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# GIBBERELLIN $A_{13}$ 7-ALDEHYDE: A PROPOSED INTERMEDIATE IN THE FUNGAL BIOSYNTHESIS OF GIBBERELLIN $A_3$

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**Key Word Index**—Partial syntheses; gibberellin $A_{13}$ 7-aldehyde and 7-alcohol; gibberellin $A_{13}$ anhydride-7-aldehyde and 7-alcohol; *Gibberella fujikuroi*; mutant B1-41a; strain CMI 58289; AMO-1618; GC/MS; non-incorporation.

**Abstract**—Gibberellin $A_{13}$ 7-aldehyde, previously proposed as an intermediate in the fungal biosynthesis of gibberellin $A_3$, has been prepared from gibberellin $A_{13}$. Neither this aldehyde and its anhydride, nor gibberellin $A_{13}$ 7-alcohol and its anhydride, were converted into gibberellin $A_3$ or other $C_{19}$-gibberellins by intact cultures of *Gibberella fujikuroi*.

## INTRODUCTION

In the biosynthetic conversion of 3-hydroxy-$C_{20}$-gibberellins into 3-hydroxy-$C_{19}$-gibberellins by *Gibberella fujikuroi*, gibberellin $A_{13}$-aldehyde (**20**, Scheme 1) was proposed [1] as a key intermediate. Incubation of *ent*-kaur-16-ene (**1**), labelled *inter alia* with $^{14}C$ at C-20, with *G. fujikuroi* gave $^{14}CO_2$, and not formaldehyde or formic acid, together with [$^{14}C$]gibberellin $A_3$ (**3**) with constant specific activity [2]. In the biosynthesis of gibberellin $A_3$ (**3**), in cultures of *G. fujikuroi*, 3-hydroxylation of gibberellin $A_{12}$ 7-aldehyde (**5**) precedes oxidation of the 7-aldehyde to the 7-oic acid [3, 4]. Incubation of [7-$^3H$, 17-$^{14}C$]gibberellin $A_{12}$ 7-aldehyde (**5**) with a crude cell-free preparation from the mycelium of *G. fujikuroi* gave [1] gibberellin $A_{14}$-aldehyde (**6**) which was further converted into gibberellin $A_3$ (**3**) and a more polar metabolite containing $^3H$ and $^{14}C$. This latter metabolite, on re-incubation with *G. fujikuroi*, also afforded gibberellin $A_3$ (**3**) (12.9% incorporation) and a mixture of gibberellins $A_4$ (**7**) and $A_7$ (**4**) (2.9% incorporation). Attempts to identify the unknown metabolite by isolation of a derivative or by MS were unsuccessful but the metabolite underwent auto-oxidation to gibberellin $A_{13}$ (**13**, Scheme 1), identified as its trimethyl ester. Gibberellin $A_{13}$ (**13**) is not an intermediate in the biosynthesis of $C_{19}$-gibberellins by *G. fujikuroi* [3, 4]. In the light of this evidence, it was tentatively suggested that the unknown metabolite was gibberellin $A_{13}$ 7-aldehyde (**20**).

In planning a partial synthesis of gibberellin $A_{13}$ 7-aldehyde (**20**) a route (Scheme 1) was devised which would also provide gibberellin $A_{13}$ 7-aldehyde anhydride (**19**). The latter compound would be an even more desirable substrate to feed to the fungus as (a) it is considerably less polar than gibberellin $A_{13}$ 7-aldehyde and thus more easily transported into the fungal cells where it could be hydrolysed to gibberellin $A_{13}$ 7-aldehyde (**20**), and (b) it might be an intermediate itself bearing in mind the reported incorporation [5] of gibberellin $A_{13}$-anhydride (**8**) into $C_{19}$-gibberellins. The proposed synthesis (Scheme 1) was also planned to provide gibberellin $A_{13}$ 7-alcohol (**17**) and gibberellin $A_{13}$ 7-alcohol anhydride (**18**) as potential and effective precursors of $C_{19}$-gibberellins by analogy with gibberellin $A_{12}$ 7-alcohol (**9**) [6, 7], and gibberellin $A_{14}$ 7-alcohol (**10**) [8]. The achievement of this partial synthesis by the Bristol group has been briefly reported [9].

## RESULTS AND DISCUSSION

Gibberellin $A_{13}$ (**13**) was treated with acetic anhydride and pyridine to give the known acetyl gibberellin $A_{13}$-anhydride (**14**) [10]. Methylation of this compound was achieved most efficiently with diazomethane in *t*-butanol. When methanol or ethanol was used appreciable alcoholysis of the anhydride was observed. Hydrolysis of the acetyl gibberellin $A_{13}$-anhydride methyl ester (**15**) with 2M NaOH in DMSO gave gibberellin $A_{13}$ 7-methyl ester (**16**). The known facility of LAH to reduce esters faster than carboxylic acids [11] was employed to reduce the 7-ester functionality selectively. More consistent results were obtained when the 19- and 20-oic acids were first converted to their salts with potassium

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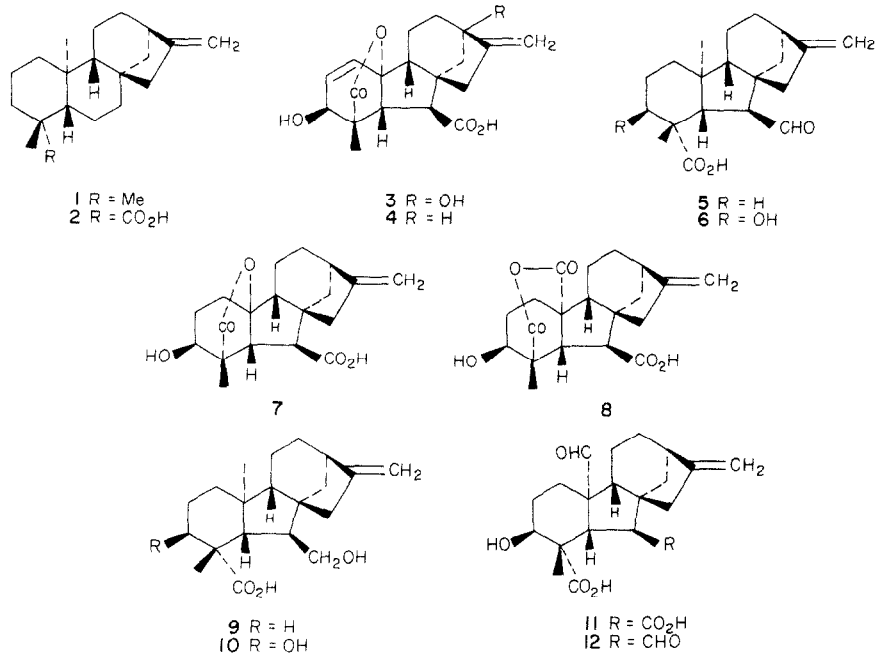
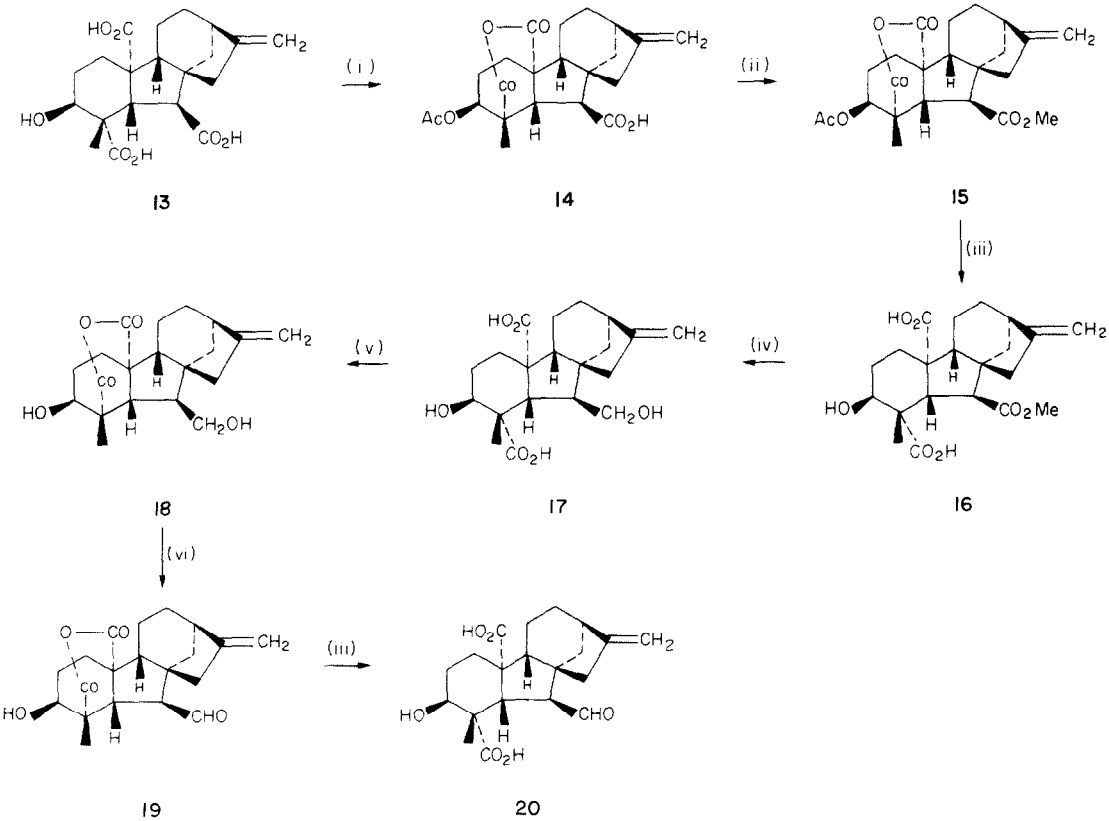


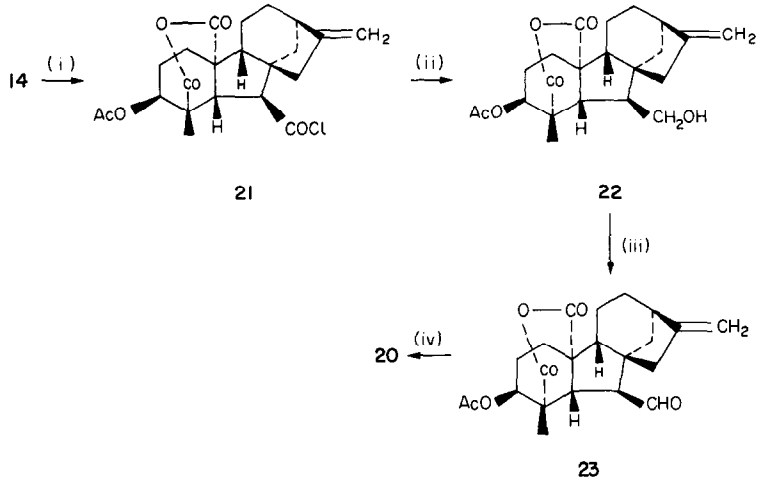

Scheme 1. Reagents: (i) $Ac_2O$–$C_5H_5N$; (ii) $CH_2N_2$–*t*-BuOH; (iii) 2M, NaOH–DMSO (iv) KH–THF–$LiAlH_4$; (v) DCC–$C_5H_5N$; (vi) pyridinium chlorochromate–$CH_2Cl_2$.

hydride. The LAH reduction proceeded smoothly to give the required gibberellin $A_{13}$ 7-alcohol (**17**). Treatment of the alcohol with dicyclohexylcarbodiimide in pyridine gave the corresponding anhydride (**18**) which was selectively oxidized to gibberellin $A_{13}$-anhydride aldehyde (**19**), without protection of the 3-hydroxyl, using buffered pyridinium chlorochromate in methylene dichloride [12]. Hydrolysis of gibberellin $A_{13}$-anhydride-7-aldehyde (**19**) to gibberellin $A_{13}$ 7-aldehyde (**20**) was achieved with 2 M NaOH in DMSO at room temperature or by autoclaving an aqueous suspension at 120° for 15 min.

An independent preparation of gibberellin $A_{13}$ 7-aldehyde (**20**), shown in Scheme 2, was subsequently achieved by the Sussex group. The reduction of the 7-carboxyl group of gibberellin $A_3$ (**3**) has been accomplished by reduction of a dimeric anhydride with disodium tetracarbonylferrate or sodium borohydride [13, 14]. In the case of 3-acetoxy-gibberellin $A_{13}$ 19,20-anhydride (**14**), the 7-acid chloride (**21**) was conveniently prepared with oxalyl chloride in benzene containing a trace of dimethylformamide [15, 16]. Reduction of the acid chloride with sodium borohydride in the presence of the cadmium-dimethylformamide solvate in dimethylformamide [17], gave the 3-acetoxy-7-alcohol (**22**) and not the anticipated 7-aldehyde. Reduction with sodium borohydride alone gave a mixture whilst the use of disodium tetracarbonylferrate or the Rosenmund reduction, were not successful. The acetoxy-alcohol (**22**) was then oxidized with pyridinium dichromate [18] in methylene dichloride to afford the C-7 aldehyde (**23**) ($\delta$ 9.75, $J = 7$ Hz). The anhydride and acetoxyl functions were then hydrolysed with methanolic potassium carbonate to give the required aldehyde (**20**). Methylation with diazomethane afforded the gummy dimethyl ester, characterized by $^1$H NMR; interestingly, in contrast to the $C_{19}$-gibberellins and to gibberellin $A_{13}$ 7-aldehyde 19,20-anhydride (**19**), the lower field signal for the 5- and 6-protons at $\delta$ 3.82 (*dd*, 7 and 13 Hz) must be assigned to the 6-proton. Because of the elapse of 4 years between the earlier [1] and present studies, a comparison of the unknown metabolite from [7-$^3$H, 17-$^{14}$C] gibberellin $A_{12}$ 7-aldehyde (**5**) and the synthetic gibberellin $A_{13}$ 7-aldehyde (**20**) was not possible.

The role of the synthetic gibberellin $A_{13}$ 7-aldehyde (**20**) in the biosynthesis of gibberellin $A_3$ (**3**) by *G. fujikuroi* was independently investigated by both groups. At Bristol the mutant B1-41a was used. This mutant is blocked [19] for gibberellin biosynthesis at the step before *ent*-kaurenoic acid (**2**); metabolites after *ent*-kaurenoic acid (**2**) do not accumulate but this acid (**2**) and later intermediates are converted [3] into gibberellin $A_3$ (**3**). Gibberellin $A_{13}$ 7-alcohol anhydride (**18**) and gibberellin $A_{13}$ 7-aldehyde anhydride (**19**) were fed to resuspension cultures of *G. fujikuroi*, mutant B1-41a, under the normal conditions and metabolites were analysed by GC/MS as previously described [3]. After 5 days at pH 4.5 gibberellin $A_{13}$ 7-aldehyde anhydride (**19**) gave gibberellin $A_{13}$-aldehyde (**20**) (80%) and gibberellin $A_{13}$ (**18**) (20%). Under these conditions gibberellin $A_{13}$ 7-alcohol anhydride (**18**) with mutant B1-41a yielded gibberellin $A_{13}$ 7-alcohol (**17**) (95%) and traces of gibberellin $A_{13}$-aldehyde (**20**) and gibberellin $A_{13}$ (**13**). In neither of these feeds were any $C_{19}$-gibberellins detected. Thus the anhydrides (**18**) and (**19**), gibberellin $A_{13}$ 7-aldehyde (**20**) and gibberellin $A_{13}$ 7-alcohol (**17**) do not act as efficient precursors to $C_{19}$-gibberellins.

At Sussex, a wild type strain CMI 58289 of *G. fujikuroi* was used in conjunction with the synthetic growth retardant, AMO-1618. This compound inhibits [20] the formation of *ent*-kaur-16-ene (**1**) in *G. fujikuroi* and hence prevents the accumulation of post-*ent*-kaurene metabolites. It does not, however, affect the post-kaurene stages in the biosynthesis, and thus in its presence, exogenous *ent*-kaur-16-ene and later intermediates, are converted into gibberellin $A_3$ (**3**). Gibberellin $A_4$ (**7**) is an efficient precursor of gibberellin $A_3$ (**3**) [3, 4] and it would be a close succeeding intermediate to the supposed gibberellin $A_{13}$




Scheme 2. Reagents: (i) $(COCl)_2$–$C_6H_6$–$HCONMe_2$; (ii) $NaBH_4$–$CdCl_2$–$HCONMe_2$; (iii) pyridinium dichromate–$CH_2Cl_2$; (iv) $K_2CO_3$–MeOH–$H_2O$.

7-aldehyde (**20**). Gibberellin $A_4$ (**7**) and gibberellin $A_{13}$ 7-aldehyde (**20**) were incubated at the same concentrations in parallel inhibited fermentations of *G. fujikuroi*. Parallel control fermentations were also fed with [2-$^{14}$C]MVA in the presence and absence of AMO-1618 to ensure both the effectiveness of the inhibitor and that the strain of *G. fujikuroi* was producing gibberellin $A_3$ (**3**). Whereas the gibberellin $A_4$ (**7**) gave gibberellin $A_3$ (**3**) (16% conversion), no gibberellin $A_3$ was detected from the gibberellin $A_{13}$ 7-aldehyde. Instead it was efficiently converted (72%) into gibberellin $A_{13}$ (**13**). Hence with strain CMI 58289, as well as the mutant B1-41a, gibberellin $A_{13}$ 7-aldehyde (**20**) was not an intermediate in gibberellin $A_3$ biosynthesis.

From these results it seems very unlikely that the metabolite, previously detected from [7-$^3$H, 17-$^{14}$C]gibberellin $A_{12}$ 7-aldehyde (**5**) and converted into gibberellin $A_3$ (**3**) in 12.9% yield, is gibberellin $A_{13}$ 7-aldehyde (**20**). Moreover, the results with the mutant B1-41a do not support the idea that gibberellin $A_{13}$ 19,20-anhydride (**8**) is an intermediate [5]. It is possible that the absence of appreciable metabolism, apart from anhydride hydrolysis of **18** and **19**, is a result of the inability of the substrates (or hydrolysis products) to reach the site of the enzyme catalysing the $C_{20}$- to $C_{19}$-gibberellin conversion. However, in the light of the recent discovery [21] that gibberellin $A_{36}$ (**11**) is metabolized to gibberellin $A_4$ (**7**) in cell-free preparations from the endosperm of seed of *Cucurbita maxima*, it is possible that the unknown metabolite [1] from gibberellin $A_{12}$ 7-aldehyde (**5**) is gibberellin $A_{36}$ 7-aldehyde (**12**).

Although the immediate precursor of the $C_{19}$-gibberellins in *G. fujikuroi* remains unidentified, its structure must meet several criteria. The mevalonoid hydrogen atoms at C-1, -5 and -9 are retained [22, 23] in gibberellin $A_4$ (**7**) which is a precursor of gibberellin $A_3$ (**3**), a result which precludes the intermediacy of compounds unsaturated at these carbons. The γ-lactone ring of gibberellin $A_3$ (**3**) lies on the same face of the molecule as the departing C-20 and both oxygens of the lactone are derived from the 19-oic acid of the $C_{20}$-precursors [24]. Thus C-10 must become an electrophilic centre in the course of the loss of C-20. A Baeyer–Villiger type of oxidation of a 10-aldehyde has been often suggested [25, 26]. However, as illustrated in Scheme 3 an unusual oxidation of the intermediate formate ester is required to generate both carbon dioxide and an electrophilic C-10; since hydrolysis of the formate, would lead to a lactone ring in which only one of the oxygen atoms was derived from the 19-oic acid of the $C_{20}$-precursor.

## EXPERIMENTAL

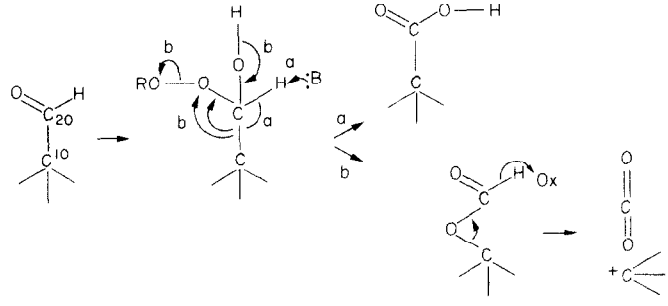
General experimental details [2, 19] and incubation procedures for *Gibberella fujikuroi*, strain CMI 58289 [4] and mutant B1-41a [3] have been described previously. For GC/MS see ref. [15].

ent-3-*Acetoxygibberell*-16-*ene*-7,19,20-*trioic acid* 19,20-*anhydride* (**14**). Gibberellin $A_{13}$ (**13**) (1.01 g) in dry pyridine (24 ml) was treated with $Ac_2O$ (6 ml) for 20 hr. Water (10 ml) was added cautiously in portions with cooling, followed by 5 M HCl (70 ml). The product was recovered in EtOAc which was washed with dil. HCl, then $H_2O$ and dried over $Na_2SO_4$. Evapn of the EtOAc afforded a crystalline residue (0.92 g) which was recrystallized from EtOAc–hexane as needles, mp 271–273° (lit. [10] 264–267°).

ent-3α-*Acetoxygibberell*-16-*ene*-7, 19, 20-*trioic acid* 19, 20-*anhydride* 7-*methyl ester* (**15**). The above anhydride (250 mg) in *t*-BuOH (50 ml) was treated with excess ethereal $CH_2N_2$. Evapn of the soln gave a foam (260 mg) which gave a single spot at $R_f$ 0.7 by TLC on Si gel HF with EtOAc–PE–AcOH (40 : 10 : 1); $^1$H NMR ($CDCl_3$): δ 1.17 (*s*, 18-$H_3$), 2.12 (*s*, OCOMe), 2.46 and 2.76 (each *d*, *J* = 11 Hz, 5- and 6-H), 3.71 (*s*, $CO_2$Me), 4.95 (*br*, 3-H), 4.95 and 5.10 (both *br*, 17-$H_2$); IR $\nu_{max}^{CCl_4}$ cm$^{-1}$: 1805, 1767, 1752, 1737, 1655 and 885; MS *m/z*: 416 [M]$^+$ (2%), 370 (33), 356 (52), 328 (100), 296 (62), 284 (87), 282 (30), 268 (73), 224 (88), 223 (44), and 43 (82).

ent-3α-*Hydroxygibberell*-16-*ene*-7,19,20-*trioic acid* 7-*methyl ester* (**16**). The preceding acetate (**15**) (670 mg) in DMSO (16 ml) was treated with 2 M NaOH (4 ml) at room temp. for 24 hr. The reaction mixture was diluted with $H_2O$ (100 ml) and adjusted to pH 2.5 with 2 M HCl. Extraction with EtOAc and evapn gave a gum (645 mg). Purification by PLC on Si gel HF with EtOAc–PE–AcOH (70 : 30 : 1) and recovery from the band at $R_f$ 0.5 gave a gum (395 mg); $^1$H NMR ($d_6$-$Me_2CO$): δ 1.21 (*s*, 18-$H_3$), 2.72 (*d*, *J* = 13 Hz, 5-H), 3.68 (*s*, $CO_2$Me), 3.94 (*m*, 3-H), 4.04 (*d*, *J* = 13 Hz, 6-H), 4.84 (*br*, 17-$H_2$); IR $\nu_{max}$ cm$^{-1}$: 3510, 3000 (*br*), 1735, 1710, 1660; MS *m/z* (probe): 374 [M − 18]$^+$ (7), 342 (81), 328 (42), 314 (100), 296 (44), 286 (48), 268 (59), 224 (22).

ent-3α, 7-*Dihydroxygibberell*-16-*ene*-19, 20-*dioic acid* (**17**). KH (2 ml, 20% suspension in oil) was added slowly to $GA_{13}$ 7-methyl ester (**16**) in dry THF (30 ml) at 0° with stirring. LAH (400 mg) was added in portions then the mixture allowed to reach room temp. After 2 hr, wet THF was added cautiously, followed by aq. KH tartrate. The mixture was concd then extracted with PE (discarded) and then with EtOAc which was evaporated to give an oil. PLC



Scheme 3.

on Si gel HF with EtOAc–PE–AcOH (40 : 10 : 1) gave a band at $R_f$ 0.5 which was extracted to yield the required 7-alcohol, crystallizing from $Me_2CO$–PE with mp 225–227°. (Found: C, 65.9; H, 7.4. $C_{20}H_{28}O_6$ requires C, 65.9; H, 7.7%.) $^1$H NMR ($C_5D_5N$): δ 2.2 (*s*, 18-$H_3$), 4.00–4.50 [*m* (*br*), 7-$H_2$], 4.64 [*t* (*br*), 3-H], 4.98 and 5.06 (each *br*, 17-$H_2$); IR $\nu_{max}$ cm$^{-1}$: 3420, 3350, 3050 (*br*), 1700, 1690 and 1655; MS *m/z* (probe): 364 [M]$^+$ (0), 346 (8) 328 (100), 300 (29), 282 (45), 256 (42), 239 (48), and 184 (53); MS *m/z* (dimethyl ester bis TMSi ether, GC/MS): 536 [M]$^+$ (2), 521 (20), 386 (51), 296 (34), 237 (40), 184 (52), 129 (100), 75 (24), and 73 (70).

ent-3α,7-*Dihydroxygibberell*-16-*ene*-19,20-*dioic acid* 19, 20-*anhydride* (**18**). The preceding alcohol (**17**) (124 mg), in dry pyridine (3 ml) was treated with dicyclohexylcarbodiimide (110 mg). After 24 hr, the filtered reaction mixture was evaporated to give a gum which was purified by PLC on Si gel HF with $Me_2CO$–PE (1 : 1). Recovery from the band at $R_f$ 0.35 gave the required anhydride (**18**) (91 mg) which was crystallized from $Me_2CO$–PE with mp 108–110°. (Found: M$^+$ 346.174. $C_{20}H_{26}O_5$ requires M$^+$ 346.178.) $^1$H NMR ($CDCl_3$): δ 1.44 (*s*, 18-$H_3$), 3.86 [(*t* (*br*) 3-H], 3.70–4.40 (*m*, 7-$H_2$) and 4.92 [*s* (*br*), 17-$H_2$]; IR $\nu_{max}$ cm$^{-1}$: 3620, 3540, 3250 (*br*), 1783, 1750, 1740 and 1655; MS *m/z* (probe): 346 [M]$^+$ (22), 344 (9), 328 (42), 318 (11), 300 (82), 282 (38), 274 (39), 256 (83), 225 (53), and 184 (100); MS *m/z* (bis TMSi ether, GC/MS): 490 [M]$^+$ (6), 372 (33), 326 (17), 282 (36), 269 (24), 238 (34), 184 (80), 129 (82), 103 (21), 75 (56), and 73 (100).

ent-3α-*Hydroxy*-7-*oxogibberell*-16-*ene*-19,20-*dioic acid* 19, 20-*anhydride* (**19**). The preceding alcohol (**18**) (72 mg) in $CH_2Cl_2$ (15 ml) was treated with powdered NaOAc (15 mg) and pyridinium chlorochromate (43 mg). After stirring at room temp. for 1.25 hr, $Me_2O$ was added and the mixture was filtered through a bed of Si gel. Evapn of the filtrate gave a solid (58 mg) which was purified by PLC on Si gel HF with $Me_2CO$–PE (1 : 1). Recovery from the band at $R_f$ 0.5 gave the required aldehyde (**19**) as a gum (33 mg). (Found: M$^+$, 344.163. $C_{20}H_{24}O_5$ requires M$^+$, 344.162.) $^1$H NMR ($CDCl_3$): δ 1.25 (*s*, 18-$H_3$), 2.52 (*dd*, *J* = 4 and 11 Hz, 6-H), 2.83 (*d*, *J* = 11 Hz, 5-H), 3.94 [*t* (*br*), 3-H], 4.98 [*s* (*br*), 17-$H_2$] and 9.64 (*d*, *J* = 4 Hz, 7-H); MS *m/z* (probe): 344 [M]$^+$ (10), 328 (18), 316 (37), 298 (100), 270 (40), 269 (25), 225 (38), 143 (28), 91 (33), 56 (42), and 43 (42); (TMSi ether, GC/MS): 416 [M]$^+$ (5), 370 (14), 269 (12), 254 (15), 225 (24), 157 (11), 129 (100), 75 (23), and 75 (85).

ent-3α-*Acetoxy*-7-*hydroxygibberell*-16-*ene*-19,20-*dioic acid* 19,20-*anhydride* (**22**). The acetoxy-anhydride (**14**) (290 mg) in dry $C_6H_6$ (10 ml), containing one drop of dry $HCONMe_2$, was treated with $(COCl)_2$ (0.25 ml) with rigorous exclusion of moisture. The suspension was shaken and left overnight at room temp. by which time the acid has dissolved. The solvent was removed *in vacuo* and the residue was immediately dissolved in dry $HCONMe_2$ (4 ml). This soln was added to an ice-cold mixture of $NaBH_4$ (28 mg), $CdCl_2$–$HCONMe_2$ solvate (131 mg) in dry $HCONMe_2$ and stirred for 20 min. The soln was acidified with dil. HCl and the product was extracted with EtOAc. The extract was washed with water, dil. HCl, and aq. $NaHCO_3$. The dried extract was evaporated and the crystalline residue (256 mg) was chromatographed on Si gel to afford the required alcohol (**22**) which was crystallized from EtOAc–hexane as needles (52 mg) mp 267–269°. (Found: C, 68.8; H, 6.9%; M$^+$ 388. $C_{22}H_{28}O_6$ requires C, 68.0; H, 7.3%, M$^+$ 388.)

ent-3α-*Hydroxy*-7-*oxogibberell*-16-*ene*-19,20-*dioic acid* (**20**). (a) The preceding alcohol (22) (351 mg) was treated with pyridium dichromate (174 mg) in dry $CH_2Cl_2$ (8 ml) and left overnight. $Et_2O$ (10 ml) was added and the soln was filtered through a short column of $MgSO_4$. The residue, obtained by evapn of the filtrate, was dissolved in MeOH (15 ml) and heated for 1 hr under reflux under $N_2$ with $K_2CO_3$ (800 mg) and $H_2O$ (2 ml). The soln was concentrated *in vacuo*, diluted with $H_2O$ and extracted with EtOAc. The aq. phase was acidified with 11 M HCl (2 ml) and the product was recovered in EtOAc. The residue (179 mg) was homogeneous by TLC on Si gel with EtOAc–$CHCl_3$–AcOH (4 : 5 : 1) and had $^1$H NMR: δ 1.26 (*s*, 18-$H_3$), 2.70 (*d*, *J* = 13 Hz, 5-H), 3.70 (*m*, 6-H and OH), 5.00 (*br*, 17-$H_2$) and 9.75 (*d*, *J* = 7 Hz, 7-H). The di-Me ester, prepared with $CH_2N_2$, had $^1$H NMR: δ 1.26 (*s*, 18-$H_3$), 2.64 (*d*, *J* = 13 Hz, 5-H), 3.48 and 3.52 (both *s*, 2 × $CO_2\underline{Me}$), 3.82 (*dd*, *J* = 7 and 13 Hz, 6-H), 3.95 [*s* (*br*), 3-H], 4.84 and 4.94 [both *s* (*br*), 17-$H_2$] and 9.75 (*d*, *J* = 7 Hz, 7-H).

(b) The anhydride (**19**) (2 mg) in DMSO (450 μl) was treated with 2 M NaOH (150 μl) as for the anhydride (**15**). The required aldehyde (**20**) was a gum. (Found: M$^+$ 362.173. $C_{20}H_{26}O_6$ requires M$^+$ 362.173.) MS *m/z* (probe): 362 [M]$^+$ (1), 344 (9), 328 (24), 316 (36), 314 (29), 298 (100), 270 (21), 254 (26), and 225 (43); MS *m/z* (di-Me ester TMSi ether, GC/MS): 462 [M]$^+$ (1.6), 447 (6), 402 (12), 370 (16), 319 (12), 312 (14), 253 (15), 160 (13), 129 (100), 75 (12), and 73 (49).

Alternatively, the anhydride (**19**) (1 mg) was dissolved in $Me_2CO$ (100 μl) and added to $H_2O$ (5 μl). The suspension was then autoclaved at 120° for 15 min. The mixture was cooled, acidified to pH 2.5 and extracted with EtOAc. The sole product was identified as the aldehyde (**20**) by TLC and GC/MS of its di-Me ester TMSi ether.

*Metabolism of the anhydrides* (**18**) *and* (**19**). The substrates (2 mg), in the minimum volume of $Me_2CO$, were added to hot autoclaved $H_2O$ (5 ml) in conical flasks (100 ml). When the flasks were cool, a mycelial suspension (20 ml) of *G. fujikuroi*, mutant B1-41a, prepared as previously described [17], was added to each flask. Incubation for 5 days at the natural pH of 4.5, then work-up and GC/MS analysis were performed as previously described [3, 19].

The acidic fraction from gibberellin $A_{13}$ 7-aldehyde 19, 20-anhydride (**19**) contained unchanged substrate (**19**) and gibberellin $A_{13}$ 7-aldehyde (**20**), each identified from the MS of their di-Me TMSi ethers and respectively accounting for 80% and 20% of the TIC of the GC/MS scans.

The acidic fraction from gibberellin $A_{13}$ 7-alcohol 19, 20-anhydride (**18**) contained gibberellin $A_{13}$ 7-alcohol (**17**) (80% of the TIC) and traces of gibberellin $A_{13}$ (**13**) and gibberellin $A_{13}$ 7-aldehyde (**20**).

*The metabolism of* ent-3α-*hydroxy*-7-*oxogibberell*-16-*ene*-19, 20-*dioic acid*. 180 flasks of sterile medium [2] (50 ml) were inoculated with *G. fujikuroi*, strain CMI 58289. AMO-1618 (35 mg) in EtOH (17 ml) was evenly distributed between 170 of these flasks. After 12 hr growth 75 of the inhibited cultures were treated with gibberellin $A_4$ (150 mg) in EtOH (19 ml), and Tween 80 (5 drops), 75 flasks were treated with gibberellin $A_{13}$ 7-aldehyde (150 mg) in EtOH (19 ml) and Tween 80 (5 drops), 10 flasks were treated with [2-$^{14}$C]MVA ($6.6 \times 10^6$ cpm) in EtOH (1 ml) whilst the remaining cultures containing the inhibitor were retained as controls. The 10 flasks without AMO-1618 were also treated with [2-$^{14}$C]MVA ($6.6 \times 10^6$ dpm) in EtOH (1 ml). The fermentations were continued for a further 5 days and then harvested. The mycelium of each group was filtered and washed with EtOAc. The broth from each group was acidified to pH 2 with dil. HCl and extracted with EtOAc. The extracts were separated into acidic and neutral fractions with aq. $NaHCO_3$ and examined as follows: (a) MVA cul-

tures: The acid fractions were separated by TLC on Si gel in $CHCl_3$–EtOAc–AcOH (5 : 4 : 1). The bands which co-chromatographed with gibberellin $A_3$ were eluted with EtOAc, diluted with gibberellin $A_3$ (10 mg), methylated with $CH_2N_2$ and crystallized to constant radioactivity. The [$^{14}$C]gibberellin $A_3$ (Me ester) from the normal culture had $8.7 \times 10^3$ dpm/mg (1.32% incorporation) whilst the gibberellin $A_3$ (Me ester) from the cultures treated with AMO-1618 had $1.1 \times 10^2$ dpm/mg (0.016% incorporation).

(b) Gibberellin $A_4$ cultures. The acidic fraction was methylated and chromatographed on Si gel. Elution with EtOAc–PE (1 : 4) gave gibberellin $A_4$ Me ester (102 mg) identified by its IR NMR spectra. Elution with EtOAc–PE (3 : 7) gave methyl gibberellate (24 mg) identified by its IR and NMR spectra.

(c) Gibberellin $A_{13}$ 7-aldehyde cultures. The acidic fraction was chromatographed on Si gel. Elution with EtOAc–PE (3 : 7) gave gibberellin $A_{13}$ 7-aldehyde (31 mg) which was methylated with $CH_2N_2$ and identified by comparison with the Me ester described above (IR and NMR). Further elution with EtOAc–PE (7:15) gave gibberellin $A_{13}$ (107 mg) which was methylated with $CH_2N_2$ and identified by comparison (IR and NMR) with an authentic sample. No gibberellic acid was detected either by TLC or during the chromatography.

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