-20-norgibberell-2-ene-7,19-dioic acid 19,10-lactone (**11**)*

CH_2I_2 (0.33 ml, 4.09 mmol), I_2 (52 mg, 0.20 mmol) and Cu powder (520 mg, 8.13 mmol) were added to a soln of GA_5 methyl ester (**9**) [11] (94 mg, 0.27 mmol) in toluene (5.0 ml) and the mixture heated at reflux, under N_2, for 21 hr. After filtering through Celite with an Et_2O wash, the solvent was removed and the residue chromatographed on silica gel. Adduct **10** (62 mg, 63%) was eluted with EtOAc-hexane (1:2) and crystallised slowly from EtOAc. ^1H NMR ($CDCl_3/d_4$-MeOH, 4:1): δ 0.23, 0.43, 0.84 (4H, 3 × *m*, H_2-17, H_2-17'), 1.20 (3H, *s*, H_3-18), 2.57 (1H, *d*, $J_{6,5}$ = 9.0 Hz, H-5), 2.68 (1H, *d*, $J_{5,6}$ = 9.0 Hz, H-6), 3.55 (3H, *s*, OMe), 5.62 (1H, *br d*, J = 9.3 Hz, H-3), 5.77 (1H, *dt*, J = 9.3, 2.9 Hz, H-2). A suspension of **10** (32.6 mg) in MeOH (2.0 ml) was treated with 2 M NaOH (2.0 ml) and the mixture heated under reflux for 15 hr. The pH was lowered to 5 by the addition of 5 M HCl and the product extracted into EtOAc. After washing with brine and drying over Na_2SO_4, the solvent was removed and the residue dissolved in glacial HOAc (0.5 ml). The soln was heated to 100° for 10 min, then the solvent removed and the residue chromatographed on silica gel. Acid **11** was eluted with EtOAc–hexane (7:3) and obtained as a white solid (25.6 mg, 81%). ^1H NMR ($CDCl_3/d_4$-MeOH, 4:1): δ 0.23, 0.46, 0.82 (4 × 1H, 3 × *m*, H_2-17, H_2-17'), 1.19 (3H, *s*, H_3-18), 2.52 (1H, *d*, $J_{6,5}$ = 9.0 Hz, H-5), 2.66 (1H, *d*, $J_{5,6}$ = 9.0 Hz, H-6), 5.61 (1H, *br d*, J = 9.3 Hz, H-3), 5.77 (1H, *dt*, J = 9.3,2.9 Hz,H-2). EIMS *m/z* 326 $[M\text{-}18]^+$ (75), 314 (35), 299 (30), 285 (55), 255 (56), 254 (100), 225.

*Cyclopropanation of ent-3α-acetoxy-13-hydroxy-20-norgibberell-1,16-diene-7,19-dioic acid 19,10-lactone 7-methyl ester (**13**)*

(a) A soln of ester **12** [23] (1.0 g, 2.48 mmol) in DME (8 ml) was added to reagent prepared from CH_2I_2 (800 ml, 10 mmol) as described for substrate **6**. After stirring under reflux for 21 hr, and work-up as before, chromatography on silica gel (EtOAc–hexane, 1:2) afforded mainly recovered **12**. (b) A mixture of Cu-bronze. (4.73 g) and I_2 (470 mg, 1.86 mmol) in dry toluene (10 ml) was stirred at room temp for 10 min then CH_2I_2 (3 ml, 37.2 mmol) was added and the resulting mixture heated under reflux with stirring under N_2 for 16 hr. The cooled mixture was filtered through Celite, washing well with Et_2O, and the solvent then removed and the residue chromatographed on silica gel as before to give an inseparable 9:6:3:2 mixture of **13**, **14**, **12** and **12**-isolactone, respectively (as estimated from the NMR spectrum).

*ent-3α-Acetoxy-13-hydroxy-16,17-methano-20-norgibberell-1-ene-7,19-dioic acid 19,10-lactone 7-methyl ester (**13**)*

^1H NMR ($CDCl_3$): δ 0.34, 0.52, 0.59, 0.90 (4 × 1H, 4 × *m*, H_2-17, H_2-17'), 1.15 (3H, *s*, H_3-18), 2.11 (3H, *s*, $OCOCH_3$), 2.76 (1H, *d*, $J_{6,5}$ = 10.6 Hz, H-6), 3.30 (1H, *d*, $J_{5,6}$ = 10.6 Hz, H-5), 3.74 (3H, *s*, CO_2Me), 5.36 (1H, *d*, J = 3.2 Hz, H-3), 5.88 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.43 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1).

*ent-3α-Acetoxy-13-hydroxy-16,17-methano-20-norgibberell-1(10)-ene-7,19-dioic acid 19,2-lactone 7-methyl ester (**14**)*

^1H NMR ($CDCl_3$): δ 0.30, 0.47, 0.55, 0.88 (4 × 1H, 4 × *m*, H_2-17, H_2-17'), 1.21 (3H, *s*, H_3-18), 2.12 (3H, *s*, $OCOCH_3$), 2.56 (1H, *d*, J = 6.0 Hz, H-6), 2.68 (1H, *br d*, J = 7.1 Hz, H-11), 3.29 (1H, *dd*, J = 6.0 Hz, J = 6.0 Hz J = 2.7 Hz, H-5), 3.70 (3H, *s*, CO_2Me), 5.02 (2H, *m*, H-2 + H-3), 5.74 (1H, *m*, H-1).

ent-3α,13-*Diacetoxy*-16β,17-*(dibromomethano)*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester (***16***)*

A soln of NaOH (50%), 2 ml) was added to a soln of ester **15** [23] (173 mg, 0.40 mmol), choline chloride (11 mg, 0.08 mmol) and $CHBr_3$ (70 μl, 0.8 mmol) in CH_2Cl_2 (4 ml) under N_2, and the mixture stirred vigorously for 2.5 hr. Further $CHBr_3$ (50 μl) was then added and stirring continued for 0.5 hr. EtOAc was added and the organic layer washed with H_2O and brine, then reduced to dryness. Chromatography of the residue on silica gel (EtOAc–hexane, 1:2) afforded the adduct **16** (103 mg, 43%). ^{1}H NMR ($CDCl_3$): δ 1.20 (3H, *s*, H-18), 1.63 (1H, *d*, $J_{17,17'}$ = 7.6 Hz, H-17), 2.04 (1H, *d*, $J_{17,17'}$ = 7.6 Hz, H'-17), 2.10 (3H, *s*, OAc), 2.27 (3H, *s*, OAc), 2.82 (1H, *d*, $J_{6,5}$ = 10.5 Hz, H-6), 3.33 (1H, *d*, $J_{5,6}$ = 10.5, Hz, H-5) 3.72 (3H, *s*, OMe), 5.30 (1H, *br s*, H-3), 5.87 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,1}$ = 3.8 Hz, H-2), 6.36 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1).

ent-13-*Acetoxy*-3α-*hydroxy*-16,17-*methano*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl Ester (***17***)*

Dibromide **16** was dissolved in benzene (10 ml), tri-*n*-butylstannane (*n*-Bu_3SnH) (340 ml, 1.26 mmol) and AIBN (10 mg, 63 mmol) were added and the mixture heated at reflux under N_2 for 3.5 hr. Additional stannane (350 ml, 1.30 mmol) was added and reflux continued for a further 18 hr. The reaction mixture was diluted with Et_2O, washed with dilute NH_3 and brine, then dried over Na_2SO_4. Chromatography on silica gel, eluting with EtOAc–hexane (1:3), afforded the debrominated diacetate (74 mg), then, on elution with EtOAc–hexane (1:1), the 13-monoacetate (**17**) (64 mg). The diacetate was dissolved in MeOH (10 ml) and treated with a soln of K_2CO_3 (200 mg) in H_2O (0.5 ml) and stirred for 16 h, affording a further 89 mg of product **17** (R=H) (total yield for two steps 51%). ^{1}H NMR ($CDCl_3$): δ 0.38, 0.49, 0.80, 0.96 (4 × 1H, 4 × *m*, H_2-17, H_2-17'), 1.22 (3H, *s*, H_3-18), 1.96 (3H, *s*, $OCOCH_3$), 2.79 (1H, *d*, $J_{6,5}$ = 10.7 Hz, H-6), 3.19 (1H, *d*, $J_{5,6}$ = 10.7 Hz, H-5), 3.70 (3H, *s*, CO_2Me), 4.15 (1H, *m*, H-3), 5.92 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.36 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1).

ent-13-*Acetoxy*-3α-*hydroxy*-16-*methyl*-20-*norgibberellane*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester (***18***)*

A soln of ester **17** (89 mg, 0.21 mmol) in EtOAc (5 ml) containing 5% Rh–Al_2O_3 (6 mg) was stirred at room temp under H_2 for 22 hr. A further sample of catalyst (6 mg) was then added and stirring continued for 50 hr. After filtration of the mixture through Celite, the solvent was removed and the residue dissolved in HOAc (3 ml). Pt_2O (10 mg) was added and the mixture stirred at 50° for 16 hr under H_2. The filtered (Celite, CH_2Cl_2 washes) mixture was evaporated to dryness and the residue chromatographed on silica gel, eluting with EtOAc–hexane (1:1). Ester **18** was obtained as a colourless solid (78 mg, 87%). ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, *s*, 16-Me), 1.11 (3H, *s*, 16-Me'), 1.18 (3H, *s*, H_3-18), 1.99 (3H, *s*, $OCOCH_3$), 2.63 (1H, *d*, $J_{6,5}$ = 11.0 Hz, H-6), 3.14 (1H, *d*, $J_{5,6}$ = 11.0 Hz, H-5), 3.72 (3H, *s*, CO_2Me), 3.82 (1H, *br s*, H-3).

ent-13-*Acetoxy*-16-*methyl*-20-*norgibberell*-2-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methyl ester (***19***)*

To a stirred soln of carbinol **18** (59 mg, 0.14 mmol) in dry CH_2CL_2 (3 ml) at 4° under N_2, was added Et_3N (78 ml, 0.56 mmol) followed by $MeSO_2Cl$ (95 ml, 1.22 mmol). After 1 hr a piece of ice was added and the mixture stirred for 15 min. Further ice was added and stirring continued for 10 min, then the temp allowed to rise to ambient. The mixture was diluted with EtOAc, washed with KH_2PO_4 (20%), brine and K_2CO_3 soln (10%), then dried over Na_2SO_4. Removal of the solvent afforded the crude 3-mesylate which was dissolved in toluene (3 ml, distilled from CaH_2) and treated with DBU (105 ml, 0.70 mmol). The soln was then heated at 120° under N_2 for 12 hr. The cooled soln was extracted with K_2CO_3 soln and the aq layer acidified with 1 M HCl, then extracted with EtOAc. The organic layer was washed with H_2O until neutral, dried ($NaSO_4$) and reduced to dryness. The residue was chromatographed on silica gel, eluting with EtOAc–hexane (3:1), to afford alkene **19** (37 mg, 61%). ^{1}H NMR ($CDCl_3$): δ 0.98 (3H, *s*, 16-Me), 1.18 (3H, *s*, 16-Me), 1.19 (3H, *s*, H_3-18), 1.98 (3H, *s*, $OCOCH_3$), 2.36 (1H, *d*, $J_{6,5}$ = 9.4 Hz, H-5), 2.73 (1H, *d*, $J_{5,6}$ = 10.5 Hz, H-6), 3.72 (3H, *s*, OMe), 5.64 (1H, *br d*, *J* = 9.3 Hz, H-3), 5.83 (1H, *dt*, *J* = 9.3, 2.9 Hz, H-2).

ent-3α,13-*Dihydroxy*-16-*methyl*-20-*norgibberell*-2-*ene*-7,19-*dioic acid* 19,10-*lactone (***4***)*

A suspension of ester **19** (30 mg, 0.075 mmol) in MeOH (2.0 ml) was treated with 2 M NaOH (2.0 ml) and the mixture heated under reflux for 15 hr. The pH was lowered to 5 by the addition of 5 M HCl and the product extracted into EtOAc. After washing with brine and drying over $NaSO_4$, the solvent was removed and the residue dissolved in HOAc (0.5 ml). The soln was heated to 100° for 10 min and then the solvent removed and the residue chromatographed on silica gel. Acid **4** was eluted with EtOAc–hexane (7:3) and obtained as a white solid (23 mg, 80%). ^{1}H NMR (d_4-MeOH): δ 1.05 (3H, *s*, 16-Me), 1.08 (3H, *s*, 16-Me), 1.24 (3H, *s*, H_3-18), 2.50 (1H, *d*, $J_{6,5}$ = 9.3 Hz, H-5), 2.72 (1H, *d*, $J_{6,5}$ = 9.3 Hz, H-6) 5.74 (1H, *br d*, *J* = 9.3 Hz, H-3), 5.90 (1H, *dt*, *J* = 9.3, 2.9 Hz, H-2). EIMS *m/z* (rel. int.): 346 $[M]^+$ (47), 328 (18), 302 (51), 289 (100), 271 (40), 241 (44), 243 (66), 225 (24), 199 (45), 197 (54), 169 (37), 155 (46).

ent-3α-*Acetoxy*-13-*hydroxy*-20-*norgibberell*-1,16-*diene*-7,19-*dioic acid* 19,10-*lactone* 7-*methoxymethyl ester* (**20**)

A soln of gibberellic acid 3-acetate [23] (23 g, 0.06 mol) in CH_2Cl_2 (250 ml) was treated with diisopropylethylamine (14 ml, 0.08 mol) and methoxymethyl chloride (4.5 ml, 0.06 mol), and the mixture kept under N_2 at 4° for 14 hr. Sat $NaHCO_3$ was added and the product extracted into CH_2Cl_2. The organic layer was washed with 2 M HCl, H_2O, $NaHCO_3$ soln and brine, then dried over Na_2SO_4. After removal of solvent, the residue was chromatographed on silica gel and acetate **20** (22 g, 86%) eluted with EtOAc–hexane (1:1). ^{1}H NMR ($CDCl_3$): δ 1.16 (3H, *s*, H_3-18), 2.10 (3H, *s*, $OCOCH_3$), 2.79 (1H, *d*, $J_{6,5}$ = 10.9 Hz, H-6), 3.33 (1H, *d*, $J_{5,6}$ = 10.9 Hz, H-5), 3.47 (3H, *s*, $CO_2CH_2OCH_3$), 4.96 (1H, *s*, H-17), 5.26 (1H, *d*, *J* = 5.4 Hz, $CO_2CH_2OCH_3$), 5.27 (1H, *s*, H-17), 5.29 (1H, *d*, *J* = 6.1 Hz, $CO_2CH_2OCH_3$), 5.33 (1H, *d*, $J_{3,2}$ = 3.8 Hz, H-3), 6.38 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1).

ent-3α-*Acetoxy*-16β,17-(*dichloromethano*)-13-*hydroxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methoxymethyl ester* (**21**)

To a soln of ester **20** (5.3 g, 0.012 mol) in $CHCl_3$ (100 ml) under N_2 was quickly added powdered NaOH (4.0 g, 0.1 mol) followed by benzyltriethylammonium chloride (100 mg) and stirring continued for 40 min. The temp. of the mixture was kept below 30° by occasional cooling with an external ice-water bath. Further powdered NaOH (2.0 g, 0.05 mol) was added and stirring continued for a further 40 min. The mixture was filtered through Celite, washed with NaH_2PO_4 soln (20%), dried over Na_2SO_4, concentrated and chromatographed on silica, eluting with EtOAc–petrol (40–60°) (1:1) to afford the adduct **21** (4.0 g, 63%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 1.17 (3H, *s*, H_3-18), 1.39 (1H, *d*, $J_{17,17}$ = 7.5 Hz, H-17), 1.74 (1H, *d*, $J_{17,17}$ = 7.5 Hz, H′-17), 2.09 (3H, *s*, $OCOCH_3$), 2.81 (1H, *d*, $J_{6,5}$ = 10.5 Hz, H-6), 3.31 (1H, *d*, $J_{5,6}$ = 10.5 Hz, H-5), 3.52 (3H, *s*, $CO_2CH_2OCH_3$), 5.28 (1H, *d*, *J* = 6.1 Hz, $CO_2CH_2OCH_3$), 5.33 (1H, *d*, *J* = 6.1 Hz, $CO_2CH_2OCH_3$), 5.34 (1H, *s*, H-3), 5.88 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.39 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1).

ent-16β,17-(*Dichloromethano*)-3α,13-*dihydroxy*-20-*norgibberell*-1-*ene*-7, *dioic acid* 19,10-*lactone* (**22**)

Me_3SiCl (40 ml), 0.31 mmol) was added to a soln of ester **21** (20 mg, 0.33 mmol) in THF (2.0 ml) containing MeOH (20 ml) and the mixture stirred for 20 hr. After dilution with EtOAc, the product was extracted into aq. Na_2HPO_4 (20%, 5 × 2 ml). The combined extracts were acidified to pH 6 with aq. NaH_2PO_4 (20%), then extracted with EtOAc–2-butanol (3×) to afford the 7-carboxylic acid (18.3 mg), which was dissolved in MeOH (1 ml) and treated with a soln of $KHCO_3$ (3.3 mg) and K_2CO_3 (9.1 mg) in H_2O (0.50 ml). After stirring for 2 hr, aq. NaH_2PO_4 (20%) was added to pH 6 and the mixture extracted with EtOAc (2×). Drying (Na_2SO_4) and removal of the solvent afforded the desired product (**22**) (13 mg, 72%). ^{1}H NMR ($CDCl_3$): δ 1.24 (3H, *s*, H_3-18), 1.39 (1H, *d*, $J_{17,17}$ = 7.5 Hz, H-17, 1.71 (1H, *d*, $J_{17,17}$ = 7.5 Hz, H′-17), 2.75 (1H, *d*, $J_{6,5}$ = 10.5 Hz, H-6), 3.13 (1H, *d*, $J_{5,6}$ = 10.5 Hz, H-5), 4.09 (1H, *d*, *J* = 3.6 Hz, H-3), 5.87 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.28 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1). EIMS (^{35}Cl) *m/z* (rel. int.): 428 $[M]^+$ (2), 410 (10), 392 (20), 382 (42), 366 (48), 330 (45), 322 (59), 291 (53), 286 (64), 241 (50), 207 (39), 195 (53), 179 (58), 165 (67), 155 (100).

ent-16β,17-(*Chloromethano*)-3α,13-*dihydroxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* (**26**)

A soln of ester **25** (65 mg, 0.087 mmol) in benzene (2 ml) was treated with *n*-Bu_3SnH (26 ml, 0.096 mmol) and a crystal of AIBN. The mixture was heated at reflux under N_2 for 1 hr. After removal of the solvent, the residue was dissolved in Et_2O and washed with aq. NH_3 (×3). The dried (Na_2SO_4) organic layer was reduced to dryness and chromatographed on silica gel. The monochloro product **25** (44 mg, 75%, single epimer with unspecified configuration) was eluted with EtOAc–hexane (1:2) and then hydrolysed to acid **25** in 83% yield as described for the formation of acid **22**. ^{1}H NMR ($CDCl_3$): δ 0.69 (1H, *m*, H-17), 1.14 (1H, *dd*, *J* = 8.1, 6.6 Hz, H′-17), 1.24 (3H, *s*, H_3-18), 2.74 (1H, *d*, $J_{6,5}$ = 10.5 Hz, H-6), 3.15 (1H, *d*, $J_{5,6}$ = 10.5 Hz, H-5), 4.07 (1H, *d*, *J* = 3.6 Hz, H-3), 5.88 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2); 6.30 (1H, *d*, $J_{1,2}$ = 9.3 Hz, H-1). EIMS *m/z* (^{35}Cl) (rel. int.): 394 $[M-1]^+$ (2), 376 (14), 348 (75), 332 (65), 313 (30), 288 (58), 275 (18), 261 (35), 245 (42), 223 (26), 207 (29), 195 (47), 179 (50), 160 (55), 155 (93), 91 (100).

ent-3α-*Acetoxy*-16β,17-(*dibromomethano*)-13-*hydroxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methoxymethyl ester* (**23**)

NaOH soln (50%, 2 ml) was added to a soln of ester **20** (173 mg, 0.40 mmol), 2-hydroxyethyltrimethylammonium chloride (11 mg, 0.08 mmol) and $CHBr_3$ (70 ml, 0.8 mmol) in CH_2Cl_2 (4 ml) under N_2, and the mixture stirred vigorously for 2.5 hr. Further $CHBr_3$ (50 ml) was then added and stirring continued for 0.5 hr. EtOAc was added and the organic layer washed with H_2O and brine, then reduced to dryness. Chromatography of the residue on silica gel (EtAc–hexane, 1:1) afforded the adduct **23** (103 mg, 43%). ^{1}H NMR ($CDCl_3$): δ 1.19 (3H, *s*, H_3-18), 1.65 (1H, *d*, $J_{17,17}$ = 7.6 Hz, H-17), 2.02 (1H, *d*, $J_{17,17}$ = 7.6 Hz, H′-17), 2.10 (3H, *s*,

$OCOCH_3$), 2.84 (1H, *d*, $J_{6,5}$ = 10.5 Hz, H-6), 3.34 (1H, *d*, $J_{5,6}$ = 10.5 Hz, H-5), 3.54 (3H, *s*, CH_2OCH_3), 5.30, 5.38 (2H, AB*d*, *J* = 6.1 Hz, CH_2OCH_3), 5.34 (1H, *br s*, H-3), 5.89 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.39 (1H, *d*, J_1 = 9.3 Hz, H-1).

ent-16β,17-*(Dibromomethano)*-3α,13-*dihydroxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* (**24**)

Ester **23** (20 mg) was hydrolysed to acid **24** in 95% yield as described for the preparation of acid **22**. ^{1}H NMR ($CDCl_3/d_4$-MeOH, 4:1): δ 1.19 (3H, *s*, H_3-18), 1.58 (1H, *d*, $J_{17,17}$ = 7.4 Hz, H-17), 1.94 (1H, *d*, $J_{17,17}$ = 7.4 Hz, H′-17), 2.69 (1H, *d*, $J_{6,5}$ = 10.1 Hz, H-6), 3.31 (1H, *d*, $J_{5,6}$ = 10.1 Hz, H-5), 4.00 (1H, *d*, *J* = 3.6 Hz, H-3), 5.82 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.23 (1H, *d*, J_1 = 9.3 Hz, H-1).

ent-16β,17-*(Bromomethano)*-3α,13-*dihydroxy*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* (**28**)

To a soln of ester **23** (120 mg, 0.2 mmol) in benzene (5 ml) was added *n*-Bu_3SnH (0.065 ml, 86% purity, 0.21 mmol) and AIBN (one crystal). After stirring under N_2 at room temp. for 5 hr, the mixture was reduced to dryness. The residue was dissolved in Et_2O and the soln washed with dil NH_3 (3×). After drying (Na_2SO_4) and concentration, the product was chromatographed on silica gel, eluting with EtOAc–hexane (1:2, then 1:1), to afford the monodebrominated ester **27** as a single epimer of unspecified configuration (74 mg, 71%). A portion of this product (20 mg) was hydrolysed to acid **28** in 62% yield as described for the preparation of acid **22**. ^{1}H NMR ($CDCl_3/d_4$-MeOH, 4:1): δ 0.72 (1H, *dd*, *J* = 7.5, 4.6 Hz, H-17), 1.18 (3H, *s*, H_3-18), 2.69 (1H, *d*, $J_{6,5}$ = 10.5 Hz, H-6), 3.12 (1H, *d*, $J_{5,6}$ = 10.5 Hz, H-5), 3.30 (1H, *dd*, *J* = 6.3, 4.5 Hz, CHBr), 4.00 (1H, *d*, *J* = 3.6 Hz, H-3), 5.82 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.24 (1H, *d*, J_1 = 9.3 Hz, H-1).

ent-3α-*Acetoxy*-13-*hydroxy*-16,17-*methano*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methoxymethyl ester* (**29**)

To a soln of ester **27** (120 mg, 0.2 mmol) in benzene (10 ml) was added *n*-Bu_3SnH (0.13 ml, 0.48 mmol) and AIBN (one crystal). After stirring under N_2 at reflux for 2 hr, a further crystal of AIBN was added, and reflux maintained for 1 hr. More stannane (65 ml) was then added and heating continued for a further 2 hr, at which point TLC indicated only a trace of starting material remained. The mixture was reduced to dryness, the residue dissolved in EtOAc and the soln washed with dilute NH_3 (3×). After drying (Na_2SO_4) and concentration, the product was chromatographed on silica gel, eluting with EtOAc–hexane (1:2, then 1:1), to afford the debrominated ester **29** (63 mg, 71%). ^{1}H NMR ($CDCl_3$): δ 0.32, 0.56, 0.89 (3 × 1H, 3 × *m*, H-16, H-17), 1.17 (3H, *s*, H_3-18), 2.11 (3H, *s*, $OCOCH_3$), 2.79 (1H, *d*, $J_{6,5}$ = 10.7 Hz, H-6), 3.32 (1H, *d*, $J_{5,6}$ = 10.7 Hz, H-5), 3.50 (3H, *s*, OCH_2OCH_3), 5.22, 5.33 (2H, AB*d*, *J* = 6.1 Hz, OCH_2OCH_3), 5.34 (1H, *s*, H-3), 5.88 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.43 (1H, *d*, J_1 = 9.3 Hz, H-1).

ent-3α,13-*Dihydroxy*-16,17-*methano*-20-*norgibberell*-1-*ene*-7,19-*dioic acid* 19,10-*lactone* (**30**)

Hydrolysis of ester **29** (21 mg, 0.047 mmol), as described for acid **22**, afforded acid **30** (11 mg, 65%). ^{1}H NMR ($CDCl_3/d_4$-MeOH, 4:1): δ 0.28, 0.49, 0.83 (4 × 1H, 3 × *m*, H-16, H-17), 1.19 (3H, *s*, H_3-18), 2.67 (1H, *d*, $J_{6,5}$ = 10.4 Hz, H-6), 3.11 (1H, *d*, $J_{5,6}$ = 10.7 Hz, H-5), 4.02 (1H, *d*, *J* = 3.6 Hz), 5.84 (1H, *dd*, $J_{2,1}$ = 9.3 Hz, $J_{2,3}$ = 3.8 Hz, H-2), 6.28 (1H, *d*, J_1 = 9.3 Hz, H-1). EIMS *m/z* (rel. int.): 360 $[M]^+$ (18), 342 (100), 314 (52), 298 (38), 269 (29), 255 (34), 240 (55), 225 (38), 209 (33), 155 (58).

ent-16β,17-*(Dichloromethano)*-13-*hydroxy*-20-*norgibberell*-2-*ene*-7,19-*dioic acid* 19,10-*lactone* 7-*methoxymethyl ester* (**31**)

A soln of GA_5 (**1**) [11] (194 mg, 0.59 mmol) in CH_2Cl_2 (10 ml) and Et_3N (250 ml, 0.18 mmol) was treated with methoxymethyl chloride (54 ml, 7.2 mmol) and the mixture stirred at room temp. for 15 min. Satd $NaHCO_3$ soln was added and the product extracted into EtOAc. After drying over Na_2SO_4, the solvent was removed and half of the residue (99 mg) dissolved in $CHCl_3$ (3 ml). Powdered NaOH (0.4 g, 0.01 mol) was quickly added under N_2, followed by benzyltriethylammonium chloride (6 mg) and stirring maintained for 8.5 hr. The mixture was filtered through Celite, washed with NaH_2PO_4 (20%), dried over Na_2SO_4, concentrated and chromatographed on silica, eluting with EtOAc–petrol (40–60°) (1:2), to afford the adduct **31** (81 mg, 67%) as a white foam. ^{1}H NMR ($CDCl_3$): δ 1.28 (3H, *s*, H_3-18), 1.42 (1H, *d*, $J_{17,17}$ = 7.3 Hz, H-17), 1.73 (1H, *d*, $J_{17,17}$ = 7.3 Hz, H′-17), 2.71 (1H, *d*, $J_{6,5}$ = 9.0 Hz, H-5), 2.79 (1H, *d*, $J_{5,6}$ = 9.0 Hz, H-6), 5.70 (1H, *br d*, *J* = 9.2 Hz, H-3), 5.82 (1H, *dt*, *J* = 9.2, 3.0 Hz, H-2).

ent-16β,17-*(Dichloromethano)*-13-*hydroxy*-20-*norgibberell*-2-*ene*-7,19-*dioic acid* 19,10-*lactone* (**32**)

A soln of methoxymethyl ester **31** (20 mg, 0.040 mmol) in CH_2Cl_2 (2 ml) was treated with TFA (0.2 ml) and the mixture stirred at room temp under N_2 for 1 hr. After removal of the solvent, acid **21** was obtained as a colourless glass. ^{1}H NMR ($CDCl_3/d_4$-MeOH, 4:1): δ 1.27 (3H, *s*, H_3-18), 1.38 (1H, *d*, $J_{17,17}$ = 7.3 Hz, H-17), 1.70 (1H, *d*, $J_{17,17}$ = 7.3 Hz, H′-17), 2.64 (1H, *d*, $J_{6,5}$ = 9.0 Hz, H-5), 2.72 (1H, *d*, $J_{5,6}$ = 9.0 Hz, H-6), 5.67 (1H, *br d*, *J* = 9.2 Hz, H-3), 5.79 (1H, *dt*, *J* = 9.2 Hz, 3.0 Hz, H-2). EIMS *m/z* ($^{35}Cl_2$) (rel. int.): 412 $[M]^+$

(1), 384 (1.5), 368 (60), 325 (90), 271 (100), 235 (62).

ent-16β,17-(*Chloromethano*)-13-*hydroxy*-20-*norgibberell*-2-*ene*-7,19-*dioic acid* 19,10-*lactone* (**33**)

A soln of ester **31** (40 mg, 0.087 mmol) in C_6H_6 (2 ml) was treated with *n*-Bu_3SnH (26 ml, 0.096 mmol) and a crystal of AIBN. The mixture was heated at reflux under N_2 for 1 hr. After removal of the solvent, the residue was dissolved in Et_2O and washed with aq. NH_3 (×3). The dried (Na_2SO_4) organic layer was reduced to dryness and chromatographed on silica gel. The monochloro product (35 mg, 95%, single epimer with unspecified configuration) was eluted with EtOAc–hexane (1:2). ^{1}H NMR ($CDCl_3$): δ 0.73 (1H, *m*, H-17), 1.27 (1H, *dd*, *J* = 8.1, 6.6 Hz, H′-17), 1.28 (3H, *s*, H_3-18), 2.69 (1H, *d*, $J_{6,5}$ = 9.0 Hz, H-5), 2.81 (1H, *d*, $J_{5,6}$ = 9.0 Hz, H-6), 3.43 (1H, *dd*, *J* = 8.1, 4.1 Hz, CHCl), 3.50 (3H, *s*, OMe), 5.28 (2H, *s*, OCH_2O), 5.69 (1H, *br d*, *J* = 9.2 Hz, H-3), 5.82 (1H, *dt*, *J* = 9.2, 3.0 Hz, H-2). A soln of this methoxymethyl ester (17 mg, 0.040 mmol) in CH_2Cl_2 (2 ml) was treated with TFA (0.2 ml) and the mixture stirred at room temp. under N_2 for 1 hr. After removal of the solvent, acid **33** was obtained as a colourless glass. ^{1}H NMR (d_8-THF): δ 0.63 (1H, *m*, H-17), 1.10 (1H, *dd*, *J* = 8.1, 6.6 Hz, H′-17), 1.18 (3H, *s*, H_3-18), 2.53 (1H, *d*, $J_{6,5}$ = 9.1 Hz, H-5), 2.71 (1H, *d*, $J_{5,6}$ = 9.1 Hz, H-6), 3.37 (1H, *dd*, *J* = 8.1, 4.1 Hz, CHCl), 5.65 (1H, *br d*, *J* = 9.2 Hz, H-3), 5.79 (1H, *dt*, *J* = 9.2, 3.0 Hz, H-2). EIMS *m/z* (^{35}Cl) (rel. int.): 378 $[M-1]^+$ (6), 331 (68), 287 (100), 259 (60), 245 (44), 207 (17).

Acknowledgements—The authors are grateful to Bruce Twitchin and Cheryl Blundell for technical assistance, and to Novartis Crop Protection Inc. (Kansas, USA) for a generous sample of trinexapac-ethyl.

REFERENCES

1. Evans, L. T., *Australian Journal of Biological Sciences*, 1964, **17,** 10.
2. Evans, L. T., *Australian Journal of Biological Sciences*, 1969, **22,** 773.
3. King, R. W., Blundell, C. and Evans, L. T., *Australian Journal of Plant Physiology*, 1993, **20,** 337.
4. Evans, L. T. and Blundell, C., *Australian Journal of Plant Physiology*, 1996, **23,** 569.
5. Pharis, R. P., Evans, L. T., King, R. W. and Mander, L. N., *Plant Physiology*, 1987, **84,** 1132.
6. Evans, L. T., King, R. W., Mander, L. N. and Pharis, R. P., *Planta*, 1994, **193,** 107.
7. Adams, R., Kerber, E., Pfister, K. and Weiler, E. W., in *Progress in Plant Growth Regulation*, ed. C. M. Karssen, L. C. Van Loon and D. Vreugdenhil. Kluwer, Dordrecht, 1992, p. 818.
8. Mander, L. N., Camp, D., Evans, L. T., King, R. W., Pharis, R. P., Sherburn, M. and Twitchin, B., *Acta Horticulturae*, 1995, **394,** 45.
9. Woodworth, C. W., Buss, V. and Schleyer, P., *Chemical Communications*, 1968, 569.
10. Simmons, H. E., Cairns, T. L. and Vladuchick, S. A., *Organic Reactions*, 1973, **20,** 1.
11. MacMillan, J., Seaton, J. E. and Suter, P. J., *Tetrahedron*, 1960, **11,** 60.
12. LeGoff, E., *Journal of Organic Chemistry*, 1964, **29,** 2048.
13. Dehmlow, E. V. and Dehmlow, S. S., *Phase Transfer Catalysis*, 2nd Ed. Verlag Chemie, Weinheim, 1983.
14. Neumann, W. P., *Synthesis*, 1987, 665.
15. Lombardo, L., Mander, L. N. and Turner, J. V., *Australian Journal of Chemistry*, 1981, **34,** 745.
16. Mander, L. N., *Chemical Reviews*, 1992, **92,** 573.
17. Evans, L. T., King, R. W., Chu, A., Mander, L. N. and Pharis, R. P., *Planta*, 1990, **182,** 97.
18. Takagi, M., Pearce, D. W., Janzen, L. M. and Pharis, R. P., *Plant Growth Regulation*, 1994, **15,** 207.
19. Junttila, O., King, R. W., Poole, A., Kretschmer, G., Pharis, R. P. and Evans, L. T., *Australian Journal of Plant Physiology*, 1997, **24,** 359.
20. Foster, K. F., Lee, I., Pharis, R. P. and Morgan, P. W., *Journal of Plant Growth Regulation*, 1997, **16,** 79.
21. Rademacher, W., in *Gibberellins*, ed. N. Takahashi, B. O. Phinney and J. MacMillan. Spriner-Verlag, New York, 1991, p. 300.
22. King, R. W., Blundell, C., Evans, L. T., Mander, L. N. and Wood, J. T., *Crop Science*, 1997, **37,** 1878.
23. Cross, B. E., *Journal of the Chemical Society*, 1954, 4670.