Fungal Products. Part XII.[1] Gibberellin A_{14}-aldehyde, an Intermediate in Gibberellin Biosynthesis in *Gibberella fujikuroi*

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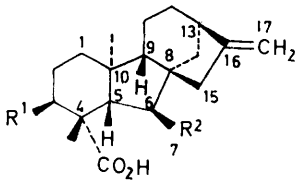
Gibberellin A_{14}-aldehyde (4) has been synthesised from 3β,7β-dihydroxykaurenolide (10). In cultures of *Gibberella fujikuroi*, mutant Bl-41a, it is shown to be formed from gibberellin A_{12}-aldehyde (1) and to be converted into fungal 3-hydroxylated gibberellins including gibberellin A_3 (8). Gibberellin A_{14} (6) and gibberellin A_{14}-alcohol (7) are also metabolised to 3-hydroxygibberellins, the former at a much lower rate. The biosynthetic pathway to gibberellins in the fungus is discussed in the light of these results.

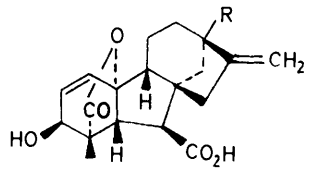
GIBBERELLIN A_{12}-ALDEHYDE (1) [2] and gibberellin A_{12} (2) [3] are normal metabolites of wild-type strains of *Gibberella fujikuroi* and are converted [4] by strain ACC 917 into gibberellin A_3 (8). However, the higher incorporation of the aldehyde, as compared with the diacid, led Cross *et al.*[4] to conclude that gibberellin A_{12}-aldehyde (1), but not gibberellin A_{12} (2), is on the direct biosynthetic

[1] Part XI, J. R. Bearder, J. MacMillan, C. M. Wels, M. B. Chaffey, and B. O. Phinney, *Phytochemistry*, 1974, in the press.
[2] J. R. Hanson, J. Hawker, and A. F. White, *J.C.S. Perkin I*, 1972, 189.
[3] B. E. Cross and K. Norton, *J. Chem. Soc.*, 1968, 1570.
[4] B. E. Cross, K. Norton, and J. C. Stewart, *J. Chem. Soc.* (*C*), 1968, 1054.

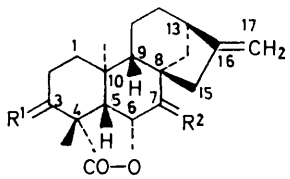
pathway to gibberellin A_3 (8). This view was supported by Bearder *et al.*[5] who, using the slow growing strain REC-193A, found that the aldehyde (1) was rapidly converted into gibberellin A_{14} (6) under conditions of culture where gibberellin A_{12} (2) was not metabolised. Both results indicated that gibberellin A_{14}-aldehyde (4) was the next intermediate after gibberellin A_{12}-aldehyde (1). This paper describes the partial synthesis of gibberellin A_{14}-aldehyde (4) and establishes its intermediacy in the biosynthetic pathway to gibberellin A_3.



(1) R^1 = H, R^2 = CHO
(2) R^1 = H, R^2 = CO_2H
(3) R^1 = H, R^2 = CH_2·OH
(4) R^1 = OH, R^2 = CHO
(5) R^1 = OTHP, R^2 = CHO
(6) R^1 = OH, R^2 = CO_2H
(7) R^1 = OH, R^2 = CH_2·OH



(8) R = OH
(9) R = H



(10) R^1 = R^2 = H, β-OH
(11) R^1 = H_2, R^2 = H, β-OH
(12) R^1 = H, R^2 = H, α-OTs
(13) R^1 = R^2 = H, β-OTHP
(14) R^1 = H, β-OH, R^2 = H, β-OTHP
(15) R^1 = H, β-OTHP, R^2 = H, β-OH

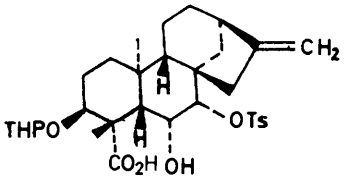
Gibberellin A_{14}-aldehyde (4) was prepared from 3β,7β-dihydroxykaurenolide (*ent*-3α,6β,7α-trihydroxykaur-16-en-19-oic acid 19,6-lactone) (10)[6,7] by following the method of Hanson and Galt[8] for the preparation of gibberellin A_{12}-aldehyde (1) from 7β-hydroxykaurenolide (11) *via* the 7α-*p*-tolylsulphonyloxy-compound (12). In the present case prior protection of the 3β-hydroxy-group was necessary. Sufficient selectivity of reaction of the 3β- and 7β-hydroxy-groups was achieved by treating the diol (10) with an equimolar amount of dihydropyran in the presence of toluene-*p*-sulphonic acid. The mixture of products was separated by t.l.c. into the starting diol (10) (50%), two diastereoisomeric pairs of the bistetrahydropyranyl (THP) ether (13) (10%), each diastereoisomer of the 7-mono-THP ether (14) (total yield 15%), and the required 3-mono-THP ether (15) as an inseparable pair of diastereoisomers (25%). This last was conveniently crystallised directly from the product mixture in 17% yield and the mixture of the mother liquors was hydrolysed by acid to give the starting diol (10) which was recycled. The 3-THP ether (15) was distinguished from the 7-mono-THP ether (14) by the high intensity of the *m/e* 129 ion in the mass spectrum of the trimethylsilyl (TMS) ether of the latter.[9]

(16) R^1 = H, β-OTHP, R^2 = O
(17) R^1 = H, β-OTHP, R^2 = H, α-OH
(18) R^1 = H, β-OH, R^2 = H, α-OTs
(19) R^1 = H, β-OTHP, R^2 = H, α-OTS
(20) R^1 = H, α-OH, R^2 = H, α-OH
(21) R^1 = R^2 = O

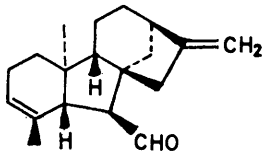
Oxidation of the 3-THP ether (15) with chromium trioxide–pyridine complex to the 7-ketone (16) and reduction of the latter compound with sodium borohydride gave the 7α-alcohol (17) in 90% overall yield. Treatment of the 7α-alcohol (17) with toluene-*p*-sulphonyl chloride in pyridine gave a mixture of the toluene-*p*-sulphonates (18) and (19). Ring contraction of the tosylate (19) with potassium hydroxide in aqueous t-butyl alcohol gave gibberellin A_{14}-aldehyde 3-THP ether (5). In several small-scale (50 mg) reactions the yield of the 3-THP ether (5) was 70% but, on a larger scale (1 g), this yield was only 20% and the main product was the hydroxy-acid (22). Acidic hydrolysis of the 3-THP ether (5) gave gibberellin A_{14}-aldehyde (4), which was also obtained in 55% yield by ring contraction of the toluene-*p*-sulphonate (18). Gibberellin A_{14}-aldehyde (4) and its 3-THP ether (5) were also obtained by ring contraction of the hydroxy-acid (22) with boiling collidine.

[5] J. R. Bearder, J. MacMillan, and B. O. Phinney, *Phytochemistry*, 1973, **12**, 713.
[6] J. H. Bateson and B. E. Cross, *J.C.S. Perkin I*, 1972, 1117.
[7] P. Hedden, J. MacMillan, and M. J. Grinsted, *J.C.S. Perkin I*, 1973, 2773.
[8] R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.*, 1965, 1565.
[9] R. Binks, J. MacMillan, and R. J. Pryce, *Phytochemistry*, 1969, **8**, 271.

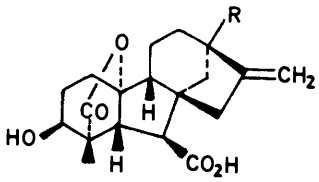
The n.m.r. spectrum of gibberellin A_{14}-aldehyde (4) was similar to that of gibberellin A_{12}-aldehyde (1) except for the 3-proton signal at τ 5·84 ($W_{\frac{1}{2}}$ 7 Hz) and the deshielding (0·56 p.p.m.) of the 5-proton (τ 7·58) by



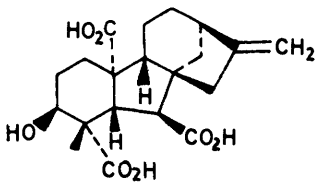
(22)



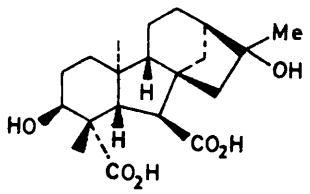
(23)



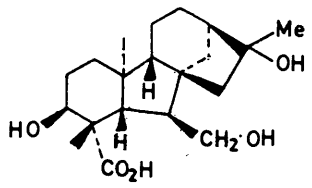
(24) R=OH
(25) R=H



(26)



(27)



(28)

the 3β-hydroxy-group. Gibberellin A_{14}-aldehyde was slowly oxidised in air and was reduced by sodium borohydride to the alcohol (7). On treatment with tritiated water and sodium methoxide in tetrahydrofuran, it gave a mixture of [6-^{3}H]gibberellin A_{14}-aldehyde and [6-^{3}H]-gibberellin A_{14}-alcohol (7), reduction presumably occurring by hydride transfer from the methoxide ion (*cf.* ref. 4).

The bistoluene-*p*-sulphonate of the 3α,7α-diol (20), on treatment with potassium hydroxide in aqueous t-butyl alcohol gave the *ent*-19-norgibberellane (23), characterised by its spectroscopic properties. The n.m.r. spectrum showed the presence of an aldehyde function (τ 0·24; *J* 4 Hz), an exocyclic methylene group (τ 5·05 and 5·19), a vinylic proton (τ 4·71), and a vinylic methyl group (τ 8·24).

To determine their role in gibberellin biosynthesis in *G. fujikuroi*, the following compounds were fed to cultures of the mutant B1—41a: gibberellin A_{14}-aldehyde (4) and its [6-^{3}H]-derivative; gibberellin A_{14}-alcohol (7) and its [6-^{3}H]-derivative; and gibberellin A_{14} (6) and its [17-^{3}H]-derivative. Bearder *et al.*[1] have shown that the mutant B1—41a is blocked for gibberellin biosynthesis at the step between *ent*-kaur-16-en-19-al and *ent*-kaur-16-en-19-oic acid and that metabolites from unlabelled substrates, normally occurring beyond the block in wild-type strains, can be conveniently identified by combined gas chromatography–mass spectrometry (g.l.c.–m.s.) in the absence of endogenous gibberellins. Similarly the origin of the metabolites from the labelled substrates can be established by tandem gas chromatography–radio counting (g.l.c.–r.c.).[10] Since the metabolic block in the mutant is known[1] to be 97·5% effective, the dilution of label by endogenous metabolites is very small. The feeds were made with both non-replacement cultures and resuspension cultures of the mutant B1—41a.[1]

In non-replacement cultures, the labelled and unlabelled substrates (4), (6), and (7) were added to cultures of B1—41a which had been growing for 3 days on (*a*) the glucose–nitrate medium containing 4·8 g l^{-1} of ammonium nitrate (100%-ICI as defined by Geissman *et al.*[11]), and (*b*) the same medium containing 0·48 g l^{-1} ammonium nitrate (10%-ICI). The cultures were then grown for a further 5 days. In the 100%-ICI medium substrates were completely unmetabolised. In the 10%-ICI medium the aldehyde (4) was completely metabolised to gibberellins A_3 (8) (15%), A_7 (9) (17%), A_1 (24) (5%), A_4 (25) (19%), A_{13} (26) (5%), and A_{14} (6) (9%). Similarly in the 10%-ICI medium the alcohol (7) was completely metabolised to the same gibberellins except that gibberellin A_1 was not detected. Although gibberellin A_{14} (6) was converted into the same gibberellins in the 10%-ICI medium, it was incompletely metabolised and 40% remained after 5 days. Each substrate gave rise to a metabolite not produced by the others. Gibberellin A_{14} (6) gave a trace of gibberellin A_{42} (27);[12] gibberellin A_{14}-aldehyde (4) gave an isomer (*ca.* 15%) of gibberellin A_1 (24) of unknown structure; and gibberellin A_{14}-alcohol (7) gave a metabolite (19%) assigned structure (28) from the mass spectrum of the tri-TMS ether of the methyl ester, which contained an intense ion of *m/e* 103 (CH_2OTMS^+), characteristic of the TMS ether of a primary alcohol. Hydration of the 16,17-double bond does not occur in the absence of the fungus.

The unlabelled and labelled forms of the aldehyde (4), the alcohol (7), and the acid (6) were also fed to resuspension cultures of the mutant B1—41a as described by Bearder *et al.*[1] After incubation for 2 days at pH 4·5 gibberellin A_3 was the major product in all cases, but again gibberellin A_{14} was incompletely metabolised. When [6-^{3}H]gibberellin A_{12}-aldehyde (1) was incubated with a resuspension culture of the mutant B1—41a for 2 h it was almost completely metabolised to [^{3}H]-gibberellin A_{14}. Unlabelled gibberellin A_{14}-aldehyde, added to the resuspension culture at this stage, was recovered, and purified by t.l.c. After crystallisation to constant specific radioactivity, it contained 0·5% of the

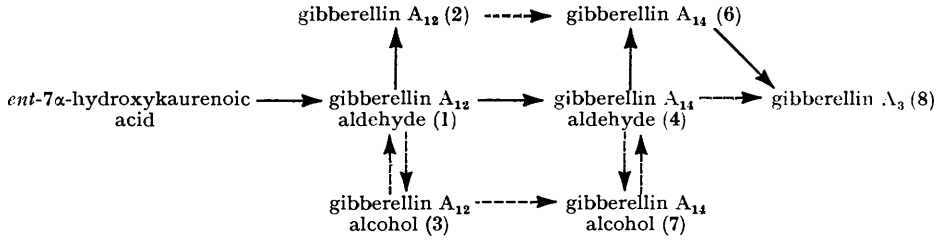
[10] J. MacMillan and C. M. Wels, *Phytochemistry*, 1974, in the press.

[11] T. A. Geissman, A. J. Verbiscar, B. O. Phinney, and G. Cragg, *Phytochemistry*, 1966, **5**, 933.

[12] J. R. Bearder and J. MacMillan, *J.C.S. Perkin I*, 1973, 2824.

total radioactivity of the fed [6-^{3}H]gibberellin A_{12}-aldehyde. Thus gibberellin A_{14}-aldehyde (4) is formed directly from gibberellin A_{12}-aldehyde and acts as an efficient precursor of gibberellins A_{14}, A_4, A_7, A_1, and A_3.

The present and previous results on gibberellin biosynthesis in *G. fujikuroi* are best discussed in terms of the metabolic grid [13] shown in the Scheme. Hanson *et al.*[2] have shown by isotope dilution that *ent*-7α-hydroxy[17-^{14}C]kaur-16-en-19-oic acid is converted into [17-^{14}C]gibberellin A_{12}-aldehyde in cultures of strain ACC 917. The present results show that gibberellin



SCHEME Metabolic grid for gibberellin biosynthesis in *G. fujikuroi*

A_{12}-aldehyde is 3-hydroxylated by the mutant B1—41a to gibberellin A_{14}-aldehyde, which is metabolised to other 3-hydroxylated gibberellins. Like gibberellin A_{14}-aldehyde, gibberellin A_{14} is a normal metabolite of the fungus and is further metabolised to 3-hydroxylated gibberellins. Its less efficient metabolism by cultures of B1—41a, as compared with the aldehyde (4) and the alcohol (7), may be due to its poor transport across the cell membranes. The aldehyde and the alcohol may be oxidised by a non-specific oxidase to gibberellin A_{14} which, once formed within the cell, is rapidly metabolised to other 3-hydroxylated gibberellins. Enzymic oxidation does occur to some extent as shown by the formation of some gibberellin A_{14} from both the aldehyde and the alcohol in the presence of the mutant B1—41a but not in blank experiments without the fungus. The alternative explanation for the slower conversion of gibberellin A_{14} into other 3-hydroxylated gibberellins is that gibberellin A_{14} is not on the direct pathway from gibberellin A_{14}-aldehyde to gibberellin A_3 and this possibility is being investigated.

Although gibberellin A_{12}-alcohol (3) [14,15] and A_{14}-alcohol (7) act as efficient precursors of 3-hydroxylated gibberellins, there is at present no evidence that they are normal metabolites of the fungus; their conversion may occur by non-specific oxidation as indicated in the Scheme.

EXPERIMENTAL

For general experimental details see Part V.[16] For g.l.c.–m.s. see Part VII.[15]

Reaction of ent-3α,6β,7α-*Trihydroxykaur*-16-*en*-19-*oic Acid* 19,6-*Lactone with Dihydropyran*.—The lactone (6·55 g, 0·0197 mol) in dry ether (500 ml) was heated under reflux for 7·5 h with freshly distilled dihydropyran (2 ml, 0·0217 mol) and a few crystals of toluene-*p*-sulphonic acid. The solution was washed with 2N-sodium hydroxide, and water; it was then dried and evaporated to dryness under vacuum to give a gum which was crystallised from a concentrated solution in ethyl acetate to provide the 3-*tetrahydropyranyl ether* (15) as a diastereoisomeric mixture (1·4 g), m.p. 186—200° (Found: M^+, 416·259. $C_{25}H_{36}O_5$ requires M, 416·256), $\nu_{max.}$ 3610, 3410br, 1770, 1658, and 885 cm^{-1}; τ 9·08 and 9·06 (each s, 20-H_3), 8·60 and 8·69 (each s, 18-H_3), 8·05 (d, J 7 Hz, 5-H), 5·39 (t, J 7 Hz, 6-H), and 5·13br and 4·99br (17-H_2).

Addition of light petroleum to the mother liquors gave crystalline starting material (2·3 g). The residue was chromatographed on a column of alumina (175 g; Laporte type C). Fractions eluted with light petroleum containing 25—45% ethyl acetate gave the 3,7-*bistetrahydropyranyl ether* (13) (diastereoisomeric mixture) as a gum (1·0 g) (Found: M^+, 500·314. $C_{30}H_{44}O_6$ requires M, 500·314), $\nu_{max.}$ (CCl_4) 1777, 1600, and 883 cm^{-1}; τ 9·05 (s, 20-H_3), 8·70 (s, 18-H_3), and 5·05 and 4·98 (17-H_2).

Fractions eluted from the alumina column with light petroleum containing 70—100% ethyl acetate gave additional amounts of the 3-tetrahydropyranyl ether (13). Fractions eluted with ethyl acetate containing 2—25% ethanol gave a mixture of starting material and the 7-tetrahydropyranyl ether (14) which was separated by preparative t.l.c. on silica gel with light petroleum–ethyl acetate (1 : 1). The diastereoisomers of the 7-tetrahydropyranyl ether were separated from each other and were recovered as gums from bands at R_F *ca.* 0·2 and 0·3 (1·5 g total). Data for one isomer: $\nu_{max.}$ 3660, 3440br, 1750, 1650, and 887 cm^{-1}; τ 9·04 (s, 20-H_3), 8·67 (s, 18-H_3), 8·05 (d, J 6 Hz, 5-H), 5·65 (d, J 6 Hz, 7-H), 5·36 (t, J 6 Hz, 6-H), and 5·14br and 5·03br (17-H_2).

ent-6β-*Hydroxy*-7-*oxo*-3-(*tetrahydropyran*-2-*yloxy*)*kaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (16).—Chromium trioxide (10 g) was added with stirring to pyridine (100 ml) and stirring was continued for 1 h. The reagent was cooled to 0° and the 3-tetrahydropyranyl ether (15) (1·6 g) in pyridine was added to it. The solution was allowed to warm to room temperature and was stirred for 24 h. Dilution with 2N-sodium hydroxide (100 ml) and recovery in ethyl acetate gave the 7-*ketone* (16) as a solid which crystallised from acetone–light petroleum as needles, m.p. 173—181° (Found: M^+, 414·240. $C_{25}H_{34}O_5$ requires M, 414·241), $\nu_{max.}$ 1785, 1722, 1660, and 890 cm^{-1}; τ 9·24 (s, 20-H_3), 8·56 (s, 18-H_3), 7·55 (d, J 6 Hz, 5-H), and 5·12br and 4·94br (17-H_2).

ent-6β,7β-*Dihydroxy*-3α-(*tetrahydropyran*-2-*yloxy*)*kaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (17).—The foregoing 7-ketone

[13] J. D. Bu'Lock, 'The Biosynthesis of Natural Products,' McGraw-Hill, London, 1965, ch. 7.
[14] J. R. Hanson and J. Hawker, *Phytochemistry*, 1973, **12**, 1073.
[15] J. R. Bearder, J. MacMillan, and B. O. Phinney, *Phytochemistry*, 1973, **12**, 2655.
[16] J. MacMillan and T. J. Simpson, *J.C.S. Perkin I*, 1973, 1487.

(16) (1·4 g) in methanol (150 ml) was treated at 0° with sodium borohydride (4 g). After 20 h at room temperature, the usual work-up gave the *alcohol* (17), which crystallised from acetone–light petroleum as needles (1·2 g), m.p. 193—198° (Found: M^+, 416·256. $C_{25}H_{36}O_5$ requires M, 416·256), $\nu_{max.}$ 3580, *ca.* 3480, 1770, 1660, and 887 cm^{-1}; τ 8·83 (s, 20-H_3), 8·66 (s, 18-H_3), 8·16 (d, J 6 Hz, 5-H), 5·98 (d, J 7 Hz, 7-H), 5·14 (dd, J 6 and 7 Hz, 6-H), and 5·14br and 5·01br (17-H_2).

Reaction of ent-6β,7β-*Dihydroxy*-3α-(*tetrahydropyran*-2-*yloxy*)*kaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (17) *with Toluene*-p-*sulphonyl Chloride*.—The alcohol (17) (1·1 g) and toluene-*p*-sulphonyl chloride (2·1 g) in dry pyridine (5·3 ml) were stirred at room temperature for 19 h. The mixture was poured into 0·1M-phosphate buffer (pH 7) and extracted with ethyl acetate to give a gum (1·43 g), which was chromatographed on a column of silica gel (75 g) made up in light petroleum–ethyl acetate (19 : 1). Elution with light petroleum–ethyl acetate (3 : 1) gave the 7-*toluene*-p-*sulphonate* (19) (750 mg), which crystallised from ethyl acetate–light petroleum as needles, m.p. 144—146°; $\nu_{max.}$ 1780, 1662, and 895 cm^{-1}; τ 9·07 (s, 20-H_3), 8·72 (3H, s, 18-H_3), 7·60 (s, ArMe), and 5·90 (d, J 7 Hz, 7-H).

Elution of the silica gel column with light petroleum–ethyl acetate (11 : 9—2 : 3) gave the 7-*toluene*-p-*sulphonate* (18) as a gum (200 mg) (Found: M^+, 486·206. $C_{27}H_{34}O_6S$ requires M, 486·207); $\nu_{max.}$ 3595, *ca.* 3440, 1770, 1660, and 895 cm^{-1}; τ 9·10 (s, 20-H_3), 8·75 (s, 18-H_3), 7·53 (s, ArMe), 5·88 (d, J 7 Hz, 7-H), 5·70 (m, 3-H), and 5·15br and 5·00br (17-H_2).

Ring Contraction of ent-6β-*Hydroxy*-3α-(*tetrahydropyran*-2-*yloxy*)-7β-(p-*tolylsulphonyloxy*)*kaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (19).—(a) *Small scale*. Potassium hydroxide (0·6 g) in water (0·5 ml) was added to the toluene-*p*-sulphonate (50 mg) in t-butyl alcohol (12 ml) and the solution was boiled for 4·5 h in a stream of nitrogen. Addition of water, removal of the t-butyl alcohol under vacuum, acidification to pH 3 with 2N-hydrochloric acid, and extraction with ethyl acetate gave a gum (40 mg) which was purified by preparative t.l.c. on silica gel with ethyl acetate–chloroform–acetic acid (15 : 5 : 1). Extraction of the band at R_F *ca.* 0·5 gave 3-O-*tetrahydropyranylgibberellin* A_{14}-*aldehyde* (5) (diastereoisomeric mixture) as an intractable foam (27 mg) (Found: M^+, 416·256. $C_{25}H_{36}O_5$ requires M, 416·256); $\nu_{max.}$ 3500, 3340—2400, 2740, 1710, 1660, and 885 cm^{-1}; τ 9·22 (s, 20-H_3), 8·80 and 8·75 (each s, 18-H_3), 7·60 and 7·65 (d, J 12 Hz, 5-H), 6·79 (dd, J 6 and 12 Hz, 6-H), 5·17 and 5·06 (17-H_2), and 0·29 (d, J 6 Hz, 7-H).

(b) *Large scale*. The experiment was repeated as in (a) with the toluene-*p*-sulphonate (1 g), potassium hydroxide (3 g), t-butyl alcohol (50 ml), and water (2 ml). The product consisted of three components which were separated by preparative layer chromatography (p.l.c.) on silica gel with ethyl acetate–light petroleum–acetic acid (25 : 75 : 1). The most mobile component was the 3-tetrahydropyranylgibberellin A_{14}-aldehyde (5) (70 mg). The least mobile component was the hydroxy-acid (22), isolated as a gum (700 mg); $\nu_{max.}$ 3520, 3400—2400, 1720, 1660, and 890 cm^{-1}; τ 8·80 (s, 20-H_3), 8·73 (s, 18-H_3), and 7·55 (s, ArMe).

Ring Contraction of ent-3α,6β-*Dihydroxy*-7β-(p-*tolylsulphonyloxy*)*kaur*-16-*en*-19-*oic Acid* 19,6-*Lactone* (18).—Treatment of the toluene-*p*-sulphonate (48 mg) in t-butyl alcohol (12 ml) with potassium hydroxide (0·6 g) in water (0·5 ml) as in the previous experiment gave a gum (36 mg) which was purified by p.l.c. on silica gel with ethyl acetate–benzene–acetic acid (24 : 16 : 1). Recovery from the band at R_F *ca.* 0·7 gave *gibberellin* A_{14}-*aldehyde* (4) (18 mg), m.p. 195—198° (from acetone–light petroleum), $[\alpha]_D^{20}$ −18·6° (*c* 0·365) (Found: M^+, 332·197. $C_{20}H_{28}O_4$ requires M, 332·199); $\nu_{max.}$ 3620, 3500br, 3400br, 3300—2400, 2730, 1720br, 1660, and 885 cm^{-1}; τ 9·21 (s, 20-H_3), 8·72 (s, 18-H_3), 7·58 (d, J 12 Hz, 5-H), 6·78 (dd, J 6 and 12 Hz, 6-H), 5·84 (m, $W_{\frac{1}{2}}$ 7 Hz, 3-H), 5·17 and 5·06 (m, 17-H_2), and 0·27 (d, J 6 Hz, 7-H). Irradiation at τ 6·8 caused the collapse of the 5- and 7-proton doublets to singlets and irradiation at τ 7·6 caused the 6-proton double doublet to simplify to a doublet.

Hydrolysis of 3-O-*Tetrahydropyranylgibberellin* A_{14}-*aldehyde* (5).—A solution of the tetrahydropyranyl ether (145 mg) in methanol (18 ml) and acetic acid (2 ml) was boiled for 11·5 h in a stream of nitrogen. Removal of the solvent, then p.l.c. of the residue on silica gel with ethyl acetate–chloroform–acetic acid (25 : 5 : 1), gave gibberellin A_{14}-aldehyde (104 mg).

Ring Contraction of ent-6β-*Hydroxy*-3α-(*tetrahydropyran*-2-*yloxy*)-7β-(p-*tolylsulphonyloxy*)*kaur*-16-*en*-19-*oic Acid* (22).—The toluene-*p*-sulphonate (680 mg) in collidine (50 ml) was boiled for 15 h. The pH was adjusted to 3·5 with 2N-hydrochloric acid and the solution was extracted with ethyl acetate. The crude product, recovered from the ethyl acetate, was subjected to p.l.c. on silica gel with ethyl acetate–light petroleum–acetic acid (25 : 75 : 1). Two bands at R_F *ca.* 0·3 and 0·5 were extracted and the recovered material from each was purified by p.l.c. on silica gel–5% silver nitrate plates with the same solvent system. The less mobile compound was gibberellin A_{14}-aldehyde (4) (48 mg) and the more mobile component was the tetrahydropyranyl ether of gibberellin A_{14}-aldehyde (38 mg).

Gibberellin A_{14}-*alcohol* (7).—A solution of gibberellin A_{14}-aldehyde (9 mg) and sodium borohydride (96 mg) in methanol (20 ml) was stirred at 20° overnight to give *gibberellin* A_{14}-*alcohol* (7), which crystallised from ethyl acetate–light petroleum as needles (6 mg), m.p. 236—238° (Found: M^+, 334·214. $C_{20}H_{30}O_4$ requires M, 334·214); τ (C_5D_5N) 8·89 (s, 20-H_3), 8·02 (s, 18-H_3), *ca.* 5·8 (m, 7-H_2), 5·29 (m, $W_{\frac{1}{2}}$ 5 Hz, 3-H), and 5·09 and 4·96 (both m, 17-H_2).

6-[^{3}H]*Gibberellin* A_{14}-*aldehyde and* -*alcohol*.—Tetrahydrofuran (5 ml; distilled from lithium aluminium hydride), gibberellin A_{14}-aldehyde (4·8 mg), sodium methoxide (1 g), and tritiated water (100 μl, *ca.* 10 mCi) were boiled for 5 h in a stream of nitrogen. The mixture was poured into M-phosphate buffer (pH 7), which was extracted with ethyl acetate. The aqueous solution was then acidified to pH 3 with 2N-hydrochloric acid and re-extracted with ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness and the residue was subjected to p.l.c. on silica gel with ethyl acetate–benzene–acetic acid (10 : 30 : 1). The upper band gave [6-^{3}H]gibberellin A_{14}-aldehyde (1·3 mg, 0·3 μCi μmol^{-1}), and the lower band [6-^{3}H]gibberellin A_{14}-alcohol (1·6 mg, 0·2 μCi μmol^{-1}).

*Non-replacement Cultures of Mutant B*1—41*a of* Gibberella fujikuroi.—Culture conditions and methods of feeding substrates were those described in Part XI.[1] The term *x*%-ICI medium refers to the glucose–ammonium nitrate medium described by Geissman *et al.*[11] in which the concentration of ammonium nitrate is 4·8 × 10^{-2} g l^{-1}.

(a) *Preliminary feeds*. Cultures (25 ml) of the mutant B1—41a in 10- and 100%-ICI medium were supplemented with gibberellin A_{14}-aldehyde (100 μg). Two blanks were included: (i) with the mutant B1—41a and without

gibberellin A_{14}-aldehyde, and (ii) without the mutant B1—41a and with GA_{14}-aldehyde. After 5 days at 25°, the contents of the flask were acidified to pH 3 with 2N-hydrochloric acid and extracted with ethyl acetate. The extracts were methylated and analysed by g.l.c. and g.l.c.–m.s.

Gibberellin A_{14}-aldehyde was unchanged in the control without an inoculation of the fungus and in the 100%-ICI medium but it was completely metabolised, mainly to gibberellins A_4 and A_7, in the 10%-ICI medium.

(b) *Feeds of gibberellin A_{14}, gibberellin A_{14}-aldehyde, and gibberellin A_{14}-alcohol in 10%-ICI medium.* Cultures (25 ml) of mutant B1—41a were supplemented with the following substrates: (i) control; (ii) gibberellin A_{14} (500 μg); (iii) [17-^{3}H]gibberellin A_{14} (500 μg; $1{\cdot}1 \times 10^6$ disint. min^{-1}); (iv) gibberellin A_{14}-aldehyde (500 μg); (v) [6-^{3}H]-gibberellin A_{14}-aldehyde (400 μg; $8{\cdot}5 \times 10^5$ disint. min^{-1}); (vi) gibberellin A_{14}-alcohol (500 μg); (vii) [6-^{3}H]GA_{14}-alcohol (500 μg; $8{\cdot}5 \times 10^5$ disint. min^{-1}). The cultures were extracted as described in (a). The metabolites were characterised by g.l.c.–r.c.[10] from the ^{3}H-labelled substrates and by g.l.c.–m.s. from the unlabelled substrates by comparison with reference spectra. The m.s. of GA_{42} (27) was identical with that previously described.[12] The new metabolite, tentatively assigned structure (28), was converted into its methyl ester TMS ether, *m/e* 582 (M^+, 0%), 567 (7), 492 (7), 477 (7), 460 (41), 436 (5), 432 (4), 402 (15), 400 (8), 361 (25), 360 (30), 349 (9), 343 (8), 310 (10), 283 (10), 282 (8), 274 (23), 273 (100), 224 (20), 214 (22), 212 (15), 186 (8), 143 (27), 129 (90), 117 (18), 103 (20), 75 (40), and 73 (100). The methyl ester TMS ether of the new metabolite from gibberellin A_{14}-aldehyde had *m/e* 506 (12%), 491 (3), 474 (22), 431 (10), 429 (6), 341 (10), 129 (30), 119 (8), 117 (7), 75 (40), and 73 (100).

Feeds with Resuspension Cultures of Mutant B1—41a.—The following substrates were fed to resuspension cultures (20 ml) as previously described in Part XI:[1] (i) blank; (ii) gibberellin A_{14} (600 μg); (iii) gibberellin A_{14}-aldehyde (400 μg); (iv) GA_{14}-alcohol (300 μg); (v) [17-$^3H_2$]gibberellin A_{14} (7×10^5 disint. min^{-1}); (vi) [6-^{3}H]gibberellin A_{14}-aldehyde ($4{\cdot}5 \times 10^5$ disint. min^{-1}), and (viii) [6-^{3}H]-gibberellin A_{14}-alcohol (7×10^5 disint. min^{-1}). The cultures were incubated for 46 h, then extracted, and the products were converted into derivatives as for the non-replacement cultures. Gibberellin A_3 was the major metabolite from all feeds, as shown by g.l.c.–r.c. and g.l.c.–m.s.

Conversion of [6-^{3}H]Gibberellin A_{12}-aldehyde into [6-^{3}H]-Gibberellin A_{14}-aldehyde by Resuspension Cultures of Mutant B1—41a.—The mycelium from a 50 ml culture was resuspended as described[1] into 0%-ICI medium (100 ml), adjusted to pH 6·0 with potassium hydroxide solution. Aliquot portions (20 ml) of this mycelial suspension were pipetted into each of two flasks (100 ml) containing 0%-ICI medium (5 ml) and (*a*) [6-^{3}H]gibberellin A_{12}-aldehyde ($6{\cdot}5 \times 10^6$ disint. min^{-1}; *ca.* 1·0 mg), or (*b*) GA_{12}-aldehyde (650 μg). After 2 h at 25° on an orbital shaker, GA_{14}-aldehyde (10 mg) was added to flask (*a*) and both cultures were extracted as before. The extract from flask (*b*) was methylated and examined by g.l.c. to show that almost all the gibberellin A_{12}-aldehyde had been converted into gibberellin A_{14}. The extract from flask (*a*) was subjected to p.l.c. with ethyl acetate–light petroleum–acetic acid (50 : 50 : 1). The recovered gibberellin A_{14}-aldehyde was crystallised to constant specific activity (2·6 mg; 3×10^3 disint. min^{-1}).

ent-19-*Norgibberella*-3,16-*diene*-7-*al* (23).—(*a*) *ent*-3β,6β,7β-Trihydroxykaur-16-en-19-oic acid 19,6-lactone (44 mg), m.p. 129—131°, prepared by reduction of the diketone (21) with sodium borohydride as described by Bateson and Cross,[6] was treated with toluene-*p*-sulphonyl chloride (706 mg) in pyridine (1·5 ml) for 4 days at 20°. Addition of 3N-hydrochloric acid to pH 3, followed by extraction with ethyl acetate, gave a gum (80 mg) which was purified by p.l.c. on silica gel with ethyl acetate–light petroleum (1 : 1). Recovery from the band at R_F *ca.* 0·5 gave a gum (41 mg); τ (60 MHz) 9·08 (s, 20-H_3), 8·67 (s, 18-H_3), 7·57 (s, 2 × ArMe), 4·95 and 5·14 (17-H_2).

(*b*) The gum (40 mg) from (*a*) in t-butyl alcohol (12) was boiled for 4 h under nitrogen with potassium hydroxide (0·6 g) in water (0·5 ml). Removal of the solvent *in vacuo*, acidification to pH 3 with 2N-hydrochloric acid, and extraction with ethyl acetate gave a gum (20 mg). P.l.c. on silica gel with ethyl acetate–light petroleum (1 : 3) and recovery from the band at R_F *ca.* 0·6 gave the *aldehyde* (23) as a gum (8·5 mg) (Found: M^+, 270·198. $C_{19}H_{26}O$ requires *M*, 270·198); $\nu_{max.}$ (CCl_4) 2710, 1722, 1658, and 882 cm^{-1}; τ 9·21 (s, 20-H_3), 8·24 (s, 18-H_3), 5·19 and 5·05 (both m, 17-H_2), 4·71 (m, 3-H), and 0·24 (d, *J* 4 Hz, 7-H).

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