Studies in Terpenoid Biosynthesis. Part IX.[1] The Sequence of Oxidation on Ring B in Kaurene–Gibberellin Biosynthesis

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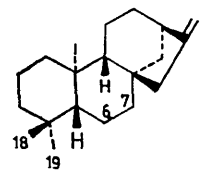
The formation of 7β-hydroxy-(−)-kaur-16-en-19-oic acid and its incorporation into gibberellic acid and the kauranoid metabolites of *Gibberella fujikuroi* are described. The results of incubation of 7β-hydroxy-[6β-^{3}H,17-^{14}C]-(−)-kaur-16-en-19-oic acid have shown that the 6β-hydrogen atom is lost in the formation of gibberellic acid. However 6β,7β-dihydroxy-(−)-kaur-16-en-19-oic acid is not incorporated into gibberellic acid. Experiments with [1-$^{3}H_{2}$,1-^{14}C]geranyl pyrophosphate suggest that the 6β-hydrogen atom migrates to C-7 during ring contraction.

We have previously [2] described the fate of mevalonoid hydrogen atoms in the major kauranoid and gibberellin metabolites of *Gibberella fujikuroi*. The diterpene hydrocarbon (−)-kaurene (1) plays a central role in gibberellin biosynthesis.[3] The results of incorporation of [2-^{14}C]mevalonate into gibberellic acid (2) established [4] that in the rearrangement of ring B to form the five-membered ring of the gibberellins, it is the kauranoid C-7 atom which is extruded. Considerable evidence has been obtained to show that C-19 is oxidized to afford (−)-kaur-16-en-19-oic acid prior to the oxidation and rearrangement of ring B.[5] The sequence of oxidation of ring B forms the subject of the present paper.[6] During these studies Lew and West reported [7] that 7β-hydroxy-(−)-kaur-16-en-19-oic acid (3; R = H) is a precursor of gibberellic acid, and Cross *et al.* reported [8] that 6β,7β-dihydroxy-(−)-kaur-16-en-19-oic acid (3; R = OH) is not a precursor. Graebe *et al.* recently described [9] the incorporation of [2-^{14}C]mevalonate into gibberellin A_{12} aldehyde (4; R = CHO) by a cell-free system from *Curcurbita pepo*.

The oxidation of ring B clearly represents a divergence in biosynthetic pathways between the kaurenolide metabolites [*e.g.* (5; R = OH)] and the gibberellins. If oxidation occurs first at C-6 then kaurenolide (5; R = H) [10] might be expected to be a precursor of 7β-hydroxykaurenolide (5; R = OH) and 7β,18-dihydroxykaurenolide. Alternatively oxidation at C-7 giving 7β-hydroxy-(−)-kaur-16-en-19-oic acid (4; R = H) [11] would furnish a potential precursor for both series. Since the occurrence of microbiological transformation of kauranoid diterpenes by *Gibberella fujikuroi* is known,[12] it is possible that the fungus might transform a putative intermediate without it actually lying on the biosynthetic pathway. First we attempted to establish the formation of either kaurenolide or 7β-hydroxy-(−)-kaur-16-en-19-oic acid, both of which were unknown as metabolites. [17-^{14}C]-(−)-Kaurene (1) was fed to *Gibberella fujikuroi*. The frementation was subjected to dilution analysis for both kaurenolide (5; R = H) and 7β-hydroxy-(−)-kaur-16-en-19-oic acid (4; R = H). Only the latter was radioactive, showing a low incorporation of 0·006%; *cf.* 7β-hydroxykaurenolide (5; R = OH) 0·8%, 7β,18-dihydroxykaurenolide 5·45%. The conversion was shown to be specific by ozonolysis of the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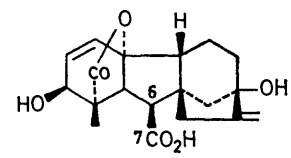
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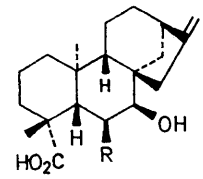
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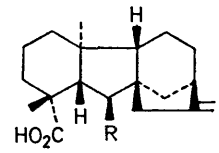
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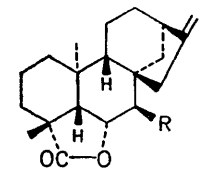
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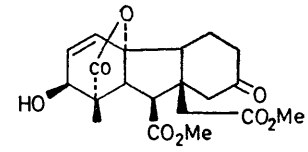
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hydroxy-acid to give the corresponding 17-nor-16-ketone, and recovery of C-17 as the formaldehyde dimedone derivative. The latter contained 99% of the radioactivity.

7β-Hydroxy-[17-^{14}C]-(−)-kaur-16-en-19-oic acid (3; R = H) was prepared from the corresponding 17-nor-16-ketone by a Wittig reaction. It was fed as its

[1] Part VIII, B. Achilladelis, P. M. Adams, and J. R. Hanson, *J.C.S. Perkin I*, 1972, 1425.

[2] (*a*) J. R. Hanson and A. F. White, *J. Chem. Soc.* (*C*), 1969, 981; (*b*) R. Evans, J. R. Hanson, and A. F. White, *ibid.*, 1970, 2601.

[3] B. E. Cross, R. H. B. Galt, and J. R. Hanson, *J. Chem. Soc.*, 1964, 295.

[4] A. J. Birch, R. W. Rickards, H. Smith, and W. B. Whalley, *Tetrahedron*, 1959, **7**, 241.

[5] For a review see B. E. Cross, *Progr. Phytochem.*, 1968, **1**, 195.

[6] Preliminary communications, (*a*) J. R. Hanson and A. F. White, *Chem. Comm.*, 1969, 410; (*b*) J. R. Hanson and J. Hawker, *ibid.*, 1971, 208.

[7] F. T. Lew and C. A. West, *Phytochem.*, 1971, **10**, 2065 and references cited therein.

[8] B. E. Cross, J. C. Stewart, and J. L. Stoddart, *Phytochem.*, 1970, **9**, 1065.

[9] J. Graebe, D. H. Bowen, and J. MacMillan, *Planta*, 1972, **102**, 261.

[10] J. R. Hanson, *Tetrahedron*, 1966, **22**, 2877.

[11] J. R. Hanson and A. F. White, *Tetrahedron*, 1969, **25**, 2743.

[12] J. R. Hanson and A. F. White, *Tetrahedron*, 1968, **24**, 6291; I. F. Cook, P. R. Jefferies, and J. R. Knox, *Tetrahedron Letters*, 1971, 2157.

potassium salt to *Gibberella fujikuroi*. The gibberellin A_{12} aldehyde (4; R = CHO) [13] and gibberellin A_{12} (4; R = CO_2H) were isolated by dilution analysis after 4 and 24 h. The results are shown in Table 1. The

TABLE 1

Incorporation of 7β-hydroxy-[17-^{14}C]-(−)-kaur-16-en-19-oic acid

	4 h fermentation	24 h fermentation
Gibberellin A_{12} aldehyde (4; R = CHO)	0·21	0·17
% in formaldehyde		96·5
% in nor-ketone	0	0
Gibberellin A_{12} (4; R = CO_2H)	0·29	0·37
% in formaldehyde		98·0
% in nor-ketone	0	0

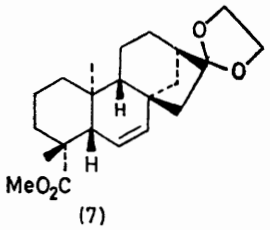
incorporation was shown to be specific by oxidizing the aldehyde to gibberellin A_{12}. The gibberellin A_{12} was then ozonized and the formaldehyde collected as its dimedone derivative. The nor-ketone was isolated as its dimethyl ester. Hence gibberellin A_{12} aldehyde (4; R = CHO) is produced by *Gibberella fujikuroi*. Cross *et al.* have demonstrated [13b] that the aldehyde is efficiently converted into gibberellic acid.

The potassium salt of 7β-hydroxy-(−)-kaur-16-en-19-oic acid (3; R = H) was fed to another fermentation of *Gibberella fujikuroi* which was then grown for a further 5 days. Gibberellic acid (2) was isolated and purified as its methyl ester. It showed an incorporation of 32·3%. Ozonolysis of the methyl gibberellate gave formaldehyde and a keto-acid which was purified as its dimethyl ester (6). The formaldehyde contained 97·6% of the radioactivity; 2·7% remained in the keto-ester. 7β-Hydroxykaurenolide showed an incorporation of 0·03% and 7β,18-dihydroxykaurenolide 0·44%. The kaurenolides were degraded to the corresponding inactive 17-nor-16-ketones. The formaldehyde from 7β,18-dihydroxykaurenolide contained 98·5% of the radioactivity in that metabolite.

A 5-*pro*-*R*-mevalonoid hydrogen atom is lost [2b] from ring B during the formation of the gibberellins. We concluded that ring contraction takes place with the displacement of the equatorial hydrogen atom from the kauranoid 6-position. Additional proof of this was obtained by use of stereospecifically labelled 7β-hydroxy-[6β-^{3}H,17-^{14}C]-(−)-kaur-16-en-19-oic acid (3; R = ^{3}H). Methyl 16,16-ethylenedioxy-17-nor-(−)-kaur-6-en-19-oate (7),[14] in which the ethylenedioxy-group served to protect ring D against labelling, was tritioboronated. The protecting group was removed from the resultant 7β-alcohol and the C-17 label was introduced by a Wittig reaction. The ester was hydrolysed with lithium iodide in collidine to give the known hydroxy-acid (3; R = ^{3}H).[11] This was fed to *Gibberella fujikuroi*, and after 20 h the gibberellic acid (2) showed a 4·5% incorporation of ^{14}C but no ^{3}H.

[13] (*a*) R. H. B. Galt and J. R. Hanson, *J. Chem. Soc.*, 1965, 1565; (*b*) B. E. Cross, K. Norton, and J. C. Stewart, *J. Chem. Soc.* (*C*), 1968, 1054.

In the light of this, 6β,7β-dihydroxy-(−)-kaur-16-en-19-oic acid (3; R = OH) seemed a logical precursor. Methyl 6β,7β-dihydroxy-16-oxo-17-nor-(−)-kauran-19-oate (8) was prepared by osmylation of methyl 16-oxo-17-nor-(−)-kaur-6-en-19-oate. The stereochemistry of this reaction has been described elsewhere.[8,14] The glycol was protected against epimerization under the basic conditions of the Wittig reaction used to introduce the C-17 label, by the formation of its isopropylidene derivative. Hydrolysis of the ester and removal of the protecting group afforded the dihydroxy-acid (3; R = OH). However when this was fed to *Gibberella fujikuroi* there was no incorporation into gibberellic acid and only a low incorporation (0·01%) into fujenal (9). At this stage Cross *et al.* reported [8] the same negative result.



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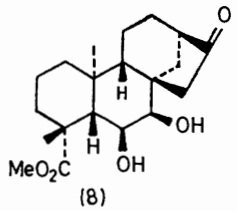

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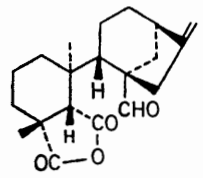

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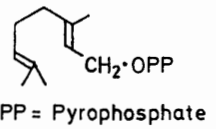

OPP = Pyrophosphate
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An alternative proposal [2b] was that abstraction of the 6β-hydrogen atom itself initiated ring contraction. [1-$^{3}H_2$,1-^{14}C]Geranyl pyrophosphate (10), which would introduce a label at this centre, was fed to *Gibberella fujikuroi* and the metabolites were isolated by dilution analysis (except for gibberellic acid). The results are given in Table 2. The incorporation was extremely low

TABLE 2

Incorporation of [1-$^{3}H_2$,1-^{14}C]geranyl pyrophosphate

	^{3}H : ^{14}C Ratio
Geranyl pyrophosphate (10)	10 : 1
(−)-Kaurene (1)	9·1 : 1
7β-Hydroxykaurenolide (5; R = OH)	6·0 : 1
Gibberellin A_{12} aldehyde (4; R = CHO) *	9·7 : 1
Gibberellic acid (2) †	5·7 : 1

* As its methyl ester semicarbazone. † As its methyl ester.

but it was specific. The gibberellin A_{12} aldehyde (4; R = CHO) was purified as its monomethyl ester semicarbazone. This was oxidized with cerium(IV) ammonium nitrate [15] to gibberellin A_{12} monomethyl ester.[13a] There was loss of tritium from the aldehydic C–H. The remaining label has already been located [2b] at C-6 in gibberellic acid (2). Consequently we suggested [6b] that the ring contraction occurs by hydroxylation at C-7 and

[14] J. R. Hanson and J. Hawker, *Tetrahedron*, 1972, **28**, 2521.
[15] J. W. Bird and D. G. M. Diaper, *Canad. J. Chem.*, 1969, **47**, 45.

a hydride shift from C-6 to C-7 as the 7,8-bond migrates to C-6. In view of the low incorporations obtained with the intact fungus, these stages, including the oxidation level of the rearrangement, are being studied with a cell-free preparation from *Gibberella fujikuroi*.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded on a Perkin-Elmer 257 spectrometer. N.m.r. spectra were determined for solutions in [^{2}H]chloroform on a Varian A60A spectrometer with tetramethylsilane as internal standard. Radioactive compounds were crystallized to constant activity (a minimum of six recrystallizations) and counted by the liquid sctintillation method (Koch–Light KL355 liquid scintillator) on a Beckmann LS100 counter, with a preset error of 1% except where stated. *Gibberella fujikuroi* (CMI 58,290) was grown as shake culture (120 rev. min^{-1}) in Erlenmeyer flasks (250 ml) at 24° on the following medium: glucose (80 g), ammonium nitrate (0·48 g), potassium dihydrogen phosphate (5·0 g) and magnesium sulphate (1·0 g) (quantities per litre of distilled water). A trace elements solution (2 ml) was added. This contained iron(II) sulphate (0·1 g), copper(II) sulphate (0·015 g), zinc sulphate (0·161 g), manganese sulphate (0·01 g), and ammonium molybdate (0·1 g) (quantities per 100 ml of distilled water). The main fermentation (100 ml per flask) was inoculated with seed (1 ml) grown on the foregoing medium for 4—5 days. The substrates were added after a further 10 days growth. The fermentations were harvested by filtration of the mycelium and the culture filtrate was acidified to pH 2 and extracted with ethyl acetate. The extract was dried and the solvent was evaporated off *in vacuo*. Carrier samples of kaurene, the kaurenolides, and gibberellin A_{13} were added at this stage. Known fungal metabolites were identified by m.p. and by t.l.c. comparison with authentic samples (5% acetic acid in trichloroethylene; silica plates; development with iodine vapour or ethanolic sulphuric acid).

Feeding of [17-^{14}C]-(−)-*Kaur*-16-*ene* (1).—[17-^{14}C]-(−)-Kaur-16-ene (10 mg; $3{\cdot}4 \times 10^5$ disint. min^{-1} mg^{-1}) in ethanol (30 ml) was distributed between 30 flasks of *Gibberella fujikuroi* at the natural pH. The fermentation was harvested after 4 days. In addition to dilution with the normal metabolites, kaurenolide (10 mg) and 7β-hydroxy-(−)-kaur-16-en-19-oic acid (10 mg) were added. Chromatography on silica (elution with 5% ethyl acetate in light petroleum) gave kaurenolide (10 mg), m.p. 202—204° (0 disint. min^{-1} mg^{-1}). Elution with 5—10% ethyl acetate in light petroleum gave 7β-hydroxykaurenolide (12 mg), m.p. 188—191° (2280 disint. min^{-1} mg^{-1}; 0·8% incorporation). Elution with 10—15% ethyl acetate in light petroleum gave 7β-hydroxy-(−)-kaur-16-en-19-oic acid (10 mg), m.p. 253—256° (19 disint. min^{-1} mg^{-1}; 0·006% incorporation). Elution with 20% ethyl acetate in light petroleum afforded 7,18-dihydroxykaurenolide (16 mg), m.p. 211—213° (11,600 disint. min^{-1} mg^{-1}; 5·45% incorporation).

Ozonolysis of 7β-*Hydroxy*-(−)-*kaur*-16-*en*-19-*oic Acid*.—Ozonized oxygen was passed through the labelled acid (7 mg) in acetic acid (3 ml) at room temperature for 0·5 min. The solution was diluted with water (10 ml) and the formaldehyde was steam distilled into a solution containing water (2 ml) and a 10% solution of dimedone in methanol (0·3 ml). The formaldehyde dimedone derivative crystallized from aqueous ethanol as needles, m.p. 187—189° [19 disint. min^{-1} mg^{-1}, 6000 disint. min^{-1} mmol^{-1} ≡ 99% of the activity of the 7β-hydroxy-(−)-kaur-16-en-19-oic acid]. The residue after steam distillation was extracted with ethyl acetate. Evaporation of the extract and recrystallization from ethyl acetate–light petroleum gave the 17-nor-16-ketone (3 mg), m.p. 239—241° (0 disint. min^{-1} mg^{-1}).

Preparation of 7β-*Hydroxy*-[17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid*.—Powdered [^{14}C]methyltriphenylphosphonium iodide (100 mg) suspended in dry ether (10 ml) was treated with *N*-butyl-lithium (0·5 ml) and the mixture was stirred for 3 h under nitrogen. 7β-Hydroxy-16-oxo-17-nor-(−)-kauran-19-oic acid (30 mg) in dry tetrahydrofuran (5 ml) was added and stirring was continued for 20 h. The ether was distilled off and the solution was heated under reflux for 3 h. Acetone (1 ml) was added and refluxing was continued for a further 30 min. The solvents were evaporated off and the residue was purified by preparative layer chromatography on silica to give 7β-hydroxy-[17-^{14}C]-(−)-kaur-16-en-19-oic acid (20 mg), which crystallized from ether–light petroleum as needles, m.p. 253—256° (176,000 disint. min^{-1} mg^{-1}).

Feeding of 7β-*Hydroxy*-[17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid; Four and Twenty-four Hour Fermentations*.—The acid (3·5 mg; 176,000 disint. min^{-1} mg^{-1}) in water (10 ml) containing 2N-potassium hydroxide (1 drop) was sterilized by filtration, made up to 30 ml with sterile water, and distributed between 30 flasks of *Gibberella fujikuroi*. After incubation at room temperature for 4 h 15 flasks were harvested; the remainder were harvested after 24 h. Both extracts were diluted with the gibberellin A_{12} aldehyde (4; R = CHO) (10 mg) and gibberellin A_{12} (4; R = CO_2H) (10 mg) and chromatographed on silica. The results are given in Table 1.

Oxidation of the Aldehydes from the Foregoing Fermentation.—The aldehydes (4; R = CHO) (7 mg) (65 disint. min^{-1} mg^{-1}, 20,000 disint. min^{-1} mmol^{-1}, and 53 disint. min^{-1} mg^{-1}, 16,700 disint. min^{-1} mmol^{-1}) from the 4 and 24 h fermentations were each dissolved in acetone (1 ml) and treated with 8N-chromium trioxide (0·03 ml) for 1 h at room temperature. Recovery in ethyl acetate and crystallization from ethyl acetate–light petroleum gave gibberellin A_{12} (63 disint. min^{-1} mg^{-1}, 21,000 disint. min^{-1} mmol^{-1} and 50 disint. min^{-1} mg^{-1}, 16,600 disint. min^{-1} mmol^{-1} for the 4 and 24 h fermentations).

Ozonolyses of Gibberellin A_{12}.—(a) *Four hour fermentation*. The gibberellin A_{12} samples (6 mg) were separately ozonized in ethyl acetate (4 ml) at room temperature for 0·5 min. The solvent was removed *in vacuo* and the residue was treated with water for 1 h. Recovery in ethyl acetate and methylation with diazomethane gave gibberellin A_{12} nor-ketone dimethyl esters, which crystallized from light petroleum as needles, m.p. 134—137° (0 disint. min^{-1} mg^{-1}).

(b) *Twenty-four hour fermentation*. Both gibberellin A_{12} samples (6 mg) were ozonized in acetic acid (3 ml) at room temperature for 0·5 min. The solution was diluted with water (10 ml) and the formaldehyde was steam distilled into water (2 ml) containing a 10% solution of dimedone in methanol (0·3 ml). The formaldehyde dimedone derivatives showed activities of 51 disint. min^{-1} mg^{-1} (16,100 disint. min^{-1} mmol^{-1} ≡ 96·5% of the activity of the gibberellin A_{12} aldehyde) and 117 disint. min^{-1} mg^{-1} (37,000 disint. min^{-1} mmol^{-1} ≡ 98% of the activity of the gibberellin A_{12}). The gibberellin A_{12} nor-ketone dimethyl esters, recovered as before, both showed 0 disint. min^{-1} mmol^{-1}.

Feeding of 7β-*Hydroxy*-[17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid; Five Day Fermentation.*—The acid (10 mg, 176,000 disint. min^{-1} mg^{-1}) as its potassium salt was distributed between 30 flasks of *Gibberella fujikuroi* and the metabolites were isolated after 5 days. The neutral fraction was chromatographed on silica gel to give 7β-hydroxykaurenolide (14 mg), m.p. 187—189° (38 disint. min^{-1} mg^{-1}; 0·03% incorporation) and 7,18-dihydroxykaurenolide (16 mg), m.p. 210—213° (486 disint. min^{-1} mg^{-1}; 0·44% incorporation). The acidic fraction was methylated with diazomethane and chromatographed on alumina. Elution with 40—60% ethyl acetate–light petroleum gave methyl gibberellate (140 mg), m.p. 206—208° (4060 disint. min^{-1} mg^{-1}; 32·3% incorporation).

Ozonolysis of the Methyl Gibberellate.—(*a*) Ozonized oxygen was passed through a solution of the foregoing methyl gibberellate (40 mg, 1·46 × 10^6 disint. min^{-1} mmol^{-1}) in acetic acid (5 ml) at room temperature for 2 min. Water (2 ml) was added and the formaldehyde was steam distilled into water (4 ml) containing a 10% solution of dimedone in methanol (1 ml) to give formaldehyde dimedone derivative (6 mg), m.p. 186—187° (4510 disint. min^{-1} mg^{-1}, 1·43 × 10^6 disint. min^{-1} mmol^{-1} ≡ 97·6% of the activity of the methyl gibberellate).

(*b*) Ozonized oxygen was passed through a solution of methyl gibberellate (53 mg) in ethyl acetate (5 ml) at −70° for 2·5 min. The solvent was removed *in vacuo*, water was added, and the solution was shaken for 24 h. The product was separated into neutral and acidic fractions with aqueous sodium hydrogen carbonate. The acidic fraction was methylated with diazomethane and purified by preparative layer chromatography with ethyl acetate–chloroform–acetic acid (15 : 5 : 1) as mobile phase. The dimethyl ester (6) (9 mg), crystallized from benzene, had m.p. 171—174° (100 disint. min^{-1} mg^{-1}, 39,200 disint. min^{-1} mmol^{-1} ≡ 2·7% of the activity of the methyl gibberellate).

Ozonolysis of 7β-*Hydroxykaurenolide.*—Ozonized oxygen was passed through a solution of the kaurenolide (7 mg) in acetic acid (3 ml) at room temperature for 0·5 min. The solution was diluted with water and shaken for 1 h. It was neutralized with aqueous sodium hydrogen carbonate and extracted with ethyl acetate. Evaporation of the ethyl acetate gave the nor-ketone, m.p. 308—310° (0 disint. min^{-1} mg^{-1}).

Ozonolysis of 7β,18-*Dihydroxykaurenolide.*—The kaurenolide was diluted and recrystallized. Ozonized oxygen was passed through a solution of the kaurenolide (30 mg; 27,200 disint. min^{-1} mmol^{-1}) in acetic acid (3 ml) for 1 min. The formaldehyde was recovered by steam distillation as formaldehyde dimedone derivative (26,800 disint. min^{-1} mmol^{-1} ≡ 98·5% of the activity of the 7β,18-dihydroxykaurenolide). The nor-ketone was recovered from the steam distillation residue by neutralization with sodium hydrogen carbonate and extraction with ethyl acetate. Evaporation of the ethyl acetate afforded the nor-ketone, m.p. 260—262° (0 disint. min^{-1} mg^{-1}).

7β-*Hydroxy*-[6β-^{3}H,17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid.*—This was prepared essentially as described previously [11,14] from methyl 16,16-ethylenedioxy-17-norkaur-6-en-19-oate. The tritiohydroboronation was carried out with diborane generated externally from sodium boro[^{3}H]hydride (100 mCi) and boron trifluoride–ether complex. The 7β-hydroxy-[6β-^{3}H,17-^{14}C]-(−)-kaur-16-en-19-oic acid crystallized from ether–light petroleum as needles, m.p. 253° (^{3}H 8·37 × 10^6 disint. min^{-1} mg^{-1}; ^{14}C 1·24 × 10^6 disint. min^{-1} mg^{-1}).

Feeding of 7β-*Hydroxy*-[6β-^{3}H,17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid.*—The acid (6·9 mg) as its potassium salt in 50% aqueous ethanol (30 ml) was distributed between 30 flasks of *Gibberella fujikuroi* 10 days after inoculation. The metabolites were isolated after a further 20 h. The gibberellic acid (44·4 mg) was methylated with diazomethane and the ester was purified by crystallization from chloroform.* It had m.p. 178° (^{3}H 0 disint. min^{-1} mg^{-1}; ^{14}C 8644 disint. min^{-1} mg^{-1}; 4·5% incorporation).

6β,7β-*Dihydroxy*-[17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid.*—Prepared as described previously,[14] this crystallized from acetone as prisms, m.p. 234—236° (lit.,[8] 234—237°) (^{14}C 2·47 × 10^6 disint. min^{-1} mg^{-1}).

Feeding of 6β,7β-*Dihydroxy*-[17-^{14}C]-(−)-*kaur*-16-*en*-19-*oic Acid.*—The acid (2·27 mg) as its potassium salt in 50% aqueous ethanol (30 ml) was distributed between 30 flasks of *Gibberella fujikuroi* 10 days after inoculation. The metabolites were isolated after a further 3 days. Fujenal (10 mg), m.p. 158—160° (^{14}C 108 disint. min^{-1} mg^{-1}; 0·02% incorporation), was isolated from the neutral fraction. The acidic fraction was methylated and the methyl gibberellate (66·0 mg) was purified by preparative layer chromatography and crystallization from chloroform.* It had m.p. 177—179° (^{14}C 0 disint. min^{-1} mg^{-1}).

Feeding of [1-^{3}H$_2$,1-^{14}C]*Geranyl Pyrophosphate.*—The pyrophosphate (9·3 mg; ^{3}H 7·5 × 10^6 disint. min^{-1} mg^{-1}; ^{14}C 0·75 × 10^6 disint. min^{-1} mg^{-1}) was distributed between 30 flasks of *Gibberella fujikuroi* 10 days after inoculation. The metabolites were isolated after a further 6 days and purified by preparative layer chromatography. (−)-Kaurene (6·7 mg) had m.p. 47—49° [^{3}H 373 disint. min^{-1} mg^{-1}; ^{14}C 41 disint. min^{-1} mg^{-1} (9·1 : 1); 0·004% incorporation]. 7-Hydroxykaurenolide (13 mg) had m.p. 186—188° [^{3}H 3814 disint. min^{-1} mg^{-1}; ^{14}C 633 disint. min^{-1} mg^{-1} (6·0 : 1); 0·117% incorporation]. The gibberellin A$_{12}$ aldehyde (4; R = CHO) was further purified as its *methyl ester semicarbazone* (4·2 mg), m.p. 196—197° (Found: C, 68·1; H, 8·6; N, 11·1. $C_{22}H_{33}N_3O_3$ requires C, 68·2; H, 8·6; N, 10·8%); $\nu_{max.}$ 3500, 3320, 1700, 1680, and 1600 cm^{-1}, τ 9·30 (3H, s), 8·83 (3H, s), 6·9 (1H, q, *J* 8 and 13 Hz), 6·35 (3H, s), 5·20 (2H, d, *J* 13 Hz), 4·40 (2H, m), 3·0 (1H, d, *J* 8 Hz), and 1·87 (1H, s) [^{3}H 953·5 disint. min^{-1} mg^{-1}; ^{14}C (98·3 disint. min^{-1} mg^{-1} (9·7 : 1); 0·005% incorporation]. The gibberellic acid (46·9 mg) gave methyl ester of m.p. 177—178° [^{3}H 1172 disint. min^{-1} mg^{-1}; ^{14}C 204·5 disint. min^{-1} mg^{-1} (5·7 : 1); 0·14% incorporation].

Oxidation of the Gibberellin A$_{12}$ Aldehyde (4; R = CHO) *Methyl Ester Semicarbazone.*—The semicarbazone (3·5 mg) was treated with cerium(IV) ammonium nitrate solution (0·1 ml) [from cerium ammonium nitrate (1·63 g) in 1·0N-nitric acid (67 ml) and ethanol (33 ml) prepared at −40° and stored below 0°] in ethanol (0·5 ml) at −20° for 1 h. The solution was allowed to attain room temperature overnight, then extracted with chloroform; the gibberellin A$_{12}$ hemiester was purified by repeated preparative layer chromatography. The total eluate from a gibberellin A$_{12}$ hemiester zone was counted for 24 h. It showed ^{3}H 19·4 disint. min^{-1}; ^{14}C 3·95 disint. min^{-1} (4·9 : 1).

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* Methyl gibberellate, when crystallized from chloroform as needles, melts at 177—178°, resets, and remelts at 208°.