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THE INHIBITION OF GIBBERELLIN PLANT HORMONE BIOSYNTHESIS BY ENT-7-NORGIBBERELLA-5,16-DIENES

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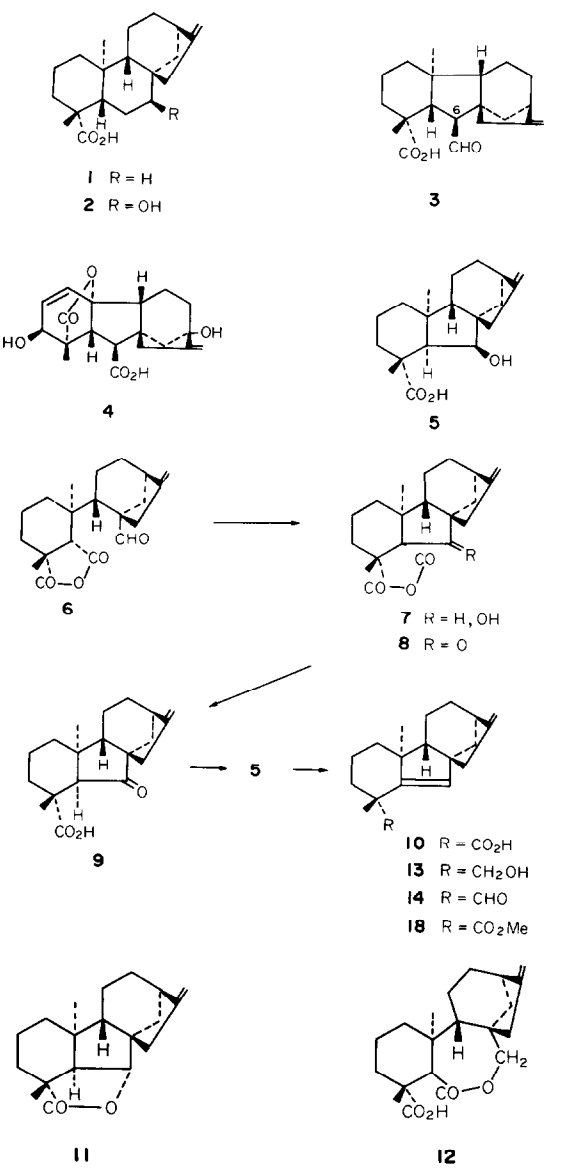
Abstract—Ent-7-norgibberella-5,16-dien-19-oic acid and the corresponding 19-alcohol and aldehyde, have been prepared from fujenal and shown to act as inhibitors of gibberellic acid biosynthesis in *Gibberella fujikuroi* They act as plant growth regulators when tested against rice seedlings

INTRODUCTION

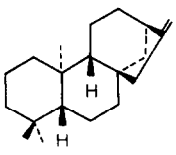
A key step in gibberellin biosynthesis involves the hydroxylation of ent-kaur-16-en-19-oic acid (**1**) at C-7 with the formation of ent-7α-hydroxykaur-16-en-19-oic acid (**2**) [1] This hydroxy-acid then forms the substrate in *G fujikuroi* for ring contraction to afford gibberellin A_{12} 7-aldehyde (**3**) which is eventually converted into gibberellic acid (**4**) Recently we have shown [2–4] that ent-6α-hydroxy-5β(H)-gibberell-16-en-19-oic acid (**5**)* and the corresponding 19-alcohol, blocked the right contraction and hence acted as inhibitors of the biosynthesis of gibberellic acid (**4**) These compounds also showed plant growth regulatory properties X-ray studies [Hanson, J, Hitchcock, P B, Parry, K P and Willis, C L, unpublished results] have revealed a close fit between the B/C/D ring system of ent-7α-hydroxykaur-16-en-19-oic acid and ent-6α-hydroxy-7-nor-5β(H)-gibberell-16-en-19-oic acid We have now examined a group of ent-7-norgibberell-5,16-dienes in which C-6 (= kaurenoid C-7) has been converted to a trigonal centre, as possible inhibitors of the hydroxylation of ent-kaur-16-en-19-oic acid and hence as gibberellin biosynthesis inhibitors A preliminary report on this work was given in ref [5]

RESULTS AND DISCUSSION

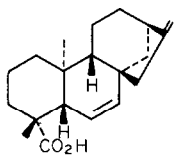
The unsaturated acid **10** was readily prepared from fujenal (**6**) which is a metabolite of *G fujikuroi* [6] Reaction of fujenal (**6**) with sodium hydride gave, *inter alia*, the hydroxy-compound **7** which was oxidized with chromium trioxide to the ketone **8** Hydrolysis and decarboxylation of this β-keto-anhydride (**8**) gave a keto-acid (**9**) which was reduced with sodium borohydride to the alcohol **5** and its C-6 epimer [7] Dehydration of the alcohol (**5**) with thionyl chloride in pyridine gave the unsaturated acid **10** together with the lactone **11** The acid (**10**) was also obtained as a significant by-product from the action of sodium hydride on fujenal (**6**) although it was



*Although these compounds have been systematically named as norgibberellenes, they may also be considered as ent-B-nor-5β(H)-kaurenes



15



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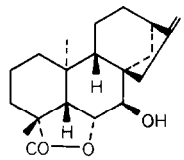
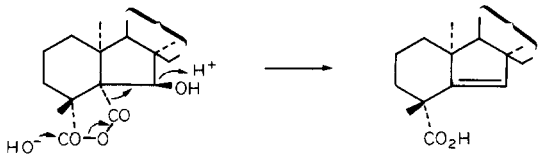


17

not separated from the condensation product (7) until the latter was oxidized to the ketone 8 The acid (10) was accompanied by a lactone which was tentatively assigned the structure 12 on the basis of its ^{1}H NMR spectrum and by analogy with some earlier transformations of fujenal [8] The acid (10) probably arises by hydrolysis of the hydroxy-anhydride (7) during work-up (see Scheme 1) The 19-alcohol (13) was obtained as a by-product from the reduction of the keto-acid (9) with lithium aluminium hydride Oxidation of the 19-alcohol (13) with chromium trioxide gave sequentially, the 19-aldehyde (14) and the 19-acid (10)



Scheme 1

Inhibition of gibberellic acid biosynthesis was revealed by the effect on the incorporation of [2-^{14}C]mevalonic acid into gibberellic acid by *G fujikuroi* (Table 1) Incubation of the 19-alcohol (13), 19-aldehyde (14) and 19-acid (10) with *G fujikuroi* at a concentration of 40 mg/l produced similar effects on gibberellin biosynthesis The production of gibberellic acid was blocked whilst ent-[^{14}C]kaurene (15) and [^{14}C]fujenal (6) accumulated These metabolites were isolated and characterized from a larger scale fermentation in the presence of the 19-alcohol (13) The latter was oxidized to the 19-acid (10) by *G fujikuroi*

Incubation of the 19-alcohol (13) and ent-[^{14}C]kaurene (15) led to an increase in the amount of recovered unmetabolized ent-kaurene and an enhancement of the incorporation into fujenal compared to controls (Table 2) Only a little [^{14}C]gibberellic acid was formed On the other hand [6α-^{3}H]gibberellin A_{12} 7-aldehyde (3) was efficiently incorporated into gibberellic acid (26% vs 1 62% in the control) in the presence of the 19-alcohol The 19-acid (10) not only inhibited the production of gibberellic acid but also reduced the growth of the fungus However the corresponding 19-methyl ester was without effect on mycelial growth or gibberellin biosynthesis The 19-hydroxy-7-norgibberell-5,16-diene (13) showed growth regulatory activity when tested on rice seedlings It also diminished the 'bakanae' effect of a *G fujikuroi* infection of the rice seedlings

In conclusion, ent-19-hydroxy-7-norgibberell-5,16-diene (13), the corresponding 19-aldehyde (14) and the 19-

Table 1 Incubation of [2-^{14}C]MVA with *G fujikuroi* in the presence of compounds 10, 13, 14 and 18

Substrate	Metabolite isolated	Age of culture (days)	Control		+ Substrate	
			Sp act 10^{-3} dpm mg^{-1}	Incorporation (%)	Sp act 10^{-3} dpm mg^{-1}	Incorporation (%)
13 (20 mg)	15	3	0 86	0 13	2 6	0 43
		5			3 5	0 53
		7	0 19	0 03	5 2	0 78
	6	3	0 15	0 023	8 7	1 3
		5	0 97	0 15	13 0	1 9
		7	2 3	0 35	18 0	2 7
10 (20 mg)	15	2	1 2	0 18	0	0
		4	0 9	0 14	0 51	0 07
		6	0 28	0 04	0 76	0 15
		10	0	0	1 9	0 29
	6	4	0 36	0 055	0 19	0 029
		6	0 81	0 12		
		10	3 5	0 53	2 9	0 44
14 (20 mg)	15	2	1 7	0 26	2 1	0 32
		4	0 62	0 09	4 8	0 73
		6			4 6	0 70
	6	2	0 41	0 062	0 64	0 097
		4			1 8	0 27
		6	3 1	0 47	4 4	0 66
18 (20 mg)	4	3	3 19	0 48	3 45	0 52
		6	4 93	0 75	5 17	0 78

Table 2 Incubation of ent-[^{14}C]kaurene with *G fujikuroi* in the presence of compound **13**

Metabolite isolated	Age of culture (days)	Control Sp act (10^{-3} dpm/mg)	Control Incorporation (%)	+ Substrate Sp act (10^{-3} dpm/mg)	+ Substrate Incorporation (%)
15*	2	345	62 5	461	83 5
	4	218	39 5	309	56 0
	6	97 9	17 7	277	50 2
6	2			16 4	3 0
	4			31 2	5 7
	6	4 91	0 89	35 6	6 4
4	2	8 93	1 62	0 58	0 11
	4	9 62	1 74	0 75	0 14
	6	28 5	5 16	1 03	0 19

*Recovered material

acid (**10**), which are readily prepared from fujenal, act as gibberellin biosynthesis inhibitors and hence as plant growth regulators The 19-alcohol appears to inhibit biosynthesis at a post-kaurene but pre-gibberellin step Unlike the incubations with the hydroxy-acid (**5**), ent-7α-hydroxykaur-16-en-19-oic acid (**2**) did not accumulate However fujenal (**6**) which is a terminal metabolite on the kaurenolide (**17**) branch of the diterpenoid pathways in *G fujikuroi* [9], accumulated This could suggest that the diene (**13**) is blocking the hydroxylation of ent-kaur-16-en-19-oic acid (**1**) and diverting material via ent-kaur-6,16-dien-19-oic acid (**16**) [10] and the kaurenolide (**17**) to fujenal (**6**)

EXPERIMENTAL

General experimental details have been described previously [3, 4]

Dehydration of the alcohol (**5**) The alcohol (**5**) [8] (460 mg) in pyridine (2 ml) was treated with $SOCl_2$ (four drops) for 5 min at 0° The mixture was poured onto ice–H_2O and the product recovered in EtOAc and chromatographed on Si gel Elution with 5% EtOAc–petrol gave an unidentified product (63 mg) mp 160–163°, IR ν_{max} cm^{-1} 1808, 1662 and 880, ^{1}H NMR δ 1 0 (3H, *s*), 1 35 (3H, *s*), 4 85 (2H, *m*) and 5 48 (1H, *s*) Elution with 10% EtOAc–petrol gave the lactone (**11**) (92 mg), mp 123–125°, identified by its IR and NMR spectra Further elution gave the ent-7-nor-gibberell-5,16-diene-19-oic acid (**10**) (140 mg) which crystallized from petrol as needles, mp 92–94° (Found C, 79 9, H, 9 0 $C_{19}H_{26}O_2$ requires C, 79 7, H, 9 1%) IR ν_{max} cm^{-1} 3000(*br*), 1698, 1655 and 875, ^{1}H NMR δ 0 98 (3H, *s*, H-20), 1 38 (3H, *s*, H-18), 4 82 (2H, *m*, H-17), 5 45 (1H, *s*, H-6) The methyl ester, prepared with CH_2N_2, was a gum, IR ν_{max} cm^{-1} 1730, 1660 and 880, ^{1}H NMR δ 0 90 (3H, *s*, H-20), 1 35 (3H, *s*, H-18), 3 61 (3H, *s*, OMe), 4 86 (2H, *m*, H-17), 5 41 (1H, *s*, H-6), MS *m/z* (rel int) 300 (30), 285 (15), 257 (14), 241 (100), 226 (24), 186 (19), 159 (18), 129 (12), 105 (8), 91 (10)

Reaction of fujenal with NaH The condensation was carried out as described previously [7] using fujenal (5 g) in dry DMF (140 ml) and NaH (1 g) The product was chromatographed on Si gel Elution with 25% EtOAc–petrol gave a gum (4 06 g) containing the anhydride (**7**) and a second component (TLC) Further elution with 30% EtOAc–petrol gave ent-7-hydroxy-6,7-seco-kaur-16-en-6,19-dioic acid 6 → 7-lactone (**12**) (150 mg) which crystallized from EtOAc–petrol as plates, mp 266–268° (Found C, 70 8, H, 8 0 $C_{20}H_{28}O_4$ $\frac{1}{2}H_2O$ requires C, 70 35, H, 8 5%) IR ν_{max} cm^{-1} 2930 (*br*), 1740, 1695, 1665 and 885, ^{1}H NMR δ 1 18 and 1 25 (each 3H, *s*), 2 92 (1H, *s*, H-5), 3 90 and 4 60 (each 1H, *dm*, J = 13 Hz, H-7), 4 85 (2H, *m*, H-17) The methyl ester, prepared with CH_2N_2, crystallized from EtOAc–petrol as needles, mp 168–169°, IR ν_{max} cm^{-1} 1730 (*br*), 1658 and 880; ^{1}H NMR δ 1 22 and 1 35 (each 3H, *s*), 3 70 (3H, *s*, OMe), 3 75 and 4 48 (each 1H, *d*, J = 13 Hz, H-7), 4 85 (2H, *m*, H-17) Oxidation of the gum (4 06 g) in Me_2CO (200 ml) with CrO_3 (3 ml) and separation of the product on Si gel gave, on elution with 10% EtOAc–petrol, the ketone (**8**) (2 80 g), mp 109–111°, identified by its IR spectrum Elution with 20% EtOAc–petrol gave the unsatd acid (**10**) (0 88 g) as a gum, identified by its IR and NMR spectra On TLC it had the same R_f and colour (H_2SO_4 spray) as the second component of the condensation reaction

Reduction of ent-6-oxo-7-nor-5β(H)-gibberell-16-en-19-oic acid (**9**) The acid (**9**) (0 7 g) in THF (150 ml) was heated with LiAl H_4 (0 7g) for 6 hr under reflux and then left to stand at room temp overnight EtOAc and H_2O were added and the products recovered in EtOAc and chromatographed on Si gel Elution with 10% EtOAc–petrol gave ent-19-hydroxy-7-norgibberell-5,16-diene (**13**) (430 mg) which crystallized from petrol as needles, mp 86° (Found C, 83 6, H, 10 4 $C_{19}H_{28}O$ requires C, 83 8, H, 10 3%) IR ν_{max} cm^{-1} 3350 (*br*), 1655 and 878, ^{1}H NMR δ 1 1 (6H, *s*, H-18, H-20), 4 85 (2H, *m*, H-17), 5 52 (1H, *s*, H-6), ^{13}C NMR δ 17 5 (C-11), 19 0 (C-2), 21 5 (C-20), 26 4 (C-18), 32 6 (C-12), 36 7 (C-14), 40 1 (C-3 and C-10), 42 0 (C-1), 46 7 (C-15), 48 5 and 53 4 (C-8 and C-4, assignment uncertain), 59 0 (C-9), 68 0 (C-19), 105 3 (C-17), 133 9 (C-6), 153 6 (C-5), 156 8 (C-16), MS *m/z* (rel int) 272 (6), 254 (8), 241 (100), 227 (15), 105 (10), 91 (18) Further elution with 20% EtOAc–petrol gave ent-6α,19-dihydroxy-7-nor-5β(H)gibberell-16-ene (29 mg) and in 30% EtOAc–petrol, the ent-6β-epimer (155 mg)

Oxidation of the alcohol (**13**) The above alcohol (**13**) (200 mg) in Me_2CO (50 ml) was treated with the 8N CrO_3 reagent for 10 min at room temp MeOH (3 ml) was added and the solvent evaporated *in vacuo* The residue was diluted with H_2O and the product recovered in EtOAc and chromatographed on Si gel Elution with 5% EtOAc–petrol gave ent-19-oxo-7-norgibberell-5,16-diene (**14**) (185 mg) as a gum, IR ν_{max} cm^{-1} 1725, 1655 and 875, ^{1}H NMR δ 0 90 (3H, *s*, H-20), 1 12 (3H, *s*, H-18), 4 92 (2H, *m*, H-17), 5 48 (1H, *s*, H-6), 9 72 (1H, *s*, H-19), MS *m/z* (rel int) 270 (45), 256 (18), 241 (100), 199 (35), 186 (30), 159 (38), 105 (53), 91 (72) The aldehyde (100 mg) was oxidized as above for

2 hr and the products were recovered in EtOAc and chromatographed on Si gel Elution with 7 5% EtOAc–petrol gave ent-7-norgibberella-5,16-dien-19-oic acid (**10**) (85 mg) which crystallized as needles, mp 92–94°, identified by its IR and NMR spectra

Incubation of ent-19-hydroxy-7-norgibberell-5,16-diene (**13**) *and* [2-^{14}C]*MVA with* G fujikuroi The alcohol (**13**) (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 and 7 days As a control another 10 flasks were used No [^{14}C]gibberellic acid was detected in the presence of the substrate (1 3% incorporation after 5 days in the control) On radio-TLC the major radioactive bands co-chromatographed with ent-kaurene and fujenal These bands were eluted from the Si gel with EtOAc, diluted with authentic material (10 mg) and crystallized to constant activity The results are given in Table 1

The expt was repeated with the alcohol (**13**) (150 mg) in EtOH (8 ml) distributed between 76 flasks of *G fujikuroi* over 4 days The metabolites were isolated and separated into acidic and neutral fractions and then purified by chromatography on Si gel The neutral fraction gave ent-kaurene (31 mg), fujenal (19 mg) and the alcohol (**13**) (97 mg) which were identified by their mps and IR spectra The acid fraction gave ent-7-norgibberell-5,16-dien-19-oic acid (28 mg) identified by its IR and NMR spectra

Incubation of ent-19-hydroxy-7-norgibberell-5,16-diene and ent-[^{14}C]*kaurene with* G fujikuroi The alcohol (**13**) and ent-[^{14}C]kaur-16-ene (2 mg, 2 76 × 10^6 dpm/mg, prepared biosynthetically from [2-^{14}C]MVA) in EtOH (2 5 ml) were incubated in 10 flasks (50 ml medium each) of *G fujikuroi* for 2, 4 and 6 days Another 10 flasks were used as a control The metabolites were isolated, separated by radio-TLC, diluted with authentic material (10 mg) and crystallized to constant activity (Table 2)

Incubation of ent-19-oxo-7-norgibberell-5,16-diene (**14**) *and* [2-^{14}C]*MVA with* G fujikuroi The aldehyde (**14**) (20 mg) and [2-^{14}C]MVA (3 μCi) in EtOH (2 5 ml) were incubated with a 36 hr culture of *G fujikuroi* (10 flasks) as above for 2, 4 and 6 days No [^{14}C]gibberellic acid was detected (1 1% incorporation in the control) The results are given in Table 1

Incubation of methyl ent-7-norgibberell-5,16-dien-19-oate and [2-^{14}C]*MVA with* G fujikuroi The 19-methyl ester (**18**) (20 mg) and [2-^{14}C]MVA (3 μCi) in EtOH (2 5 ml) were incubated with a 36-hr-old culture of *G fujikuroi* (10 flasks) as above for 3 and 6 days There was no apparent effect on the mycelial growth or on gibberellic acid biosynthesis (Table 1)

Incubation of ent-19-hydroxy-7-norgibberell-5,16-diene (**13**) *and ent-*[6α-^{3}H]*-7-oxogibberell-16-en-19-oic acid* (**3**) *with* G fujikuroi The alcohol (**13**) (20 mg) and the aldehyde (**3**) (5 mg, 5 2 × 10^4 dpm/mg) in EtOH (2 5 ml) were incubated in 10 flasks (50 ml medium each) of *G fujikuroi* (Table 3) The cultures were harvested after 3 and 5 days Another 10 flasks were used as a control The metabolites were isolated and gibberellic acid (**4**) (10 mg) was added to each acid fraction These were methylated with CH_2N_2 and chromatographed in Si gel The methyl gibberellate was crystallized to constant radioactivity

The effect of ent-7-norgibberell-5,16-dien-19-oic acid (**10**) *on the mycelial dry wt of* G fujikuroi *G fujikuroi* was grown in shake culture for 4 hr in 80 flasks each containing 50 ml of medium The acid (40 mg) in EtOH (10 ml) was evenly distributed between 40 flasks and the remainder were retained as a control Each day the mycelium from five flasks was filtered, washed with H_2O (30 ml) and dried overnight in an oven at 60° (see Table 4)

Plant growth regulatory activity of ent-19-hydroxy-7-norgibberell-5,16-diene (**13**) Rice seedlings (cv Crueso Ballila C) were grown in John Innes No 1 compost with *ca* 10 seedlings/pot When the shoots were *ca* 1 cm high, they were treated with the 19-alcohol (**13**) (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/determination) was measured (1) 400 μg, 7 day expt control 7 60 (s d ±1 46) cm, 19-

Table 3 Incubation of ent-[6α-^{3}H]-7-oxogibberell-16-en-19-oic acid (**3**) with *G fujikuroi* in the presence of **13**

	Incorporation into gibberellic acid			
	Control		+ Substrate	
Age of culture (days)	Sp act (10^{-3} dpm/mg)	Incorporation (%)	Sp act (10^{-3} dpm/mg)	Incorporation (%)
3	0 35	1 3	2 9	11
5	0 42	1 6	6 8	26

Table 4 Mycelial dry wts (g/l)

Age of culture (days)	Expt 1		Expt 2	
	Control	+ Substrate	Control	+ Substrate
1	1 35	0 51	0 5	0 2
2	4 06	1 86	2 64	1 97
3	7 13	3 92	6 0	3 3
4	8 84	4 51	8 18	3 95
5	9 86	6 97	10 0	6 81
6	10 59	8 12	10 75	7 93
7	10 96	8 99	10 9	8 9
8	11 30	9 84	11 13	9 43

alcohol, 4·61 (s.d. ±1·56) cm (39% reduction in height), control + *G. fujikuroi* 16·07 (s.d. ±1·4) cm, 19-alcohol + *G. fujikuroi*, 12·19 (s.d. ±2·05) cm (24% reduction in height) (2) 200 μg, 7 days expt control 7·75 (s.d. ±1·13) cm, 19-alcohol 5·77 (s.d. ±1·69) cm (25% reduction in height), control + *G. fujikuroi*, 15·19 (s.d. ±4·02) cm, 19-alcohol + *G. fujikuroi*, 10·85 (s.d. ±2·95) cm (29% reduction in height) (3) 200 μg 12 day expt control 12·70 (s.d. ±1·85) cm, 19-alcohol, 11·25 (s.d. ±1·94) cm (11% reduction in height) control + *G. fujikuroi*, 30·3 (s.d. ±4·14) cm, 19-alcohol + *G. fujikuroi*, 26·34 (s.d. ±5·46) cm (13% reduction in height)

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