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THE INHIBITION OF GIBBERELLIN PLANT HORMONE BIOSYNTHESIS BY *ENT*-6-HYDROXY-5β(H)-7-NORGIBBERELL-16-ENES

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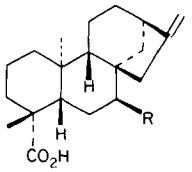
Key Word Index—*Gibberella fujikuroi*; Hypocreales; biosynthesis; gibberellins; inhibition; *ent*-6α-hydroxy-5β(H)-7-norgibberell-16-en-19-oic acid; ent-6α,19-dihydroxy-5β(H)-7-norgibberell-16-ene; plant-growth regulators.

Abstract—*ent*-6α-Hydroxy-5β(H)-7-norgibberell-16-en-19-oic acid and the corresponding diol but not the *ent*-6β-epimers are shown to be inhibitors of gibberellin biosynthesis at the ring contraction stage and to be potential plant-growth regulators. Their metabolism by *Gibberella fujikuroi* has been examined.

INTRODUCTION

Selective inhibitors of gibberellin plant hormone biosynthesis may provide novel plant-growth regulators[1]. With this object in mind, we have prepared a number of compounds which resemble key biosynthetic intermediates but lack essential structural features. The *in vivo* oxidative modification of the six-membered ring B of *ent* - kaur - 16 - en - 19 - oic acid (**1**) involves hydroxylation to form *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) which is a substrate in *Gibberella fujikuroi* and in cell-free enzyme preparations from seeds of higher plants for ring-contraction to form gibberellin A_{12} aldehyde (**3**) [2, 3]. *ent* - 6α - Hydroxy - 5β(H) - 7 - norgibberell - 16 - en - 19 - oic acid (**4**) [4] is comparable in its ring B/C/D structure to the hydroxy-acid **2** but differs from it in possessing an A/B *cis* geometry and in lacking the methylene of C-6. In this paper, we describe the effect of this acid (**4**), the corresponding 6, 19-diol (**5**), their 6-epimers (**6** and **7**) and 19-esters (**8–10**) on gibberellin biosynthesis (part of this work has appeared in the preliminary communication[5]). The effect of each compound on the metabolism of [2 – ^{14}C]MVA by *G. fujikuroi*, including its incorporation into gibberellic acid (**11**) and the accumulation of other labelled metabolites were compared to controls. Where appropriate, the metabolism of *ent* - [^{14}C] - kaur - 16 - ene (**12**) and [6α – ^{3}H]gibberellin A_{12} 7-aldehyde (**3**) was also examined. The plant-growth regulatory activity of selected compounds was also tested on young rice seedlings.



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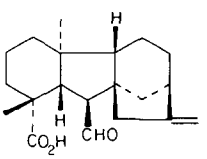

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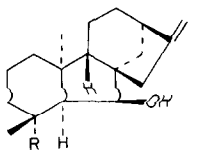

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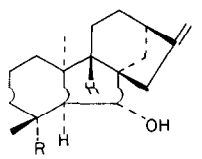
5 R = CH_2OH
8 R = CH_2OAc
9 R = CO_2Me



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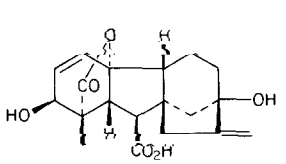
7 R = CH_2OH
10 R = CO_2Me



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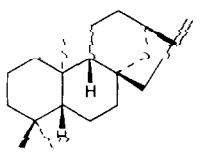

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RESULTS

The biosynthesis of gibberellic acid (**11**) from [2 – ^{14}C]MVA was completely blocked when *ent* - 6α, 19 - dihydroxy - 5β(H) - 7 - norgibberell - 16 - ene (**5**)[4] was incubated with *G. fujikuroi* at a concentration of 40 mg/l which is comparable to the amount of gibberellic acid which is produced by the fungus under our fermentation conditions (see Experimental). Although *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) is normally a transient intermediate in gibberellin biosynthesis, in this instance it accumulated (0.6% incorporation of (±)-[2 – ^{14}C]MVA). In a larger-scale fermentation, it was isolated and identified as its methyl ester[6]. Even at the lower concentration of 4 mg/l, the diol reduced the incorporation of (±)-[2 – ^{14}C]MVA into gibberellic acid by 24% when compared to the controls. Again there was an accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid.

The stage at which the biosynthesis of gibberellic acid was blocked, was examined by incubation of *ent* - [^{14}C] - kaur - 16 - ene (**12**) (prepared biosynthetically from [2 – ^{14}C]MVA) and the diol (**5**) with *G. fujikuroi*. This led to an accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) (11.7% incorporation) whilst the incorporation of *ent* - [^{14}C] - kaur - 16 - ene into gibberellic acid fell from 3.96% in the control to 0.09%. The compound which follows *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) in the biosynthetic sequence is gibberellin A_{12} 7-aldehyde (**3**). [6α – ^{3}H]Gibberellin A_{12} 7-aldehyde was prepared by carrying out the ring-contraction of the toluene - *p* - sulphonate (**13**) of 7α - hydroxykaurenolide by KOH-*t*-BuOH in the presence of $^{3}H_2O$. Incubation of the diol **5** and this substrate had no inhibitory effect on its incorporation into gibberellic acid (29% vs 2.4% in the control). The increase in incorporation reflects the lack of dilution by endogenous material. Hence the diol is acting as an inhibitor of the oxidative ring-contraction of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid.

The incubation of *ent* - 6α - hydroxy - 5β(H) - 7 - norgibberell - 16 - en - 19 - oic acid (**4**) (40 mg/l) and [2 – ^{14}C]MVA with *G. fujikuroi* led to a sparse white, globular mycelial growth with a poor metabolism of [2 – ^{14}C]MVA and no gibberellic acid production. Even at the lower concentration of 4 mg/l, there was a 95% reduction in the incorporation of [2 – ^{14}C]MVA into gibberellic acid. Dilution studies with *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) showed that this metabolite was accumulating. Both the hydroxy-acid **4** and the diol **5** had a marked effect on mycelial growth. This effect (see Fig. 1) decreased with time suggesting that these compounds were being slowly metabolized to biologically less-active material. These metabolites were identified as follows. The hydroxy-acid was recovered unchanged after incubation for 6 days with the medium alone. AMO-1618 is known to prevent[6] the formation of endogenous kaurenoid metabolites by *G. fujikuroi* thus facilitating the identification of the metabolites of exogenous substrates. Incubation of the diol **5** with *G. fujikuroi* in the presence of AMO-1618 led to the isolation of the triol, $C_{19}H_{32}O_3$ (**14**), together with the hydroxy-acid **4**. The hydroxy-acid **4** afforded a metabolite (**15**) which was characterized as its methyl ester, $C_{20}H_{32}O_4$ (**16**). The appearance of new ^{1}H NMR signals at δ 1.42 and 1.40 respectively in these metabolites and the disappearance of signals at *ca.* 5 ppm associated with the $=CH_2$ group showed that in each case, the 16, 17-olefin had been hydrated. Incubation of these metabolites (**14** and **15**) with *G. fujikuroi* and [2 – ^{14}C]MVA produced only a slight drop in the incorporation into gibberellic acid (triol: 1.11% vs 1.23% in the control; dihydroxy-acid, 1.1% vs 1.26% in the control).

Whilst the 19 - mono - acetate (**8**) had no effect on gibberellic acid production, incubation of the 19-methyl ester (**9**) and [2 – ^{14}C]MVA with *G. fujikuroi* also led to an inhibition of gibberellic acid biosynthesis (complete at 40 mg/l; 23% at 4 mg/l). However, the major metabolite which accumulated was ^{14}C-labelled ergosterol. The ester produced a 50% drop in the incorporation of *ent* - [^{14}C] - kaur - 16 - ene into gibberellic acid and caused an accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (0.24% incorporation). [6α – ^{3}H]Gibberellin A_{12} 7-aldehyde was incorporated into gibberellic acid to the extent of 31% compared with 2.2% in the control.

Incubation of the epimeric 6-alcohols, *ent* - 6β - hydroxy - 5β - 7 - norgibberell - 16 - en - 19 - oic acid (**6**), the ent - 6β, 19 - diol **7** and the 19 - methyl ester **10** with [2 – ^{14}C]MVA and *G. fujikuroi* produced only a small decrease in the incorporation into gibberellic acid. There was no apparent accumulation of *ent* - 7α - hydroxykaur-16-en-19-oic acid. Interestingly, the hydroxy-acid (**6**) and the diol (**7**) at somewhat higher concentrations, both afforded an enhanced incorporation of [2 – ^{14}C]MVA into the kaurenolide lactones and a reduction of the incorporation into gibberellic acid. Thus 7, 18 - dihydroxykaurenolide (**17**) showed an incorporation of 1.4% compared with 0.7% in the control in the presence of the diol.

Incubation of the *ent* - 6β, 19 - diol **7** with *G. fujikuroi* in the presence of AMO 1618 gave a hemiacetal, $C_{19}H_{30}O_3$ (**18**). The presence of an additional ^{1}H

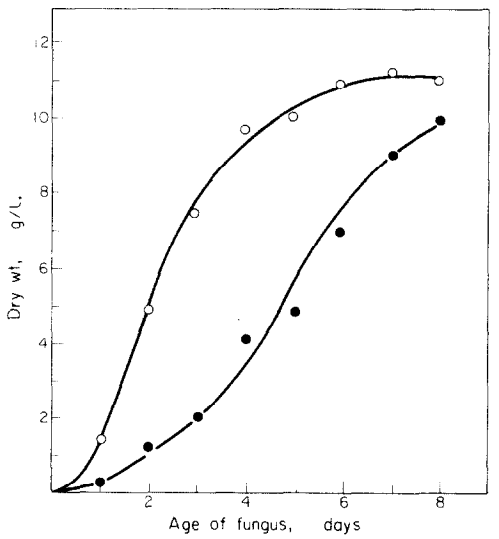
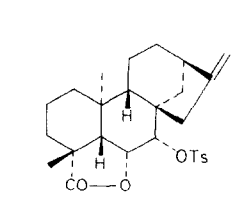


Fig. 1. Effect of the hydroxy-acid **4** on the mycelial dry wt of *G. fujikuroi*. ○, control; ●, **4**.



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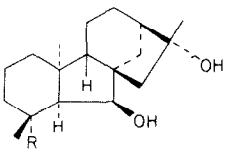
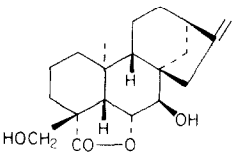


14 R = CH_2OH
15 R = CO_2H
16 R = CO_2Me



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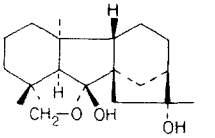

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NMR signal at δ 1.32 and the absence of olefinic signals at δ 5.0 indicated that hydration of the 16, 17-double bond had occurred. The 5-H proton resonance was a singlet (δ 2.01) whilst the hemiacetal structure was confirmed by the absence of carbonyl absorption in the IR spectrum.

The plant-growth regulatory activity of the hydroxy-acid **4** and the diol **5** were examined with rice seedlings. Rice (Crueso Ballila C cultivar) seeds were germinated and when the seedlings were *ca.* 1 cm high, they were treated by droplet application of the 7 - norgibberellene at concentrations of *ca.* 200 μg and 400 μg/seedling in the presence and absence of *G. fujikuroi.* The height of the shoots was measured at 7 days, and also in the case of the 200 μg series, 12 days after application. The results are given in Table 1. It can be seen that the compounds act both as plant-growth regulators and also reduce the 'bakanae' effect of a *G. fujikuroi* infection.

DISCUSSION

The following conclusions may be drawn from this work. *ent* - 6α - Hydroxy - 5β(H) - 7 - norgibberell - 16 - en - 19 - oic acid (**4**) and the corresponding *ent* - 6α, 19 - diol (**5**) are effective inhibitors of gibberellic acid biosynthesis at the ring contraction stage. Hitherto, no other group of plant-growth regulators has been shown to act at this step in the biosynthesis. Inhibition of this step in the biosynthesis shows considerable structure-specificity requiring a 6β - oriented (*ent* - 6α -)hydroxyl group, a free 19 - hydroxyl or carboxyl group and a 16, 17-olefin. The structural resemblance of the fragment containing the ring B hydroxyl group and the B/C/D ring junction, to *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) suggests that this determines the binding to the ring-contraction enzyme system. The different stereochemistry of the A/B ring junction, the different size of ring B and in particular the lack of a centre equivalent to C-6 then block the ring contraction. The role of hydration of the 16, 17-olefin as a possible detoxification or dumping mechanism is also apparent. The formation of ^{14}C-labelled ergosterol and the lesser accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid in the case of the 19-methyl ester, suggests that this compound might be acting at an earlier stage, for example the formation of geranylgeranyl pyrophosphate from farnesyl pyrophosphate, as well as at the ring-contraction stage. The effect of the *ent* - 6β, 19 - diol and the corresponding acid on the balance between the kaurenolide and gibberellin pathways suggests that there may be a potential regulatory point at the divergence of these pathways in the metabolism of *ent* - kaur - 16 - en - 19 - oic acid. Finally, these compounds are acting as gibberellin biosynthesis inhibitors at concentrations that are comparable with those used with the established plant growth regulator, AMO-1618. They may be prepared in 4–5 steps from a natural product.

EXPERIMENTAL

General procedure to investigate the effect of 7 - norgibberellenes on the production of ^{14}C-labelled gibberellic acid. *G. fujikuroi* (CMI 58289) was grown in shake culture at 25° for 36 hr in 20 conical flasks each containing sterile medium (50 ml)[9]. The 7 - norgibberellene (20 mg) in EtOH (2.5 ml) and Tween 80 and [2 – ^{14}C]MVA (3 μCi in EtOH, 1 ml) were evenly distributed between 10 flasks. The remaining 10 flasks were treated with EtOH (2.5 ml), Tween 80 and [2 – ^{14}C]MVA (3 μCi in EtOH, 1 ml) and retained as a control. The incubation was contained for the stated time. The mycelium was filtered, washed with EtOAc (30 ml) and dried in the oven at 60° overnight and then weighed. The broth was acidified to pH 2 with dil. HCl and the metabolites recovered in EtOAc, and separated into acidic and neutral fractions with aq. $NaHCO_3$. The neutral fraction was separated by TLC (Si gel, EtOAc–petrol, 1:1), and compared by radio-chromatogram scanning with the control. The acid fraction was separated by TLC (Si gel, $CHCl_3$–EtOAc–HOAc, 5:4:1) and also compared by radio-chromatogram

Table 1. Plant growth regulatory activity of 7-norgibberellenes on rice seedlings

Treatment	7-day treatment		12-day treatment	
	Height (cm ± s.d.)	Reduction in height (%)	Height (cm ± s.d.)	Reduction in height (%)
None (control)	7.75 (1.13)	—	12.70 (1.85)	—
200 μg diol **5**	6.19 (1.5)	20	11.21 (2.1)	12
G. fujikuroi	15.19 (4.02)	—	30.3 (4.14)	—
200 μg diol **5** and *G. fujikuroi*	14.08 (3.25)	7	25.62 (5.84)	15
None (control)	7.60 (1.46)	—		
400 μg diol **5**	5.10 (1.61)	33		
400 μg diol **7**	7.55 (1.02)	1		
G. fujikuroi	16.07 (1.4)	—		
400 μg diol **5** and *G. fujikuroi*	13.93 (1.66)	14		
400 μg diol **7** and *G. fujikuroi*	15.25 (1.78)	5		

scanning with the control. The band which co-chromatographed with gibberellic aicd was eluted from the Si gel with EtOAc, diluted with gibberellic acid (10 mg), methylated with CH_2N_2, and recrystallized to constant radioactivity from EtOAc–petrol.

Incubation of ent - 6α, 19 - *dihydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene and* [2 – ^{14}C]*MVA with* G. fujikuroi. The diol (**5**) and [2 – ^{14}C]MVA were incubated with *G. fujikuroi* for 3, 5, and 7 days as described above. There were no visible effects on the mycelium. No ^{14}C-labelled gibberellic acid was detected by radio-TLC. The band which co-chromatographed with *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid was eluted from the Si gel with EtOAc and diluted with authentic material (10 mg). It was methylated with CH_2N_2 and further purified by TLC (Si gel, EtOAc–petrol, 1:1) and crystallized to constant radioactivity from Et_2O–petrol (Table 2). No *ent* - [^{14}C] - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) was detected in the control. A second unidentified compound, absent from the controls and possessing a R_f value similar to the above acid was also detected.

The above experiment was repeated with the diol (**5**) (2 mg) distributed between 10 flasks (Table 2).

Identification of ent - 7α - *hydroxykaur* - 16 - *en* - 19 - *oic acid.* The diol (**5**) (150 mg) in EtOH (80 ml) and Tween 80 (5 drops) was evenly distributed between 76 flasks of a 1-day-old culture of *G. fujikuroi*, and the incubation then continued for a further 4 days. The metabolites were isolated as above and chromatographed on Si gel. Elution with EtOAc–petrol (3:19) gave a mixture which was methylated with CH_2N_2 and further purified by TLC (Si gel, EtOAc–petrol, 1:1). The less polar band (R_f 0.6–0.7) was eluted with EtOAc to give methyl *ent* - 7α - hydroxykaur - 16 - en - 19 - oate which crystallized from EtOAc–petrol as needles (15 mg), mp 144–146°, (lit. [6] 145–146°). It was identified by comparison of its MS and NMR with those of an authentic sample. The more polar band contained the diol (**5**) and a further unidentified product.

Incubation of the diol (**5**) *and* ent - [^{14}C] - *kaur* - 16 - *ene with* G. fujikuroi. The diol (**5**) (20 mg) and *ent* - [^{14}C] - kaur - 16 - ene (2 mg, 2.76×10^6 dpm/mg) were evenly distributed between 10 flasks of a 36-hr culture of *G. fujikuroi.* A control fermentation (10 flasks) was also grown. The metabolites were isolated as described above. Radio-TLC indicated an accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid and a diminution in the production of gibberellic acid. Both were isolated as above (Table 2). No other metabolites were detected, suggesting that the above unidentified compound was not a kaurenoid metabolite.

[6α – ^{3}H]*Gibberellin* A_{12} 7-*aldehyde.* The 7 - toluene - *p* -

Table 2

Time (days)	Incorporation into G.A. (**11**) Control (10^{-3} × dpm/mg)(%)		+ Substrate (10^{-3} × dpm/mg)(%)		Incorporation into **2** (10^{-3} × dpm/mg)(%)		Mycelial dry wt Control (g/l)	+ Substrate (g/l)
Incubation of **5** (20 mg expt) with [2 – ^{14}C]MVA								
3			not detected		1.9	0.28		
5	8.6	1.3	not detected		3.5	0.53		
7			not detected		4.2	0.63		
Incubation of **5** (2 mg expt) with [2 – ^{14}C]MVA								
3	1.8	0.28	1.8	0.28	0.98	0.15		
5	3.7	0.57	2.7	0.41	2.1	0.32		
7	6.53	0.99	4.9	0.75	1.5	0.23		
Incubation of **5** with *ent* - [^{14}C] - kaurene								
2	8.91	1.60	0.19	0.034	27.1	4.9		
4	15.5	2.83	0.493	0.084	65.9	11.9		
6	21.9	3.96	0.515	0.093	64.7	11.7		
Incubation of **5** with [6α-^{3}H]gibberellin A_{12} 7-aldehyde								
4	0.38	1.46	4.5	17.3				
6	0.61	2.35	7.7	29.6				
Incubation of **4** (20 mg expt) with [2 – ^{14}C]MVA								
2	0.78	0.12	not detected		not detected			
4	3.6	0.54	not detected		not detected			
6	8.8	1.33	not detected		0.156	0.024		
10	8.9	1.35	not detected		0.713	0.11		
Incubation of **4** (2 mg) with [2 – ^{14}C]MVA								
2	0.9	0.13	not detected		not detected		2.86	1.59
4	4.1	0.62	not detected		0.56	0.84	7.53	2.08
6	9.8	1.48	0.65	0.09	0.83	0.13	9.15	4.95
10	9.5	1.44	0.83	0.12	4.3	0.52	8.99	8.73

sulphonate of 7α-hydroxykaurenolide[10] (150 mg) in dry *t*-BuOH (15 ml, freshly distilled) was refluxed for 1 hr under N_2 with KOH (0.5 g) and ^3H_2O (0.2 ml, 40 mCi). The *t*-BuOH was removed *in vacuo* and the mixture diluted with H_2O, neutralized with dil. HCl and the product recovered in EtOAc to give the aldehyde which was crystallized from EtOAc–petrol as needles (35 mg), mp 165–166° (lit. [11] 159–163°) (5.2×10^4 dpm/mg). It was identified by its NMR spectrum.

Incubation of the diol (**5**) *and* [6α − ^{3}H]*gibberellin* A_{12} *7-aldehyde with* G. fujikuroi. The aldehyde (10 mg; 5.2×10^4 dpm/mg) was evenly distributed between 20 flasks of a 36-hr-old culture of *G. fujikuroi*. The diol (**5**) (20 mg) was added to 10 flasks and the remainder retained as a control. The metabolites were isolated as above. Gibberellic acid (10 mg) was added to each acidic extract which was then methylated with CH_2N_2 and the methyl gibberellate purified by further chromatography on TLC (Si gel, EtOAc–petrol, 3:7). It was crystallized to constant radioactivity from EtOAc–petrol (Table 2).

Incubation of ent - 6α - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid and* [2 − ^{14}C]MVA *with* G. fujikuroi. The hydroxy-acid (**4**) and [2 − ^{14}C]MVA were incubated with *G. fujikuroi* for 2, 4, 6 and 10 days as described above. A white, sparse globular mycelial growth was observed. After 2 and 4 days the [2 − ^{14}C]MVA had not been sufficiently well incorporated into any metabolites for comment. The band from the 6- and 10-day fermentations which co-chromatographed with *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid was eluted from Si gel with EtOAc, diluted with authentic material (10 mg) and crystallized to constant radioactivity (Table 2). No [^{14}C]gibberellic acid was detected in the fermentations containing the hydroxy-acid. The experiment was repeated with the hydroxy-acid (2 mg) distributed between 10 flasks of a 36-hr culture of *G. fujikuroi* (Table 2). The mycelium was white but only a slight reduction in dry wt was noted. The metabolites were isolated as above.

The effect of the hydroxy-acid mycelial dry weight of G. fujikuroi. *G. fujikuroi* was grown on shake culture for 4 hr in 80 conical flasks containing sterile medium (50 ml). The hydroxy-acid (40 mg) in EtOH (10 ml) and Tween 80 was evenly distributed between 40 flasks. The remaining 40 flasks were treated with EtOH (10 ml) and Tween 80 and retained as a control. Each day the mycelium from five flasks of each group was filtered, washed with EtOAc (30 ml) and H_2O (30 ml) and dried overnight at 60°. The experiment was repeated and the results are given in Table 3.

Incubation of ent - 6α - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid with* G. fujikuroi *in the presence of AMO* 1618. The hydroxy-acid (**4**) (250 mg) in EtOH (20 ml) was distributed between 80 flasks of a 12-hr-old culture of *G. fujikuroi* containing AMO 1618 (35 mg/l) and the incubation continued for 10 days. The metabolites were extracted as above and chromatographed on Si gel. Elution with EtOAc–petrol (3:19) gave the starting material (115 mg) identified by its IR spectrum. Further elution with EtOAc–petrol (9:11) gave *ent* - 6α, 16β - dihydroxy - 5β(H) - 7 - norgibberellan - 19 - oic acid which was methylated with CH_2N_2 to afford the corresponding 19-methyl ester which crystallized from EtOAc–petrol as needles (35 mg), mp 152–154°. (Found: C, 71.3; H, 9.5. $C_{20}H_{32}O_4$ requires C, 71.4 H, 9.5%.) IR $\nu_{max}^{cm^{-1}}$ 3450 (*br*), 1700; ^{1}H NMR δ 0.85 (3H, *s*, H-20) 1.31 (3H, *s*, H-18), 1.40 (3H, *s*, H-17), 2.47 (1H, *d*, *J* = 5 Hz, H-5), 3.65 (3H, *s*, OCH_3), 3.98 (1H, *d*, *J* = 5 Hz, H-6). Irradiation at δ 3.98 caused the doublet at δ 2.47 to collapse to a singlet. MS *m/z* (rel. int.): 318 $[M—H_2O]^+$ (7), 302 (4), 300 (6), 260 (16), 241 (14), 201 (19), 168 (11), 150 (48), 122 (58), 109 (100).

Incubation of ent - 6α, 19 - *dihydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene with* G. fujikuroi *in the presence of AMO* 1618. The diol (**5**) (250 mg) in EtOH (20 ml) was distributed between 80 flasks of *G. fujikuroi* containing AMO 1618 (35 mg/l) and the incubation continued for 8 days. The metabolites were isolated as above. Chromatography of the neutral fraction on Si gel gave, in the fraction eluted with EtOAc–petrol (3:19), the starting material (122 mg). Further elution with EtOAc–petrol (9 ; 11) gave *ent*-6α, 16β, 19 - trihydroxy - 5β(H) - 7 - norgibberellane which crystallized from EtOAc–petrol as needles (31 mg), mp 184–186°. (Found: C, 74.2; H, 10.5. $C_{19}H_{32}O_3$ requires C, 74.0; H, 10.4%.) IR $\nu_{max}^{cm^{-1}}$ 3580, 3350(*br*); ^{1}H NMR(CD_3OD): δ 1.12 (3H, *s*, H-20), 1.39 (3H, *s*, H-18), 1.42 (3H, *s*, H-17), 1.72 (1H, *d*, *J* = 5 Hz, H-5), 3.38 (2H, *s*, H-19), 3.78 (1H, *d*, *J* = 5 Hz, H-6). Irradiation at δ 3.78 collapsed the doublet at δ 1.72 to a singlet. The acid fraction was chromatographed on Si gel. Elution with EtOAc–petrol (3:19) gave the hydroxy-acid (**4**) (12 mg), mp 141–144°, identified by its IR and NMR spectra.

Incubation of ent - 6α, 16β - *dihydroxy* - 5β(H) - 7 - *norgibberellan* - 19 - *oic acid and* [2 − ^{14}C]*MVA with* G. fujikuroi. The dihydroxy-acid (**15**) and [2 − ^{14}C]MVA were incubated with *G. fujikuroi* for 3, 5, and 7 days as above. The mycelium was white but there was no significant reduction in dry wt (Table 4). No *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid was detected on the radiochromatogram.

Incubation of ent - 6α, 16β, 19 - *trihydroxy* - 5β(H) - 7 - *norgibberellane and* [2 − ^{14}C]*MVA with* G. fujikuroi. The triol (**14**) and [2 − ^{14}C]MVA were incubated with *G. fujikuroi* for 3, 5, and 7 days as above. There were no significant differences between the control and the treated fermentations (Table 4).

Incubation of ent - 19 - *acetoxy* - 6α - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene and* [2 − ^{14}C]*MVA with* G. fujikuroi. The acetate (**8**) and [2 − ^{14}C]MVA were incubated with *G. fujikuroi* for 3 and 6 days as described above. There were no significant differences between the control and the treated fermentation (Table 4).

Incubation of ent - 6α - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid* 19 - *methyl ester and* [2 − ^{14}C]*MVA with* G. fujikuroi. The ester (**9**) and [2 − ^{14}C]MVA were incubated with *G. fujikuroi* for 2, 3, 5 and 7 days as described above. There was no visible effect on the mycelium although the production of gibberellic acid was completely inhibited (Table 5). Radio-TLC showed an accumulation of ^{14}C-labelled ergosterol in the neutral fraction. The band which co-chromatographed with ergosterol was eluted from the Si gel with EtOAc, diluted with authentic material (10 mg) and crystallized to constant activity from EtOAc–petrol (Table 5). No ^{14}C-labelled ergosterol was detected in the control extract. The experiment was repeated with the ester (**9**) (2 mg) distributed between 10 flasks of *G. fujikuroi* (Table 5).

Identification of ergosterol. The ester (**9**) (150 mg) in EtOH (8 ml) and Tween 80 (5 drops) was evenly distributed between 76 flasks of a 1-day-old culture of *G. fujikuroi* and the incubation continued for 4 days. The metabolites were isolated as described above and chromatographed on Si gel. Elution with EtOAc–petrol (1:19) gave ergosterol which crystallized from EtOAc–petrol as needles (28 mg), mp 158–162° (lit. [12] 163°) identified by its IR and NMR spectrum. Further elution with EtOAc–petrol (1:9) gave the starting material (103 mg).

Table 3

Age of fungus (days)	Mycelial dry wt (g/l)					
	Expt 1		Expt 2		Average	
	Control	6β-Hydroxy-19-acid	Control	6β-Hydroxy-19-acid	Control	6β-Hydroxy-19-acid
1	1.40	0.08	1.35	0.22	1.38	0.15
2	4.15	1.35	5.67	1.07	4.91	1.21
3	6.92	2.11	7.94	1.98	7.43	2.05
4	8.75	3.97	9.70	4.23	9.72	4.10
5	9.76	4.92	10.26	4.85	10.01	4.89
6	10.54	6.87	11.41	7.17	10.97	7.02
7	10.98	9.11	11.52	9.06	11.25	9.09
8	11.17	9.87	10.84	10.01	11.00	9.94

Table 4

Time (days)	Incorporation into G.A. (11) Control (10^{-3} × dpm/mg)	(%)	+ Substrate (10^{-3} × dpm/mg)	(%)	Mycelial dry wt Control (g/l)	+ Substrate (g/l)
Incubation of **15** with [2 – ^{14}C]MVA						
3	3.2	0.48	1.6	0.24	6.86	6.53
5			6.3	0.95	8.91	8.71
7	8.3	1.26	7.2	1.1	9.34	9.50
Incubation of **14** with [2 – ^{14}C]MVA						
3	2.8	0.42	3.1	0.46	4.62	3.95
5	7.5	1.14	6.9	1.04	7.05	6.71
7	8.1	1.23	7.3	1.11	9.54	8.43
Incubation of **8** with [2 – ^{14}C]MVA						
3	1.5	0.23	2.3	0.35	7.24	7.05
6	4.1	0.62	4.3	0.65	9.61	10.12
Incubation of **10** with [2 – ^{14}C]MVA						
3	5.6	0.84	4.9	0.74	6.86	5.46
4	8.1	1.22	7.2	1.09	9.23	8.49
6	8.4	1.27	8.5	1.3	10.15	9.62

Table 5

Time (days)	Incorporation into G.A. (11) Control (10^{-3} × dpm/mg)	(%)	+ Substrate (10^{-3} × dpm/mg)	(%)	Incorporation into ergosterol (10^{-3} × dpm/mg)	(%)	Mycelial dry wt Control (g/l)	+ Substrate (g/l)
Incubation of **9** (20 mg expt) with [2 – ^{14}C]MVA								
2	0.59	0.09	not detected		9.78	1.48	6.01	5.94
3	2.2	0.34	not detected		16.6	2.52	7.52	7.67
5	5.15	0.78	not detected		29.8	4.50	8.87	8.52
7	7.98	1.21	not detected		32.3	4.91	9.96	9.03
Incubation of **9** (2 mg expt) with [2 – ^{14}C]MVA								
3	1.45	0.22	1.65	0.25	6.13	0.93		
5	4.95	0.75	4.09	0.62	5.49	0.83		
7	9.83	1.49	7.52	1.14	5.69	0.86		
Incubation of **9** with *ent*-[^{14}C]-kaurene			Incorporation into **2**					
2	5.13	0.93	1.61	0.29				
4	9.81	1.77	5.95	1.08	0.91	0.016		
6	19.5	3.50	8.27	1.5	1.34	0.24		
Incubation of **9** with [6α – ^{3}H] gibberellin A_{12} 7-aldehyde								
3	0.25	0.96	4.7		18			
6	0.57	2.2	8.1		31			

Incubation of ent - 6α - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid* 19 - *methyl ester and* ent - [^{14}C] - *kaur* - 16 - *ene with* G. fujikuroi. The ester (**9**) (20 mg) and *ent* - [^{14}C] - kaur - 16 - ene (2 mg, 2.76×10^4 dpm/mg) in EtOH (2.5 ml) were incubated with 10 flasks of a 36-hr-old culture of *G. fujikuroi.* A control fermentation was also grown. After a further 2 days growth, the metabolites were isolated as described above (Table 5). The experiment was repeated with 4- and 6-day incubations. In the latter experiments, there was a low incorporation into *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid which was not detected in the controls.

Incubation of ent - 6α - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid* 19 - *methyl ester and* [6α - ^{3}H]*gibberellin* A_{12} 7 - *aldehyde with* G. fujikuroi. [6α - ^{3}H]Gibberellin A_{12} 7-aldehyde (10 mg, 5.2×10^4 dpm/mg) in EtOH (2.5 ml) and Tween 80 was equally distributed be-

Table 6

Time (days)	Incorporation into G.A. (11)				Incorporation into 7-hydroxykaurenolide				Incorporation into 7, 18-dihydroxykaurenolide			
	Control		+ Substrate		Control		+ Substrate		Control		+ Substrate	
	$(10^{-3} \times$ dpm/mg)	(%)	$(10^{-3} \times$ dpm/mg)	(%)	$(10^{-3} \times$ dpm/mg)	(%)	$(10^{-3} \times$ dpm/mg)	(%)	$(10^{-3} \times$ dpm/mg)	(%)	$(10^{-3} \times$ dpm/mg)	(%)
Incubation of **6** (20 mg expt) with [2 – ^{14}C]MVA												
3	2.5	0.39	2.05	0.31								
5	5.3	0.81	3.83	0.58								
7	9.37	1.42	8.13	1.24								
Incubation of **6** (50 mg expt) with [2 – ^{14}C]MVA												
2	0.82	0.12	0.31	0.047	0.15	0.02	0.81	0.12	1.6	0.24	2.7	0.30
4			1.0	0.15	0.38	0.05	1.4	0.21	4.1	0.62	5.9	0.89
7	5.8	0.88	2.5	0.38	0.26	0.04	2.2	0.33	5.2	0.79	8.7	1.32
Incubation of **7** (20 mg expt) with [2 – ^{14}C]MVA												
3	2.5	0.37	1.9	0.28								
5	4.9	0.74	3.2	0.48								
7	5.6	0.85	5.05	0.76								
Incubation of **7** (50 mg expt) with [2 – ^{14}C]MVA												
2			1.5	0.16					0.92	0.14	3.9	0.59
4	6.5	0.98	4.1	0.62					3.6	0.54	7.1	1.08
6	7.0	1.06	5.2	0.79					4.6	0.70	9.3	1.41

tween 20 flasks of a 36-hr-old culture of *G. fujikuroi*. The 19-methyl ester (**9**) (20 mg) was distributed between 10 flasks whilst the remaining cultures were retained as a control. The incubations were continued for 3 and 6 days and then the metabolites were recovered as above. Gibberellic acid (10 mg) was added to each of the acid fractions which were then methylated with CH_2N_2 and chromatographed on Si gel. Elution with EtOAc–petrol (3:7) gave methyl gibberellate which was crystallized to constant radioactivity from EtOAc–petrol (see Table 5).

Incubation of ent - 6β - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid and* [2 – ^{14}C]*MVA with* G. fujikuroi. The hydroxy-acid (**6**) and [2 – ^{14}C]MVA were incubated with *G. fujikuroi* for 3, 5, and 7 days as above. There was no apparent effect on the mycelium and on the distribution of radioactivity in the metabolites (Table 6). The experiment was repeated with the hydroxy-acid (**6**) (50 mg) in 10 flasks for 2, 4 and 7 days. In all cases, there was a reduction in the incorporation into gibberellic acid and enhancement of the incorporation into the kaurenolides (Table 6).

Incubation of ent - 6β - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid* 19 - *methyl ester and* [2 – ^{14}C]*MVA with* G. fujikuroi. The hydroxy-ester (**10**) and [2 – ^{14}C]MVA were incubated with *G. fujikuroi* as above for 2, 4 and 6 days. After 2 days, there was an enhanced incorporation into 7 - hydroxykaurenolide (0.53% vs 0.18%) which was not apparent after 4 and 6 days (Table 4).

Incubation of ent - 6β, 19 - *dihydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene and* [2 – ^{14}C]*MVA with* G. fujikuroi. The diol (**7**) and [2 – ^{14}C]MVA were incubated with *G. fujikuroi* as above for 3, 5 and 7 days. There was no apparent effect on the mycelium and on the distribution of radioactivity in the metabolites (Table 6). The experiment was repeated with the diol (**7**) (50 mg) in 10 flasks for 2, 4 and 6 days. There was a slight reduction in the incorporation into gibberellic acid and an enhancement in the incorporation into 7, 18 - dihydroxykaurenolide (Table 6).

Incubation of ent - 6β, 19 - *dihydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene with* G. fujikuroi *in the presence of AMO* 1618. The diol (**7**) was incubated with a 12-hr-old culture (76 flasks) of *G. fujikuroi* in the presence of AMO 1618 (35 mg/l) for 8 days. The metabolites were isolated as above. The neutral fraction was chromatographed on Si gel. Elution with EtOAc–petrol (3:7) gave the starting meterial (120 mg), mp 159–161°. Further elution with EtOAc–petrol (3:2) gave *ent* - 16β, 19 - dihydroxy - 6 - oxo - 5β(H) - 7 - norgibberellane 19 - 6 hemiacetal which crystallized from EtOAc–petrol as needles (21 mg), mp 210–212°. (Found: C, 74.2; H, 9.8 $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.9%) IR ν_{max} cm^{-1}: 3380 (sharp), 3175 (*br*), 1055 and 1020; ^{1}H NMR δ 0.87 (3H, *s*, H-20), 1.23 [6H, *s*, H(3)-17 and H(3)-18], 2.01 (1H, *s*, H-5), 4.02 and 4.15 (2H, AB*q*, *J* = 7 Hz, H-19); MS *m/z* (rel. int.): 306 (10), 288, 270, 257, 240, 239, 189 (100), 121, 119, 109, 107, 105 and 91. No acidic transformation products were detected.

Plant growth regulatory activity of 7 - *norgibberellenes.* Rice seedlings (Crueso Ballila C cultivar) were grown in John Innes No. 1 compost with about 10 seedlings/pot. When the shoots were *ca* 1 cm high, they were treated with the 7 - norgibberellene dissolved in the minimum of Me_2CO–H_2O. The compounds were applied at a concentration of 2 or 4 mg/pot (200 or 400 μg/seedling). The height of the shoots (average of 120 seedlings/compound) were measured 7 days or 7 and 12 days after application.

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REFERENCES

1. Hanson, J. R., Parry, K. P. and Willis, C. L. (1980) *Phytochemistry* **19**, 2323.
2. For a review, see Hedden, P., MacMillan, J. and Phinney, B. O. (1978) *Annu. Rev. Plant Physiol.* **19**, 149.
3. Hanson, J. R., Hawker, J. and White, A. F. (1972) *J. Chem. Soc. Perkin Trans* 1, 1892.
4. Hanson, J. R., Parry, K. P. and Willis, C. L. (1981) *J. Chem. Soc. Perkin Trans* 1, 3020.
5. Hanson, J. R., Parry, K. P. and Willis, C. L. (1981) *J. Chem. Soc. Chem. Commun.* 285.
6. Hanson, J. R. and White, A. F. (1969) *Tetrahedron* **25**, 2743.
7. Cross, B. E. and Myers, P. L. (1969) *Phytochemistry* **8**, 79.
8. Barnes, M. F., Light, E. N. and Lang, A. (1969) *Planta* **88**, 172.
9. Dockerill, B. and Hanson, J. R. (1978) *Phytochemistry* **17**, 701.
10. Galt, R. H. B. and Hanson, J. R. (1965) *J. Chem. Soc.* 1565.
11. Cross, B. E., Norton, K. and Stewart, J. C. (1968) *J. Chem. Soc.* 1054.
12. Fieser, L. F. and Fieser, M. (1959) *Steroids* p. 100. Reinhold, New York.