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Synthesis of gibberellin GA_6 and its role in flowering of *Lolium temulentum*

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Abstract

The induction of flowering by one long day (LD) in the grass *Lolium temulentum* is most closely mimicked by application of the gibberellins (GAs) GA_5 or GA_6, both of which occur naturally. These gibberellins promote floral development but have little effect on stem elongation. Endogenous GA_5 and GA_6 contents in the shoot apex double on the day after the LD and, for GA_5 (and we presume for GA_6 as well) reach a concentration known to be inductive for the excised shoot apex in vitro. They are, therefore, strong candidates as LD floral stimuli in this grass. The synthesis of GA_6 and an examination of its florigenic properties in *L. temulentum* are described.

Keywords: *Lolium temulentum*; Gramineae; Darnel; Flowering; Stem elongation; Shoot apex; Gibberellin A_5; Gibberellin A_6

1. Introduction

Gibberellins (GAs) are the only plant growth regulators known to replace the single long day (LD) required for flowering in the grass *Lolium temulentum L.* (Evans and King, 1985). While the LD has no immediate effect on stem elongation, however, application of some GAs promote flowering and cause an early enhancement of stem elongation. Such GAs should therefore be ruled out as early-acting endogenous LD florigenic messengers. On the other hand, at low doses, several GAs including GA_{32} (Pharis et al., 1987) and 3-epi GA_3 (Evans et al., 1994a) cause flowering without increasing stem elongation while the synthetic derivative, 16–17-dihydro GA_5 causes flowering and inhibits stem elongation (Evans et al., 1994b). Although these GAs have not yet been identified as endogenous in *L. temulentum*, some native GAs, including GA_5, induce flowering while causing only a moderate increase in stem length (Evans et al., 1990, 1994b; King et al., 2001).

Currently, for *L. temulentum*, three lines of evidence point to GA_5 as a possible "floral" message transported from the photoinduced LD leaf to the shoot apex. First, leaf-applied GA_5 is exported to the shoot apex without metabolic alteration and the flowering response is proportional to the amount reaching the apex (King et al., 2001). Secondly, at the time of LD-induced floral evocation (see McDaniel et al., 1991), the endogenous GA_5 content of the shoot apex doubles (King et al., 2001). Lastly, its endogenous concentration (based on tissue water content) approaches very closely the GA_5 concentration sufficient to induce flowering when vegetative shoot apices from plants in short days (SD) are excised and cultured on an agar medium containing GA_5 (King et al., 1993).

Although GA_5 is a strong candidate as a LD flowering stimulus in *L. temulentum*, other GAs might also be involved especially if they are biosynthetically related to GA_5. One such candidate is GA_6 (**5**), a 2β-3β epoxide that may be derived from GA_5 (Hedden and Kamiya, 1997). Here we describe the chemical synthesis of GA_6,

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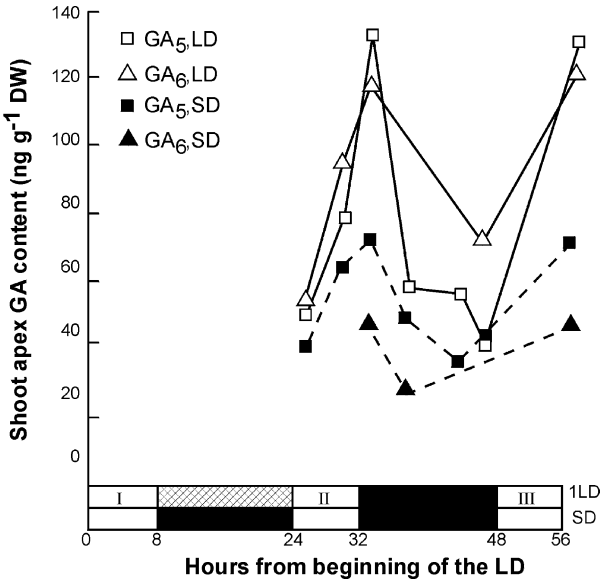


Fig. 1. Changes over time in the content of GA_5 (■, □) and GA_6 (▲, △) in shoot apices of *L. temulentum* in short days throughout (solid symbols) or following exposure to one long day (open symbols). All estimates from three experiments combined. The daily light and dark periods are shown as open and closed boxes, respectively, and the single LD exposure as a hatched box.

its effect when applied to *L. temulentum*, and its natural occurrence in shoot apices.

2. Results and discussion

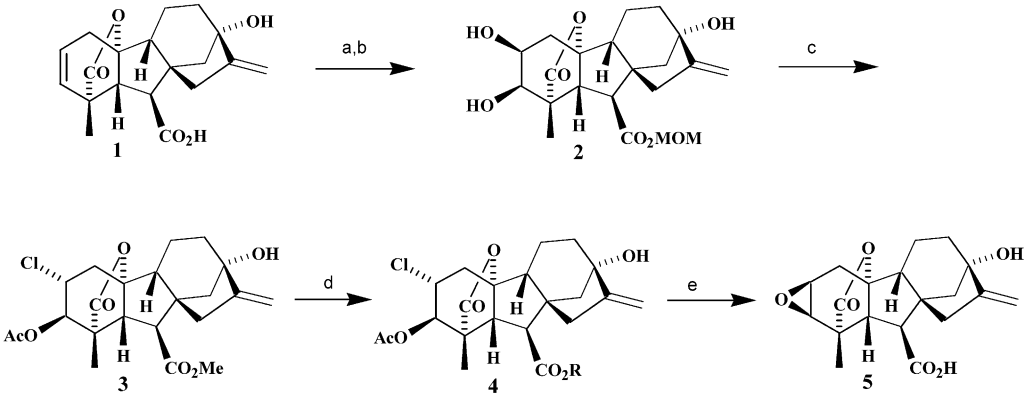
2.1. Changes at the shoot apex of endogenous GA_5 and GA_6

As we have already reported (King et al., 2001), and present here for comparative purposes (Fig. 1), there is a diurnal cycle in the content of GA_5 in shoot apices of *L. temulentum*. The peak in short day (SD) of 71 ± 5 ng g^{-1} dry weight (wt.) is reached at the end of the daily period in sunlight, after which it falls to about half that level by the end of the night. Following exposure to one LD this diurnal trend is maintained, but the peak of GA_5 content is almost double that in SD, reaching an average of 131 ± 4 ng g^{-1} dry weight across three experiments.

Although our data for GA_6 in the same experiments are not so extensive, it is clear in Fig. 1 that GA_6 is present in the shoot apex at levels similar to those of GA_5, and follows a similar diurnal trend. As with GA_5, the GA_6 content reached a maximum at the end of the day after the LD (120 ± 10 ng g^{-1} dry wt.) and remained high on the following SD (GA_6 121 ng g^{-1} dry wt.; cf GA_5 132 ng g^{-1} dry wt.). The exact pattern of change cannot be guaranteed because many of the data points were obtained from only one or two of the three experiments but, at the peak time at 32 h (i.e. the afternoon of Day II) all three experiments were represented and there was significantly more GA in apices in LD than SD (e.g. for LD and SD respectively, 131 ± 4 vs. 71 ± 5 ng g^{-1} dry wt. for GA_5 and 120 ± 10 vs. 47 ± 10 ng g^{-1} dry wt. for GA_6). Also, the SE of these peak values were small which gives considerable confidence in the data points at other times.

2.2. GA_6 synthesis

GA_6 (**5**) is available from natural sources in only trace amounts (MacMillan et al., 1962). Therefore, it was necessary to synthesize this GA and we developed the approach outlined in Scheme 1. The 16-ene functionality in GA_5 derivatives is more reactive than the Δ^2 olefinic bond towards electrophiles, so selective epoxidation of the A-ring double bond is not feasible. However, treatment with osmium tetroxide results in dihydroxylation of the 2-ene function with reasonable selectivity and has been utilised in the preparation of isotopically labelled GA_8 (Murofushi et al., 1974). Some years ago we described the conversion of a GA_8 derivative to a GA_6 type (Mander and Turner, 1981), but the procedure (involving *N*-bromosuccinimide) was not



Scheme 1. Reagents: (a) $MeOCH_2Cl$, Hunig's base; (b) O_5O_4, NMMO; (c) $Me_2C(OAc)COCl$; (d) H^+, MeOH; (e) K_2CO_3, MeOH.

expected to be applicable in the present circumstances. Instead, we turned to an alternative procedure (Greenberg and Moffatt, 1973) whereby the 2,3-diol (**2**) was treated with 2-acetoxy-isobutyryl chloride, thereby affording chloro acetate (**3**). Partial removal of the labile methoxymethyl ester protecting group was also observed, but this was of no consequence as this was required in order to complete the preparation of GA_6. Accordingly, the crude mixture was exposed to acidic methanol, affording acid (**4**), and thence GA_6 (**5**) by exposure to base.

2.3. GA_5 and GA_6 action on stem elongation and flowering

Given the increase in GA_6 content in the shoot apex of *L. temulentum* immediately after the LD, the question arises as to whether exogenous GA_6 promotes flowering and/or stem elongation. We examined both flowering and stem elongation when GA_6 was applied to the uppermost leaf blade of vegetative plants held in non-inductive SD, or when applied just before exposure to a florally inductive LD.

Three weeks after treatment, all GA-treated plants had commenced flowering as indicated in Fig. 2 by the increase in shoot apex length. GA-promotion of flowering was consistent whether it caused flowering in SD (Fig. 2a) or was additive to the induction of flowering by a LD (see below, Fig. 2b). Stem elongation was far more responsive to GA_3 than to the other two GAs, while at non-saturating doses, GA_3 was less effective for flowering (Fig. 2) as we had found previously (Evans et al., 1990). With SD plants the controls remained vegetative, so the applied GA_5 and GA_6 induced flowering although not as effectively as exposure to a single LD (Fig. 2a). However, in our previous studies, the higher doses of GA_3 or GA_5 have sometimes given as great a flowering response as the LD control (Evans et al., 1990).

The result of one of four experiments involving application prior to a LD is presented in Fig. 2b and comparable results were obtained in the others. Even with a dose of only 1 μg per plant, GA_6 very significantly promoted flowering without affecting stem elongation, whereas GA_3 and GA_5 were less promotive of flowering, but more effective for stem elongation. With greater doses, however, GA_6 also caused some stem elongation, a response also seen in SD (Fig. 2a).

As GA_5 may be converted in planta to GA_3 (Hedden and Kamiya, 1997), it is possible that as little as 10% conversion of a 25 μg dose would account for the weak effect of GA_5 on stem elongation (Fig. 2). However, the same argument cannot account for the flowering responses because, at intermediate doses, GA_5 was more effective than GA_3. Thus, GA_5 does not require conversion to GA_3 for activity. On the other hand, in planta, GA_5 may also be converted to GA_6 (Hedden and Kamiya, 1997) which is probably active per se, there being no known active metabolite of this GA.

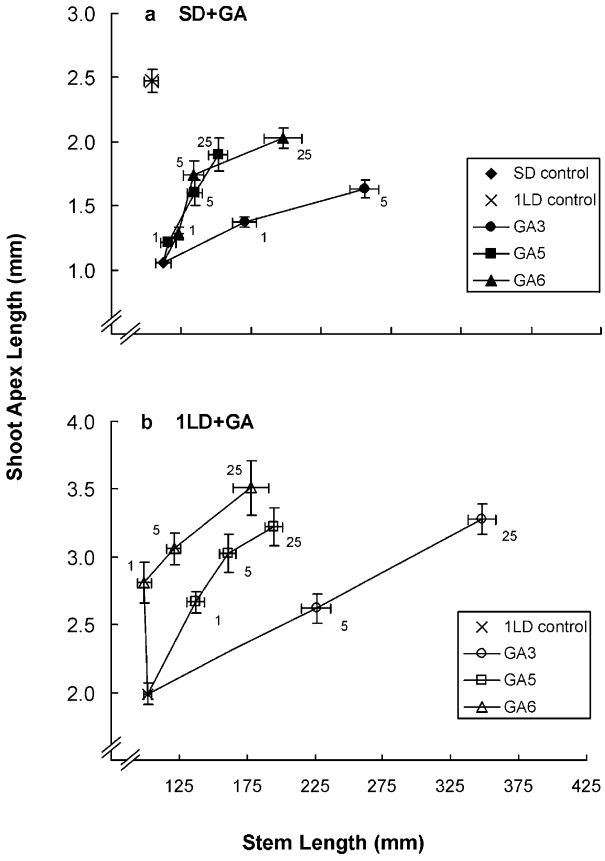


Fig. 2. Effect on the relationship between flowering (shoot apex length [mm]) and stem length (mm) when GA_3 (•, ○), GA_5 (■, □) or GA_6 (▲, △) was applied once to the leaves of *L. temulentum*. As indicated on the fig. the doses were 1, 5, or 25 μg per plant in 10 μl of 95% ethanol–water. Controls [LD (×); SD (◆)] were treated with 10 μl of 95% ethanol–water, all plants being held in SD [closed symbols (a)] or exposed to one long day [open symbols (b)] and then returned to SD. GA application was at the sixth h of the main daily 8-h light period. Shoot apex length of LD-induced plants is directly related to floral score (McDaniel et al., 1991) with all these measurements taken 3 weeks after treatment. Bars represent the SE of the mean.

Assuming that the leaf is the source of shoot apex GAs, it is interesting to speculate on how much leaf-applied GA_6 might reach the shoot apex. Previous experiments with the application of ^{32}P-phosphate, ^{35}S-sulfate (Evans and Rijven, 1967) and [$^2H_4$]-GA_5 (King et al., 2001) to leaves of *L. temulentum* have all indicated that, because of the minute size of the shoot apex, only about one part per million of a labelled compound reaches the shoot apex. On this basis, and since the shoot apex weighed between 3 and 5 μg (dry wt.) for apices examined here, the application of 1 μg of GA_6 to*L. temulentum* leaves, which produced a highly significant flowering response (Fig. 2b), should result in

a GA_6 content of approximately 200 ng g^{-1} dry wt. of shoot apex tissue. Thus, the endogenous GA_6 contents reached in *L. temulentum* apices on the day after the LD (average of 120 ± 10 ng g^{-1} see Fig. 1) are likely to have been inductive of flowering.

Based on our findings here and those reported previously (King et al., 2001), there is a strong case for GA_5 and GA_6 being involved in floral evocation of *L. temulentum*. On the day after the LD their concentration at the apex increases (King et al., 2001 and Fig. 2) just at the time when floral evocation occurs at the shoot apex (McDaniel et al., 1991; King et al., 1993). Furthermore, based on measurements of apex water content, we calculate that GA_5 reaches a concentration which matches the requirement for floral induction in vitro for apices excised from vegetative plants in SD (King et al., 1993). One reservation is that both GAs may cause some stem elongation whereas the single LD may not. However, as shown here (Fig. 2), a 1 or even a 5 μg per plant dose of GA_6 caused flowering with little or no stem elongation. Thus, either or both GA_5 or GA_6 may be agents of LD-induced floral evocation in *L. temulentum*.

Overall, GA_6 might be favoured as a naturally occurring floral stimulus in *L. temulentum* because: (1) it can induce flowering at low doses without causing stem elongation and, (2) it is likely a direct metabolite of GA_5 (Hedden and Kamiya, 1997). However, while leaf-applied [$^2H_4$]-GA_5 is translocated intact to the shoot apex (King et al., 2001), we were unable to detect any [$^2H_4$]-GA_6 product in apex extracts. Furthermore, based on studies of Spray et al. (1996) with GA-biosynthetic mutants of *Zea mays*, GA_5 maybe active per se and not require conversion to another GA. For *L. temulentum*, we know nothing of the sites of synthesis or metabolism of GA_5 or GA_6 or of GA_{20} the immediate precursor of GA_5. We assume that LD exposure enhances the production and export of GA_{20} from leaves since, in dicotyledonous species, it increases expression of a GA_{20} biosynthetic gene (e.g. Xu et al., 1997) but such information is not yet available for *L. temulentum*.

It is likely that, because of their ease of catabolism, GAs lacking a C-2 functional group, including GA_1 GA_4 and GA_{20} would be inactivated before entering the apex. This claim is based on the findings of Sakamoto et al. (2001) with rice. They found that, in vegetative plants, mRNA for a GA-2-oxidase, a GA catabolic enzyme, is expressed in a small band (ring) of cells in the terminal vascular tissue below the vegetative shoot apex. Further, this ring disappears at inflorescence formation. Consequently, GA_{20} might not reach the apex, being inactivated to GA_{29}. Similarly, GA_1 would be inactivated by conversion to GA_8, and GA_4 inactivated by conversion to GA_{34}. In contrast, GA_5, as well as GA_6, would be florigenic, being protected from C-2 hydroxylation by the Δ^2-double bond or the $2\beta,3\beta$-epoxide, as would GA_3 by its Δ^1-double bond.

Our earlier findings that GAs differ in their effect on flowering versus stem elongation provide convincing support for this scenario. For example, GA_4 and 2,2 dimethyl GA_4, are obviously active in stem tissue just below the shoot apex since both stimulate stem elongation (Evans et al., 1990). However, they differ for flowering with only the latter compound being florigenic (Evans et al., 1990). An obvious conclusion is that GA_4 is rendered inactive in sub-apical tissues, but the C-2 methyl groups of 2,2 dimethyl GA_4 protect this GA_4 derivative from 2-hydroxylation, so allowing its passage intact into the shoot apex. Interestingly, some days later as the inflorescence begins to develop, the ring of 2-oxidase disappears in rice (Sakamoto et al., 2001). At this time, but not earlier, GA_4 can be detected in apical tissue (King et al., 2001; King, Moritz and Evans, unpublished). Furthermore, at this same time applied GA_4 becomes effective in enhancing flowering of plants of *L. temulentum* induced to flower by prior exposure to a LD. The, potential structural hindrance to C-2 hydroxylation, as with GA_5 and GA_6, allows early florigenic activity as reported here, while GAs which are readily 2β-hydroxylated show no early activity because they are potentially excluded from the shoot apex due, we assume, to localized expression of the 2-oxidase. Given the early increase in shoot apex content of GA_5 and GA_6 with LD exposure, we suggest that GA_5 and GA_6 are part of the natural floral stimuli of *L. temulentum*.

3. Experimental

3.1. Plants

Plants of *Lolium temulentum L.*, strain Ceres, were grown vegetatively in 8 h short days in sunlit controlled environment cabinets as described previously (Evans et al., 1994a). Floral induction by LD involved one exposure to a 16 h extension of the 8 h of daily sunlight using light from incandescent lamps at a low intensity (10 μmol m^{-2} s^{-1}). Three weeks later, the flowering response was scored for both stage of morphogenesis (floral score) and shoot apex length (mm), these two measures being closely related (Evans et al., 1990). All GA applications were made to the uppermost expanded leaf blade in a 10 μl drop of 95% ethanol–water, the controls being treated with 95% ethanol–water.

3.2. Gibberellin assays

For gibberellin assays, the techniques were identical to those already described (King et al., 2001). In brief, homogenized tissue from samples of 45 apices, each about 5 μg dry wt., was extracted with 80% aqueous

MeOH with 30 pg of [17,17-$^2H_2$]-GAs added as internal standards to give a deutero:protio ratio close to 1.0. The GAs were purified by partitioning and solid phase extraction. The samples were methylated, further purified, and then trimethylsilylated before injection into a Hewlett-Packard 5890 GC equipped with a fused silica capillary column with a chemically bonded DB-5 MS stationary phase (J&W Scientific, Folsom, CA, USA). The column effluent was introduced into the ion source of a Jeol JMS-SX/SX102A four-sector tandem mass spectrometer of B_1E_1–B_2E_2 geometry (Jeol, Tokyo, Japan). The interface and the ion source temperatures were 270 and 250 °C, respectively. Ions were generated with 70 eV at an ionization current of 600 μA. Measurements were performed by GC/MS-selected reaction monitoring (SRM).

3.3. Synthesis of GA_6

3.3.1. ent-2α,3α,13-Trihydroxy-20-norgibberell-16-en-19-oic acid 19,10-lactone 7-methoxymethyl ester (2)

A mixture of GA_5 methyl ester (Murofushi et al., 1974) (265 mg, 0.77 mmol) in MeOH (12 ml) and 2 M NaOH (60 ml) was heated overnight under gentle reflux. After cooling to room temp, most of the MeOH was removed under reduced pressure and the residue acidified with 10 M HCl and extracted with EtOAc. The aqueous phase was concentrated and then extracted with EtOAc. The combined organic extracts were washed with water (×4) and brine and concentrated to dryness. Without further purification the residue (237 mg) was dissolved in CH_2Cl_2 (5 ml) and cooled to 0 °C under an atmosphere of nitrogen. Hunig's base (iPr_2NEt) (119 mg, 0.92 mmol) and methoxymethyl chloride (74 mg, 0.92 mmol) were then added and the mixture stirred at 0 °C for 2 h, after which EtOAc (30 ml) was added and the mixture washed with 1 M HCl, sat. $NaHCO_3$ and dried (Na_2SO_4). A portion of the resulting methoxymethyl ester (115 mg, 0.31 mmol), water (150 μl), *N*-methylmorpholine *N*-oxide (40 mg, 0.34 mmol), *t*-butanol (300 μl) and *p*-toluenesulfonic acid (29 mg, 0.15 mmol) in acetone (1.2 ml) was cooled in an ice bath and treated with osmium tetroxide (36 mg, 0.14 mmol). The solution was stirred at 0 °C for 40 min, then diluted with butan-2-ol/EtOAc (10 ml, 1:4) and washed with saturated $NaHCO_3$ (×3) and brine. The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Chromatography on silica gel (1, 2-dichloroethane/EtOAc 1:3) gave the starting alkene (27 mg, 24% recovery) and triol **2** (49 mg, 51% at 76% conversion). ^1H NMR ($CDCl_3$/d_4-MeOH) δ 1.04 (3H, *s*, 4-Me), 2.52 (1H, *d*, *J* = 10.2 Hz, H-6), 3.14 (1H, *d*, *J* = 10.2 Hz, H-5), 3.33 (3H, *s*, $OCH_2O\underline{Me}$), 3.50 (1H, *d*, *J* = 4.2 Hz, H-3), 3.63 (1H, *m*, H-2), 3.92 (1H, *br s*, OH), 4.80 (1H, s, H′-17), 5.08 (1H, *s*, H-17), 5.07, 5.15 (2×H, *ABd*, *J* = 6.0 Hz, $OC\underline{H_2}OMe$).

3.3.2. ent-3α-Acetoxy-2α-chloro-13-hydroxy-20-norgibberell-16-en-19-oic acid 19,10-lactone 7-methoxymethyl ester (3)

A solution of diol **2** (165 mg, 0.40 mmol) in MeCN (2.2 ml) was heated to 40 °C and treated with 2-acetoxyisobutyrylchloride (90 μl, 0.61 mmol). The mixture was heated for a further 5 min, then diluted with EtOAc and washed repeatedly with water until the pH was neutral. The organic phase was dried (Na_2SO_4), filtered and concentrated under reduced pressure. Chromatography on silica gel (1, 2-dichloroethane/EtOAc 1:3) afforded ester **3** (60 mg, 32%) in addition to a lower R_f material. This was re-chromatographed (AcOH/MeOH/1,2-dichloroethane/EtOAc 0.05:0.5:1:3) to yield acid **4** (56 mg, 33%). Ester **3**: ^1H NMR ($CDCl_3$) δ 1.08 (3H, *s*, 4-Me), 2.13 (3H, *s*, OAc), 2.49 (1H, *d*, *J* = 15.4 Hz, H-14), 2.73 (1H, *d*, *J* = 10.4 Hz, H-6), 3.17 (1H, *d*, *J* = 10.4 Hz, H-5), 3.44 (3H, *s*, $OCH_2O\underline{Me}$), 4.18 (1H, *d*, *J* = 6.0 Hz, H-2), 4.94 (1H, *s*, H′-17), 5.09 (1H, *s*, H-3), 5.24 (3 × H, *s*, H-17, $OC\underline{H_2}OMe$). Acid **4**: ^1H NMR ($CDCl_3$) δ 1.10 (3H, *s*, 4-Me), 2.14 (3H, *s*, OAc), 2.33 (1H, *d*, *J* = 15.3 Hz, H-15), 2.47 (1H, *d*, *J* = 15.3 Hz, H-14), 2.71 (1H, *d*, *J* = 10.5 Hz, H-6), 3.12 (1H, *d*, *J* = 10.5 Hz, H-5), 4.19 (1H, *d*, *J* = 6.5 Hz, H-2), 4.95 (1H, *s*, H′-17), 5.09 (1H, *s*, H-3), 5.28 (1H, *s*, H-17).

3.3.3. ent-3α-Acetoxy-2α-chloro-13-hydroxy-20-norgibberell-16-en-19-oic acid 19,10-lactone (4)

Ester **3** (60 mg, 0.13 mmol) was dissolved in THF (1.2 ml) and treated with MeOH (90 μl) and Me_3SiCl (278 μl, 2.18 mmol). The mixture was stirred at room temp for 3 h, then diluted with EtOAc, washed with water (until the pH was neutral) and brine, dried (Na_2SO_4), filtered and concentrated to dryness. Chromatography on silica gel (AcOH/MeOH/1,2-dichloroethane/ EtOAc 0.05:0.5:1:3) gave the title compound **4** (42 mg, 78%).

3.3.4. ent-2α,3α-Epoxy-13-hydroxy-20-norgibberell-16-en-19-oic acid 19,10-lactone (GA_6) (5)

Acid **4** (98 mg, 0.23 mmol) was dissolved in MeOH (2.8 ml) and treated with K_2CO_3/KOH solution (1.4 ml of a stock solution prepared from 1.38 g of K_2CO_3 and 150 mg of KOH in 10 ml of water). The mixture was stirred overnight at room temp. and then most of the MeOH removed under reduced pressure. The residue was acidified with 1 N HCl and extracted with EtOAc. The combined organic layers were washed with water until neutral, then dried (Na_2SO_4), filtered and concentrated under reduced pressure to give GA_6 (**5**) (65 mg, 81%). ^1H NMR ($CDCl_3$) δ 1.33 (3H, *s*, 4-Me), 2.64 (1H, *d*, *J* = 10.0 Hz, H-6), 3.02 (1H, *d*, *J* = 10.0 Hz, H-5), 3.13–3.18 (2H, *m*, H-2, H-3), 4.94 (1H, *s*, H′-17), 5.22 (1H, *s*, H-17), 6.40 (1H, *br s*, CO_2H). ^{13}C NMR ($CDCl_3$) δ 14.6 (C-18), 17.5 (C-11), 31.8 (C-1), 38.0 (C-12), 42.6 (C 14), 45.1 (C_15), 48.2 (C-8), 48.4, 49.6, 49.7 (C-2, C-3, C-6), 50.2 (C-4), 53.3 (C-9), 56.7 (C-5), 78.2

(C-13), 91.2 (C-10), 107.8 (C-17), 155.5 (C-16), 175.7, 176.3 (C-7, C-19).

3.4. 17,17–d_2 Gibberellin standards

17,17–d_2 GA_5 was prepared in 32% yield by Lombardo methylenation with Zn-$TiCl_4$-d_2-CH_2Br_2 (Lombardo, 1982) of 17-nor-GA_5-16-one methyl ester (Lombardo et al., 1981), (43% recovered starting material) followed by alkaline hydrolysis as described earlier.

17,17-d_2–GA_6 was obtained from 17,17-d_2–GA_5 as described for the parent compound. Thus, the 17,17-d_2 analogue of diol **2** was prepared from 17,17-d_2–GA_5 methoxymethyl ester (226 mg, 0.60 mmol) by treatment with acetone (2.4 ml), water (300 μl), *N*-methylmorpholine *N*-oxide (80 mg, 0.68 mmol), *t*-BuOH (600 μl), *p*-toluenesulfonic acid (four crystals) and osmium tetroxide (38 mg, 0.15 mmol). Chromatography on silica gel (CH_2Cl_2/EtOAc 1:2) afforded starting material (20 mg, 9% recovery) and the desired diol (75 mg, 33% at 91% conversion). This product (75 mg, 0.18 mmol) was converted into the 17,17-d_2 analogue of **4** by reaction with acetoxyisobutyryl chloride (40 μl, 0.28 mmol) in acetonitrile (1.8 ml), followed by chromatography, giving a mixture of ester (54 mg, 63%) and acid (11 mg, 14%). After hydrolysis of the ester (54 mg, 0.11 mmol) with Me_3SiCl (243 μl, 1.91 mmol) in MeOH (0.8 ml) and THF (1.1 ml), followed by chromatography on silica gel, the desired acid **4** (R = H) was obtained in 49% yield and then converted into GA_6(65% yield) by treatment in MeOH (0.7 ml) with K_2CO_3/KOH solution (0.34 ml).

^{1}H-NMR spectra of all deuterated intermediates and the final products were identical with those of the parent series **1–5**, apart from the absence of signals from the 17-protons and the removal of allylic coupling to the 15-protons.

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References

Evans, L.T., King, R.W., 1985. Lolium temulentum. In: Halevy, A.H. (Ed.), CRC Handbook of Flowering 3. CRC Press, Boca Raton, Florida, pp. 306–323.

Evans, L.T., King, R.W., Chu, A., Mander, L.N., Pharis, R.P., 1990. Gibberellin structure and florigenic activity in *Lolium temulentum*, a long-day plant. Planta 182, 97–106.

Evans, L.T., King, R.W., Mander, L.N., Pharis, R.P., 1994a. The relative significance for stem elongation and flowering in *Lolium temulentum* of 3β-hydroxylation of gibberellins. Planta 192, 130–136.

Evans, L.T., King, R.W., Mander, L.N., Pharis, R.P., Duncan, K.A., 1994b. The differential effects of C-16,17-dihydro gibberellins and related compounds on stem elongation and flowering in *Lolium temulentum*. Planta 193, 107–114.

Evans, L.T., Rijven, A.H.G.C., 1967. Inflorescence initiation in *Lolium temulentum*. XI. Early increases in the incorporation of phosphorus-32 and sulfur-35 by shoot apices during induction. Aust. J. Biol. Sci. 20, 1033–1042.

Greenberg, S., Moffatt, J.G., 1973. Reactions of 2-acyloxyisobutyryl halides with nucleosides. I. Reactions of model diols and of uridine. J. Am. Chem. Soc. 95, 4016–4025.

Hedden, P., Kamiya, Y., 1997. Gibberellin biosynthesis: enzymes, genes and their regulation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 48, 431–460.

King, R.W., Blundell, C., Evans, L.T., 1993. The behavior of shoot apices of *Lolium-temulentum* in-vitro as the basis of an assay system for florigenic extracts. Aust. J. Plant Physiol. 20, 337–348.

King, R.W., Moritz, T., Evans, L.T., Junttila, O., Herlt, A.J., 2001. Long-day induction of flowering in *Lolium temulentum* involves sequential increases in specific gibberellins at the shoot apex. Plant Physiol. 127, 624–632.

Lombardo, L., 1982. Methylenation of carbonyl compounds with Zn–CH_2Br_2–$TiCl_4$. Applications to gibberellins. Tetrahedron Lett. 23, 4293–4296.

Lombardo, L., Mander, L.N., Turner, J.V., 1981. Improved procedures for the degradation of gibberellic acid. The preparation of substrates for synthetic studies. Aust. J. Chem. 34, 745–753.

MacMillan, J., Seaton, J.C., Suter, P.J., 1962. Plant hormones. II. Isolation and structures of gibberellin A_6 and gibberellin A_8. Tetrahedron 18, 349–355.

Mander, L.N., Turner, J.V., 1981. Chloroethoxy(trimethyl)silane: a hard base trap which preserves TMS ether groups and improves the Wittig methylenation of gibberellins. Tetrahedron Lett. 22, 4149–4152.

McDaniel, C.N., King, R.W., Evans, L.T., 1991. Floral determination and in vitro floral differentiation in isolated shoot apices of *Lolium temulentum L*. Planta 185, 9–16.

Murofushi, N., Durley, R.C., Pharis, R.P., 1974. Preparation of radioactive gibberellins A_{20}, A_5, and A_8. Agric. Biol. Chem. 38, 475–476.

Pharis, R.P., Evans, L.T., King, R.W., Mander, L.N., 1997. Gibberellins, endogenous and applied, in relation to flower induction in the long-day plant *Lolium temulentum*. Plant Physiol. 84, 1132–1138.

Sakamoto, T., Kobayashi, M., Itoh, H., Tagiri, A., Kayano, T., Tanaka, H., Iwahori, S., Matsuoka, M., 2001. Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. Plant Physiol 125, 1508–1516.

Spray, C.R., Kobayashi, M., Suzuki, Y., Phinney, B.O., Gaskin, P., MacMillan, J., 1996. The *dwarf-1* (*d1*) mutant of *Zea mays* blocks three steps in the gibberellin-biosynthetic pathway. Proc. Nat. Acad. Sci. 93, 10515–10518.

Xu, Y.L., Gage, D.A., Zeevaart, J.A.D., 1997. Gibberellins and stem growt.h in *Arabidopsis thaliana*—effects of photoperiod on expression of the GA_4 and GA_5 loci. Plant Physiol. 114, 1471–1476.