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7-HYDROXY-6,7-SECO-KAURENES AS INHIBITORS OF GIBBERELLIN BIOSYNTHESIS IN *GIBBERELLA FUJIKUROI*

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Key Word Index—*Gibberella fujikuroi*; gibberellic acid biosynthesis; 7-hydroxy-6,7-*seco*-kaurenes; plant growth regulators.

Abstract—Some 7-substituted-6,7-secokaurenes have been shown to be inhibitors of gibberellic acid biosynthesis from [2-^{14}C] mevalonic acid in the fungus *Gibberella fujikuroi*.

INTRODUCTION

The development of inhibitors of gibberellin biosynthesis through the synthesis of mimics of key biosynthetic intermediates has formed a rational approach to the development of novel plant growth regulators [1–3]. The oxidative ring contraction of *ent*-7α-hydroxykaurenoic acid (**1**) to gibberellin A_{12} 7-aldehyde (**2**) [4, 5] is a biosynthetic step which, whilst being mechanistically very unusual, is nevertheless common to all the gibberellins such as **3**. Labelling studies [5] have shown that the ring contraction is accompanied by the abstraction of the equatorial 6β-hydrogen atom. Furthermore 'analogue biosynthesis' has revealed [4] that this step is not completely structure specific and hence it is a step which is open to inhibition by active site directed substrate mimics. In our previous studies we have shown [1] that the 7-hydroxy-B-norkaurenes (**4**, R = CH_2OH and CO_2H) lacking C-6, inhibit this stage. The inhibition was very dependant on the stereochemistry of the hydroxyl group. In this paper we describe our work on a series of 6,7-*seco*-kaurenes derived from the readily available *Gibberella fujikuroi* metabolite, fujenal (**5**). These compounds were selected for study because, whilst they possess a partial formal similarity to *ent*-7α-hydroxykaurenoic acid, they lack an essential component, namely the 6,7-bond, for the ring contraction.

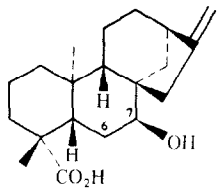
RESULTS AND DISCUSSION

Reduction of fujenal (**5**) with sodium borohydride or lithium aluminium hydride gave, *inter alia*, the alcohol (**6**) [6]. This compound, which possesses the 7-hydroxyl and rings C and D of *ent*-7α-hydroxykaurenoic acid (**1**), proved to be an efficient inhibitor of gibberellic acid biosynthesis from [2-^{14}C] mevalonic acid in the fungus, *Gibberella fujikuroi* (Table 1). Consequently we examined some structure: activity relationships in this series. The proposed mechanism for the oxidative ring contraction envisages the abstraction of the 7-hydroxyl proton by a base. This might be alkylateable by a substrate mimic with a C-7 leaving group or it might condense to form a Schiff's base with a formyl group leading to irreversible inhibition of the biosynthesis. Hence we examined some simple structural variations at C-7.

The alcohol (**6**) readily gave a mono-acetate (**7**) with acetic anhydride in pyridine and a monomethanesulphonate (**8**) on treatment with methanesulphonyl chloride. When this reaction was worked up under acidic conditions, it was accompanied by some isomerization of the Δ^{16}-double bond to form the endocyclic Δ^{15}-isomer (**13**) (δ5.40, 1H, *q*, *J* = 1.5 Hz, H-15; 1.70, 3H, *d*, *J* = 1.5 Hz, H-17). Treatment of the alcohol with triphenylphosphine in carbon tetrachloride gave the chloride (**9**) (δ3.46 and 4.08, *d*, *J* = 11 Hz, H-7) whilst oxidation with the 8 N

Table 1. Inhibition of incorporation of [2-^{14}C]mevalonic acid into gibberellic acid

Compound	Control (10^{-4} dpm/mg)	Control % incorporation	Putative inhibitor (10^{-4} dpm/mg)	Putative inhibitor % incorporation	% inhibition
6	10.01	0.45	0.890	0.04	91.1
7	63.08	7.7	26.89	3.3	57
8	40.42	4.96	36.20	4.0	19
9	25.81	1.16	15.58	0.07	94
10	27.82	1.25	26.04	1.17	6
11	63.08	7.7	24.78	3.04	61
12	63.08	7.7	29.58	3.6	53
13	40.42	4.96	40.34	4.95	0



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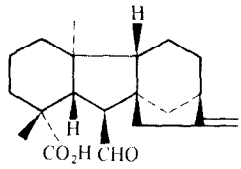

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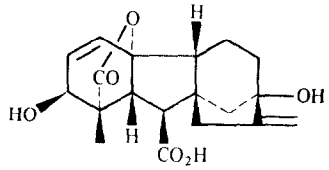

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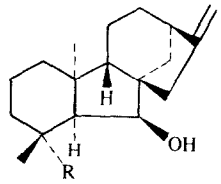

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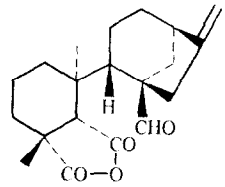

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chromium trioxide reagent gave the known aldehyde (**10**) [6].

Some modifications of C-16 were also carried out. Oxidation with sodium periodate and a catalytic amount of osmium tetraoxide gave the 17-nor-16-ketone (11) (ν_{max} 1750 cm^{-1}). Hydrogenation over 10% Pd-C gave the dihydro-compound (**12**) (δ1.03, 3H, *d*, $J = 7$ Hz, H-17). These compounds possessed spectroscopic properties in accord with their structures. The ^{13}C NMR assignments are given in Table 2.

Table 2. ^{13}C NMR signals of ring B *seco*-kaurenes (determined at 90.55 MHz in $CDCl_3$, ppm from TMS)

Carbon atom	**6**	**7**	**9**	**10**	**11**	**12**	**13**
1	35.82	36.14	36.1	34.15	36.06	35.22	35.00
2	17.75	17.57	17.6	17.63	17.55	17.68	17.65
3	31.80	31.62	31.7	31.50	31.51	31.66	31.71
4	38.75	38.40	38.6	38.44	38.60	38.43	38.46
5	53.75	53.44	53.05	53.68	53.37	54.21	53.72
6	179.58	178.60	178.3	178.20	179.69	180.16	178.93
7	71.45	72.46	55.6	204.6	69.98	72.12	73.45
8	50.79	48.20	50.4	59.27	48.76	51.29	52.99
9	45.21	45.62	46.2	47.27	47.60	45.81	45.05
10	41.80	42.19	42.1	41.44	41.91	41.99	42.81
11	21.32	21.37	21.7	19.91	21.40	22.05	22.47
12	33.12	32.97	32.8	32.08	32.90	25.95	24.72
13	44.52	44.17	45.2	44.30	44.59	40.38	40.42
14	36.34	35.42	38.0	32.74	28.88	37.58	39.00
15	48.04	48.01	48.3	43.00	52.68	47.95	130.79
16	155.27	154.58	154.2	151.55	221.31	35.40	142.13
17	102.91	103.06	103.3	104.75	–	15.20	14.88
18	30.07	30.95	30.6	31.38	30.02	30.97	31.33
19	76.13	75.69	75.7	75.75	76.08	76.07	75.81
20	23.87	23.20	23.5	23.30	23.85	23.15	23.14
Me CO.O		20.75					
MeC O.O		171.31					

Inhibition of gibberellic acid biosynthesis was examined in *Gibberella fujikuroi*. The incorporation of [2-^{14}C] mevalonic acid into gibberellic acid in the presence of the putative inhibitor was compared with the incorporation in parallel control fermentations. The putative inhibitors were initially examined at a concentration of 40 mg/l (*ca* 10^{-4}M) which is approximately the concentration of gibberellic acid produced by our strain of *Gibberella fujikuroi* when grown on shake culture. The results are given in Table 1.

The triol (**14**) [6] was also examined but it proved to be insufficiently soluble for a meaningful result to be obtained. The 6,7-*seco*-alcohol (**6**) is clearly an efficient inhibitor and hence the effect of concentration was examined (Table 3) from which it can be seen that the alcohol is still showing approximately 50% inhibition at 10^{-7}M. In comparison various plant growth retardents containing the triazole moiety are reported to give a 50% reduction in gibberellin production by *Gibberella fujikuroi* at concentrations of 10^{-6}–10^{-9} M [7].

Although the alcohol (**6**) inhibited the incorporation of *ent*-kaurene (0.29% in the presence of the inhibitor, 1.1% in the control), it did not inhibit the incorporation of gibberellin A_{12} 7-aldehyde (**2**) into gibberellic acid (20.0%; 19.1% in control). This suggests that the alcohol (**6**) is blocking the oxidative metabolism of *ent*-kaurene.

The partial inhibition of the biosynthesis by the acetate (**7**) was traced by TLC examination of the neutral extract of the fermentation, to enzymatic hydrolysis of the acetate (**7**) to the alcohol (**6**) by *G. fujikuroi*. Incubation of the acetate (**7**) with the medium alone produced no hydrolysis. On the other hand, the chloro compound (**9**) which also showed significant activity, did not appear to be hydrolysed. The failure of the aldehyde (**10**) to inhibit the biosynthesis probably reflects the importance of the C-7 hydroxyl group in binding to the active site. The inhibition of the incorporation of [2-^{14}C] mevalonate into gibberellic acid by the alcohol (**6**) could not always be reproduced. This was traced, at least in part, to a fungal isomerization of the lactone (**6**) to its isomer (**15**) [8]. This isomerization did not take place in the medium alone over the time scale of the experiment. It could possibly be mediated by either the ring contracting system or by a system related to that which cleaves ring B to form fujenal. (see Scheme 1). In conclusion we have shown that appropriately substituted 6,7-*seco*-kaurenes may be inhibitors of gibberellic acid biosynthesis affecting the oxidative metabolism of the kaurenoid ring B.

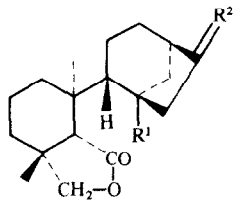
EXPERIMENTAL

General experimental details have been described previously [1]. The *ent*-7,19-dihydroxy-6,7-*seco*-kaur-16-en-6-oic acid 6→19-lactone (**6**) used in this work, was prepared by the reduction of fujenal (**5**) with $NaBH_4$ [6] and had mp 150–152° (lit. [6], 149–151°). The 7-aldehyde (**10**), obtained by oxidation with 8 N chromium trioxide in Me_2CO, had mp 155–157° (lit. [6], 156–157°). The acetate (**7**), prepared with Ac_2O in pyridine, crystallized from petrol as needles, mp 115–116°. (Found: C, 73.6; H, 8.8. $C_{22}H_{32}O_4$ requires C, 73.5; H, 8.6%). IR ν_{max} cm^{-1}: 1750, 1735, 1660, 880 cm^{-1}; ^{1}H NMR ($CDCl_3$) (360 MHz): δ1.13 (3H, *s*, H-20), 1.20 (3H, *s*, H-19), 2.13 (3H, *s*, OAc), 2.72 (1H, *s*, H-5), 3.75 and 4.04 (each 1H, *d*, J = 9 Hz, H-19), 3.84 and 4.45 (each 1H, *d*, J = 11.5 Hz, H-7), 4.73 and 4.82 (each 1H, *m*, H-17). The methanesulphonate (**8**) prepared with methanesulphonyl chloride in pyridine, crystallized from MeOH as needles, mp 114–115.5°. (Found, C. 63.3; H, 7.9. $C_{21}H_{32}O_5S$ requires C, 63.6; H, 8.1%). IR ν_{max} cm^{-1}: 1750, 1660, 1170, 880; ^{1}H NMR ($CDCl_3$) (360 MHz): δ1.14 (3H, *s*, H-20), 1.30 (3H, *s*, H-18), 3.15 (3H, *s*, OMs) 3.75 and 4.60 (each 1H, *d*, J = 11 Hz), 4.02 and 4.13 (each 1H, *d*, J = 9 Hz,) (H-7 and H-19), 4.77 and 4.85 (each 1H, *m*, H-17). On one occasion when the pyridine was removed by an acid wash rather than by treatment with aqueous $CuSO_4$, the 15-ene isomer (**13**) was obtained as needles, mp 120–121.5°. (Found: C, 63.4; H.7.7. $C_{21}H_{32}O_5S$ requires C, 63.6; H, 8.1%). IR ν_{max} cm^{-1}: 1750, 1170; ^{1}H NMR ($CDCl_3$): δ1.12 (3H, *s*, H-20), 1.18 (3H, *s*, H-18), 1.70 (3H, *d*, J = 1.5 Hz, H-17), 3.07 (3H, *s*, OMs), 3.74 and 4.06 (each 1H, *d*, J = 9 Hz), 4.12 and 4.42 (each 1H, *d*, J = 9.5 Hz) (H-7 and H-19), 5.40 (1H, *q*, J = 1.5 Hz, H-15).

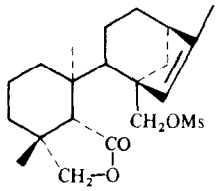
Chlorination of the hydroxy-lactone (**6**). The hydroxy-lactone (500 mg) in CCl_4 (100 ml) was heated with triphenylphosphine (600 mg) and pyridine (5 ml) under reflux for 3 days. The solvent was evapd and the residue chromatographed on silica gel. Elution with petrol–EtOAc (19:1) gave *ent*-7-chloro-19-

Table 3. Effect of concentration of compound **6** on gibberellic acid biosynthesis

Concentration of **6**	% incorporation of ^{14}C MVA into gibberellic acid	% inhibition
Expt. 1 Control	1.026	—
1.23 10^{-4} M	0.072	93
6.13 10^{-5} M	0.380	63
1.29 10^{-5} M	0.500	51
1.23 10^{-6} M	0.680	34
8.18 10^{-8} M	0.730	29
Expt. 2 Control	1.127	—
1.27 10^{-4} M	0.041	96
2.58 10^{-5} M	0.390	65
1.93 10^{-5} M	0.460	59
6.45 10^{-6} M	0.570	49
2.45 10^{-6} M	0.630	44
4.09 10^{-7} M	0.790	30



	R^1	R^2
6	CH_2OH	CH_2
7	CH_2OAc	CH_2
8	CH_2OMs	CH_2
9	CH_2Cl	CH_2
10	CHO	CH_2
11	CH_2OH	O
12	CH_2OH	H,Me



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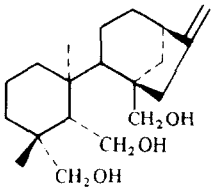

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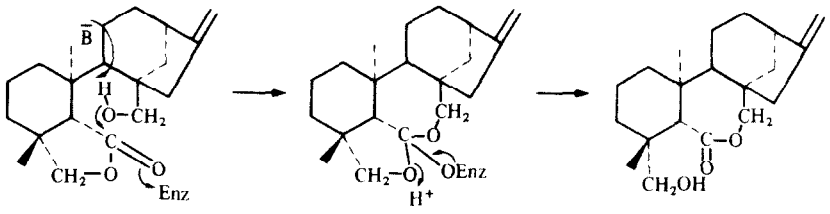

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Scheme 1.

hydroxy-6,7-*seco*-kaur-16-en-6-oic acid 6→19-lactone (**9**) (211 mg) which crystallized from EtOAc–petrol as needles, mp 177–178.5°. (Found: C, 71.2; H, 8.5. $C_{20}H_{29}O_2Cl$ requires C, 71.3; H, 8.7%). IR ν_{max} cm^{-1}: 1765, 1655, 880, 730; ^1H NMR ($CDCL_3$) (360 MHz): δ 1.15 (3H, *s*, H-20), 1.34 (3H, *s*, H-18) 2.21 (1H, *s*, H-5), 3.46 and 4.08 (each 1H, *d*, $J = 11$ Hz, H-7), 3.76 and 4.05 (each 1H, *d*, $J = 9$ Hz, H-19), 4.75 and 4.82 (each 1H, *m*, H-17).

Oxidation of the hydroxy-lactone (**6**) *with* OsO_4 *and* $NaIO_4$. The hydroxy-lactone (1 g) in aq. THF (1:1) (100 ml) was treated with OsO_4 (100 mg). $NaIO_4$ (1.25 g) was added in portions over 30 min. After a further 1 hr, the soln was concentrated and poured into aq. NaCl. The products were recovered in EtOAc and chromatographed on silica gel. Elution with EtOAc–petrol (1:1) gave *ent*-7,19-dihydroxy-16-oxo-17-nor-6,7-*seco*-kauran-6-oic acid 6→19-lactone (**11**) (291 mg) as needles, mp 148–149°. (Found: C, 71.1; H, 8.8% $C_{19}H_{28}O_4$ requires C, 71.2; H, 8.8%). IR ν_{max} cm^{-1}: 3420, 1750, 1735; ^1H NMR ($CDCl_3$) (360 MHz): δ 1.17 (3H, *s*, H-20), 1.34 (3H, *s*, H-18), 3.54 and 4.10 (each 1H, *d*, $J = 11.5$ Hz, H-7) 3.80 and 4.03 (each 1H, *d*, $J = 9$ Hz, H-19).

Catalytic hydrogenation of the hydroxy-lactone (**6**). The hydroxy-lactone (1 g) in EtOAc (20 ml) containing Pd-C (10%, 200 mg) was stirred in an atmosphere of H_2 until the rapid uptake of H_2 ceased (30 min.) The soln was filtered, the solvent evapd and the residue chromatographed on silica gel to afford *ent*-7,19-dihydroxy-6,7-*seco*-kauran-6-oic acid 6→19-lactone (**12**) (671 mg) as a mixture of prisms and needles which both melted at 150–152°. (Found: C, 74.9; H, 9.7. $C_{20}H_{32}O_3$ requires C, 75.0; H, 10.1%). IR ν_{max} cm^{-1}: 3550, 1740; δ^1H NMR ($CDCl_3$) (360 MHz): δ 1.03 (3H, *d*, $J = 7$ Hz, H-17), 1.12 (3H, *s*, H-20), 1.28 (3H, *s*, H-18), 3.24 and 4.12 (each 1H, *d*, $J = 11.5$ Hz), 3.78 and 4.04 (each 1H, *d*, $J = 9$ Hz,) (H-7 and H-19).

Incubation experiments. Gibberella fujikuroi (ACC 917) (40 flasks, 50 ml medium in each) was grown as described previously [1]. The 6,7-*seco*-kaurene (40 mg/l, culture broth) in EtOH (5 ml) and [2-^{14}C]MVA (10 μCi) in EtOH (5 ml) was evenly distributed over 20 flasks. The remaining 20 flasks (control) were treated with [2-^{14}C] MVA (10 μCi) in EtOH (5 ml). The flasks were then shaken for 4–5 days. The mycelium was filtered and the broth was acidified to pH 2 with dil. HCl, extracted with EtOAc and the extract separated into acidic and neutral fractions with aq. $NaHCO_3$. The acid fraction was concentrated to a gum. Gibberellic acid (20 mg) was added to the acid fraction which was then methylated with CH_2N_2 in MeOH. The metabolites were separated by prep. TLC on silica gel in EtOAc–petrol (1:1). Methyl gibberellate was eluted from the plate and crystallized to constant radioactivity. % inhibition is defined as

$$100 - \frac{\text{(\% incorporation in presence of substrate)}}{\text{\% incorporation in control}} 100.$$

The results are given in Tables 1 and 3.

Incubation of ent-[^{14}C]*kaurene. Gibberella fujikuroi* (6 flasks, 100 ml in each flask) was grown for 7 days. *ent*-[17-^{14}C]-Kaurene (10 mg, 4.98, 10^4 dpm/mg) in EtOH was distributed evenly

between the flasks. The alcohol (**6**) (12 mg in EtOH) was distributed over 3 flasks and 3 flasks were retained as a control. The fermentation was grown for a further 4 days. It was then worked up as above. Methyl gibberellate carrier (20 mg) was added to each of the methylated acid fractions and re-isolated by prep. TLC. The incorporations were: control, 2759 dpm = 1.108% incorporation, + substrate 739. dpm = 0.297% incorporation, 73% inhibition.

Incubation with gibberellin A_{12} 7-aldehyde. The [17-^{14}C] aldehyde (3.703×10^6 dpm) was evenly distributed between two groups of 5 flasks of *Gibberella fujikuroi* as above. The alcohol (**6**) (20 mg) was added to one group. The methyl gibberellate, isolated as above, showed the following incorporation: control, 7.063×10^5 dpm, 19.1% incorporation, + substrate, 7.408×10^5 dpm, 20.0% incorporation.

Fungal isomerization of the lactone **6**. The lactone (**6**) (90 mg) was incubated with *Gibberella fujikuroi* (5 flasks) grown as above for 5 days. The broth was harvested and split into acidic and neutral fractions. The neutral fraction was carefully chromatographed on silica gel. Elution with EtOAc afforded the lactone (**15**), (32 mg) identified by its IR and NMR spectra [8]. When the lactone **6** was shaken with the medium alone for a similar period and the broth extracted with EtOAc, no isomerization could be detected by TLC analysis.

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