Phytochemistry, Vol. 21, No. 8, pp. 1955–1958, 1982.
Printed in Great Britain.

0031-9422/82/081955-04$03.00/0
Pergamon Press Ltd.

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

JAMES R. HANSON, KEITH P. PARRY* and CHRISTINE L. WILLIS

The School of Molecular Sciences, The University of Sussex, Brighton, BN1 9QJ, U.K.; *I.C.I. Plant Protection Ltd., Jealott's Hill, Bracknell, RG12 6EY, U.K.

(*Received* 30 *October* 1981)

Key Word Index—*Gibberella fujikuroi*; Hypocreales; biosynthesis; gibberellic acid; plant growth regulator.

Abstract—*Ent*-19-hydroxy-6-oxo-5β(H)-7-norgibberell-16-ene and the corresponding 19-carboxylic acid are shown to be inhibitors of gibberellin biosynthesis in the fungus, *Gibberella fujikuroi*, at stages involved in the oxidative modification of ring B of the kaurenoid precursors.

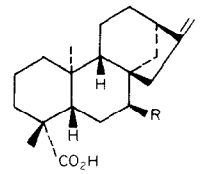
INTRODUCTION

The biosynthesis of the gibberellin plant growth hormones involves the oxidation of C-19 of a kaurenoid precursor followed by hydroxylation of **1** at C-7β to form *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**). Ring contraction then occurs to afford gibberellin A_{12} 7-aldehyde (**3**) [1, 2]. Dehydrogenation of ring B of *ent* - kaur - 16 - en - 19 - oic acid (**1**) and subsequent oxidative lactonization may form 7-hydroxykaurenolide (**4**) [3]. In the previous papers [4, 5] we showed that *ent* - 6α - hydroxy - 5β(H) - 7 - norgibberell - 16 - en - 19 - oic acid (**5**) and the corresponding 6, 19-diol acted as gibberellin inhibitors by blocking the ring contraction step. Following this precedent, a group of *ent* - 6 - oxo - 5β(H) - 7 - norgibberellenes (**6–8**) [6] were examined as inhibitors of gibberellin biosynthesis in the context of two stages: firstly as inhibitors of 7β-hydroxylation and thus altering the balance between the kaurenolide and gibberellin pathways, and secondly as inhibitors of the steps involving C-6 such as ring contraction. The chemistry of these compounds [6] indicates that there is considerable participation between the C-6 carbonyl group and C-19. Thus the 19-alcohol does not show a C-6 carbonyl band in the IR spectrum but exists as a hemi-acetal (**9**). In view of the results obtained with these compounds and their possible relationship to the kaurenolides, the corresponding 19-6 ether (**10**) and lactone (**11**) were also examined. In each case the substrates were incubated with *G. fujikuroi* in the presence of [2-^{14}C]mevalonic acid and its incorporation into the metabolites compared to controls.

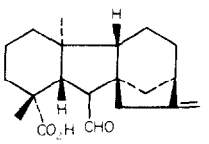
RESULTS AND DISCUSSION

Incubation of the 6 - oxo - 19 - alcohol (**6**) with *G. fujikuroi* at a concentration of 40 mg/l. led to the formation of a sparse, non-pigmented globular mycelium and a marked inhibition of gibberellic acid (**12**) biosynthesis. After 3 and 5 days, [2-^{14}C]MVA had not been incorporated sufficiently well to comment on its metabolism. After 7 and 10 days, there was only a low incorporation into gibberellic acid (0.09% vs 1.32% in the control). The incorporation into 7, 18-dihydroxykaurenolide (**13**) was also reduced but to a slightly lesser extent. At the concentration of 4 mg/l., there was only a slight reduction in the dry weight of the mycelium which was produced. However, there was a substantial reduction in the incorporation of [2-^{14}C]MVA into gibberellic acid (**12**) (0.53% vs 1.18% in the control) and an enhancement of the incorporation into 7, 18-dihydroxykaurenolide (**13**) (1.21% vs 0.90% in the control). The 6 - oxo - 19 - acid (**7**) when incubated with *G. fujikuroi* at a concentration of 40 mg/l. also produced a sparse mycelial growth and effectively blocked the biosynthesis of gibberellic acid from [2-^{14}C]MVA. Even at lower concentrations (4 mg/l.) there was only a low (0.23% vs 1.1%) incorporation of [2-^{14}C]MVA into gibberellic acid. In this case there was an accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) [7] which at the higher concentrations, was the only detectable metabolite. In contrast the 19-methyl ester (**8**) was without effect. Even at high concentrations (100 mg/l.) there was only a slight diminution (1.19% vs 1.35% in the control) in the incorporation of [2-^{14}C]MVA into gibberellic acid. The inhibitory effects of the 6 - oxo - 19 - alcohol (**6**) and 6 - oxo - 19 - acid (**7**) on the biosynthesis of gibberellic acid may be an effect of the hemi-acetal (**14**) and the lactol (**9**) both of which possess a 6β-hydroxyl group. This had previously been shown to be an important feature of the 7-norgibberellene inhibitors [4, 5].

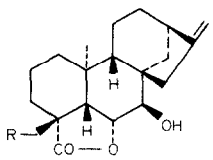
The ether (**10**) and the lactone (**11**) resemble the hemi-acetal and the lactol respectively but lack the 6β-hydroxyl group. Incubation of the ether (**10**) at a concentration of 40 mg/l. and at the higher concentration of 100 mg/l. produced no effect on gibberellic acid biosynthesis from [2-^{14}C]MVA in *G. fujikuroi*. In contrast the 19-6 lactone (**11**) produced a slight diminution in gibberellic acid biosynthesis (0.89% vs 1.16% in the control) and an increase in the in-



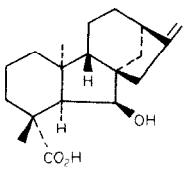
1 R = H
2 R = OH
15 $\Delta^{6,7}$, R = H



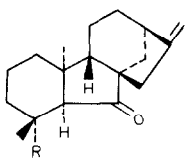
3



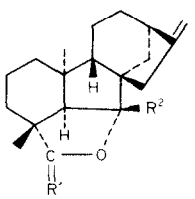
4 R = H
13 R = OH



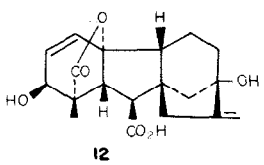
5



6 R = CH_2OH
7 R = CO_2H
8 R = CO_2Me



9 R' = O, R^2 = OH
10 R' = H_2, R^2 = H
11 R' = O, R^2 = H
14 R' = H_2, R^2 = OH



12

corporation of [2-^{14}C]MVA into the kaurenolides **4** and **13**.

When tested against rice seedlings, at concentrations of 200 and 400 μg per seedling, in the presence and absence of *G. fujikuroi* infections, the 6-oxo-19-acid showed both plant growth inhibitory action and a diminution of the 'bakanae' effect of the *G. fujikuroi* infection.

Since the 6 - oxo - acid (**7**) restricted the growth of *G. fujikuroi*, it was incubated with *Gliocladium virens* and *Trichothecium roseum* to see if this was a general fungicidal action. However it did not show the same inhibitory effect on the growth of these mycelia. Hence this secondary metabolite biosynthetic inhibitor may show some selectivity in the control of fungal growth.

The 6 - oxo - 19 - alcohol (**6**) and 6 - oxo - 19 - acid (**7**) are effective inhibitors of gibberellin biosynthesis in *G. fujikuroi*. The accumulation of *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) in the presence of the 6 - oxo - acid (**7**) suggests that it might be blocking the ring contraction stage in the biosynthesis. However, the accumulation of the kaurenolides, which was not observed in the case of the 6-alcohols, may also be a reflection of inhibition at a different step in gibberellin biosynthesis. There is now some evidence that the kaurenolides (e.g. **4**) are formed mainly via *ent* - kaur - 6, 16 - dien - 19 - oic acid (**15**) rather than via *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) [3]. Thus the main point of divergence between the kaurenolide and gibberellin pathways occurs in the immediate metabolism of *ent*-kaur-16-en-19-oic acid by dehydrogenation or, alternatively, 7β-hydroxylation. The action of the 6-oxo compounds in blocking 7β-hydroxylation would divert material into the kaurenolide pathway via the *ent*-kaur-6,16-dien-19-oic acid.

EXPERIMENTAL

General experimental details have been described previously [5].

Incubation of ent - 6 - *oxo* - 19 - *hydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene and* [2-^{14}C]*MVA with* G. fujikuroi. The 6 - oxo - 19 - alcohol (**6**) (20 mg) in EtOH (2.5 ml) and [2-^{14}C]MVA (3 μCi) in EtOH (1 ml) were incubated in 10 flasks (50 ml medium each) of *G. fujikuroi* for 3, 5, 7 and 10 days. Ten flasks were also used as controls. A white, sparse globular mycelial growth was observed. After 3 and 5 days, the [2-^{14}C]MVA had not been incorporated sufficiently well to comment on its metabolism. After 7 and 10 days, the radiochromatogram revealed a low incorporation of [2-^{14}C]MVA into gibberellic acid and 7, 18-dihydroxy-kaurenolide. The results are given in Table 1.

The experiment was repeated with the 6 - oxo - 19 - alcohol (**6**) (2 mg) and [2-^{14}C]MVA (3 μCi) distributed between 10 flasks of a 36 hr old culture of *G. fujikuroi*. The results are given in Table 1.

Effect of ent - 19 - *hydroxy* - 6 - *oxo* - 5β(H) - 7 - *norgibberell* - 16 - *ene on the growth of* G. fujikuroi. The fungus was grown in shake culture for 4 hr in 80 flasks containing sterile medium (50 ml). The alcohol (**6**) (40 mg) in EtOH (10 ml) and Tween 80 was distributed between 40 flasks. The remainder were treated with EtOH (10 ml) and Tween 80 and retained as a control. The mycelium from the control and the alcohol (**6**) incubation (five flasks each) was filtered at daily intervals, washed with EtOAc (30 ml), H_2O and dried overnight at 60°. The expt was repeated and the results are presented in Table 2.

Incubation of ent - 6 - *oxo* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid and* [2-^{14}C]*MVA with* G. fujikuroi. The 6 - oxo - 19 - acid (**7**) (20 mg) in EtOH (2.5 ml) and [2-^{14}C]MVA (3 μCi) in EtOH (1 ml) were incubated with 36 hr old cultures (10 flasks, 50 ml medium each) of *G. fujikuroi* for 3, 5, 7 and 10 days. A white sparse mycelial growth was observed. After 3 and 5 days, the [2-^{14}C]MVA had not been incorporated sufficiently well to comment on its metabolism. However after 7 and 10 days growth, the major metabolite was *ent* - 7α - hydroxykaur - 16 - en - 19 - oic acid (**2**) (0.15% incorporation after 10 days). No gibberellic acid was detected. The expt was repeated using the 6 - oxo - 19 - acid (**7**) (2 mg) in 10 flasks of *G. fujikuroil* The results are given in Table 1.

Effect of ent - 6 - oxo - 5β(H) - 7 - *norgibberell*- 16 - *en* - 19 - *oic acid on the growth of* G. fujikuroi. The procedure described above for the 19-alcohol, was repeated for the 6 - oxo - 19 - acid (40 mg) in 40 flasks of *G. fujikuroi* (Table 3).

Incubation of ent - 6 - *oxo* - 5β(H) - 7 - *norgibberell* - 16 - *en* - 19 - *oic acid* 19-*methyl ester and* [2-^{14}C]*MVA with* G. fujikuroi. The ester (**8**) (20 mg) and [2-^{14}C]MVA (3 μCi) were incubated with *G. fujikuroi* for 2, 4 and 6 days as above. There was no effect on mycelial growth or on the distribution of radioactivity in the fungal metabolites when compared with radiochromatograms of the controls. The

Table 1. Incubation of [2-^{14}C]MVA with *G. fujikuroi* in the presence of compounds **6–8**, **10** and **11**

Compound incubated with *G. fujikuroi*	Compound incorporated into	Time (days)	Control		+ Substrate	
			10^3 dpm	%	10^3 dpm	%
6(20 mg)	**12**(GA)	3	—	—	0	0
		5	4.2	0.64	0	0
		7	8.6	1.3	0.46	0.07
		10	8.7	1.32	0.59	0.09
	13	3	—	—	0	0
		5	3.6	0.54	0.29	0.04
		7	6.9	1.05	0.77	0.12
		10	7.3	1.10	0.96	0.15
6(2 mg)	**12**(GA)	3	2.85	0.43	0.36	0.05
		6	4.12	0.62	0.91	0.14
		9	7.8	1.18	3.49	0.53
	13	3	1.49	0.23	1.95	0.29
		6	2.35	0.36	3.28	0.50
		9	5.94	0.90	7.99	1.21
7(2 mg)	**2**	5	—	—	0.13	0.02
		7	—	—	0.69	0.10
		10	—	—	2.0	0.30
	12(GA)	3	2.3	0.34	0	0
		5	4.3	0.65	0	0
		7	7.8	1.20	0	0
		10	7.3	1.10	1.50	0.23
8(50 mg)	**12**(GA)	3	1.4	0.21	1.40	0.21
		5	3.6	0.54	3.00	0.45
		7	8.9	1.35	7.90	1.19
10(20 mg)	**12**(GA)	2	1.2	0.19	0.53	0.08
		5	4.5	0.67	3.89	0.59
		7	8.3	1.25	7.13	1.08
11(20 mg)	**4**	3	0.61	0.09	0.83	0.12
		7	0.35	0.05	1.70	0.26
	12(GA)	3	4.5	0.68	3.20	0.48
		7	7.7	1.16	5.90	0.89
	13	3	1.45	0.22	2.86	0.43
		7	4.69	0.71	6.74	1.02

incorporation into gibberellic acid after 6 days was 1.29% compared to 1.24% in the control. The expt was repeated with the ester (**8**) (50 mg) and [2-^{14}C]MVA (3 μCi) evenly distributed between 10 flasks of a 36 hr culture of *G. fujikuroi*. The results are given in Table 1.

Incubation of ent - 6β, 19 - *dihydroxy* - 5β(H) - 7 - *norgibberell* - 16 - *ene*, 19 - 6 - *ether and* [2-^{14}C]MVA *with G. fujikuroi*. The ether (**10**) (20 mg) in EtOH (2.5 ml) and [2-^{14}C]MVA (3 μCi) were incubated with *G. fujikuroi* as above for 2, 5 and 7 days. Although a deep red mycelial growth was observed, there was little difference in the dry weight of mycelium produced or in the distribution of radioactivity in the metabolites. The results are given in Table 1.

Incubation of ent-6β-*hydroxy*-5β(H)-7-*norgibberell*-16-*en*-19-*oic acid*, 19-6 *lactone and* [2-^{14}C]*MVA with* G. fujikuroi. The lactone (**11**) (20 mg) in EtOH (2.5 ml) and [2-^{14}C]MVA (3 μCi) in EtOH (1 ml) were incubated with a 36 hr culture (10 flasks) of *G. fujikuroi* as above for 3 and 7 days. The results are given in Table 1.

Plant growth regulatory activity of the 6 - *oxo* - 19 - *acid* (**7**). Rice seedlings (Crueso Ballila C cultivar) were grown in John Innes No. 1 compost with *ca* 10 seedlings per pot. When the shoots were *ca* 1 cm high, they were treated with the 6 - oxo - acid (**7**) (2 or 4 mg/pot; 200 or 400 μg/seedling) in the minimum of aq. Me_2CO. The height of the shoots (average of 120 seedlings per determination) was measured. (i) 400 μg, 7 days, expt: control 7.60 (s.d. 1.46) cm; 6 - oxo - 19 - acid, 4.37 (s.d. 1.3) cm (43% reduction in height); control + *G. fujikuroi* 16.87 (s.d. 1.4) cm; 6 - oxo - 19 - acid + *G. fujikuroi*, 12.51 (s.d. 2.11) cm (22% reduction in height). (ii) 200 μg, 7 days expt: control 7.75 (s.d. 1.13) cm; 6-oxo-19-acid, 5.95 (s.d. 1.86) cm (23% reduction in

Table 2. The effect of *ent* - 19 - hydroxy - 6 - oxo - 5β(H) - 7 - norgibberell - 16 - ene (**6**) on the growth of *G. fujikoroi*. The mycelial dry weights are given in g/l.

Age of fungus (days)	Experiment 1		Experiment 2		Average	
	Control	6-Oxo-19-alcohol (**6**)	Control	6-Oxo-19-alcohol (**6**)	Control	6-Oxo-19-alcohol (**6**)
1	1.05	0.18	1.72	0.13	1.38	0.15
2	3.98	1.45	5.86	1.00	4.92	1.23
3	6.34	2.67	7.37	1.93	6.56	2.30
4	8.75	4.93	9.14	4.77	8.94	4.85
5	9.82	6.53	9.60	6.81	9.71	6.67
6	10.35	8.54	9.72	8.50	10.03	8.52
7	10.95	9.62	11.35	8.71	11.15	9.17
8	11.12	10.51	11.06	11.18	11.09	10.84

Table 3. The effect of *ent* - 6 - oxo - 5β(H) - 7 - norgibberell - 16 - en - 19 - oic acid (**7**) on the growth of *G. fujikuroi*. The dry weight of the mycelium is given in g/l.

Age of fungus (days)	Experiment 1		Experiment 2		Average	
	Control	6-Oxo-19-acid (**7**)	Control	6-Oxo-19-acid (**7**)	Control	6-Oxo-19-acid (**7**)
1	1.53	0.08	1.39	0.16	1.46	0.12
2	4.82	1.10	6.66	1.71	5.74	1.40
3	7.69	1.95	9.36	2.77	8.52	2.36
4	9.71	4.83	9.42	5.56	9.56	5.20
5	10.02	6.71	10.73	7.02	10.38	6.87
6	10.95	7.92	11.15	7.90	11.05	7.91
7	11.56	9.93	11.78	8.56	11.67	8.70
8	11.62	9.15	11.27	9.00	11.44	9.07

height); control + *G. fujikuroi*, 15.19 (s.d. 4.02) cm; 6 - oxo - 19 - acid + *G. fujikuroi*, 12.23 (s.d. 3.28) cm (20% reduction in height). (iii) 200 μg, 12 days expt: control 12.70 (s.d. 1.85) cm; 6 - oxo - 19 - acid, 11.43 (3.64) cm (10% reduction in height); control + *G. fujikuroi* 30.3 (s.d. 4.14) cm; 6 - oxo - 19 - acid + *G. fujikuroi* 25.23 (s.d. 5.38) cm (17% reduction in height).

Acknowledgements—We thank the S.R.C. and I.C.I. Plant Protection Ltd. for a C.A.S.E. studentship (for C.L.W.) and Mrs. N. Dransfield for growing the fermentations.

REFERENCES

1. Hedden, P., MacMillan, J. and Phinney, B. O. (1978) *Annu. Rev. Plant Physiol.* **29**, 149.
2. Hanson, J. R., Hawker, J. and White, A. F. (1972) *J. Chem. Soc., Perkin Trans.* 1 1892.
3. Graebe, J. E., Hedden, P. and Rodemacher, W. (1980) in *Gibberellins, Chemistry, Physiology and Use*, No. 5, p. 31. British Plant Growth Regulator Group Monograph.
4. Hanson, J. R., Parry, K. P. and Willis, C. L. (1981) *J. Chem. Soc. Chem. Commum.* 285.
5. Hanson, J. R., Parry, K. P. and Willis, C. L. (1982) *Phytochemistry* (in press).
6. Hanson, J. R., Parry, K. P. and Willis, C. L. (1982) *J. Chem. Soc. Perkin Trans.* 1 (in press).
7. Hanson, J. R. and White, A. F. (1969) *Tetrahedron* **25**, 2743.