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3-HYDROXYLATION OF GIBBERELLIN A_{12}-ALDEHYDE IN *GIBBERELLA FUJIKUROI* STRAIN REC-193A*

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Abstract—When grown on PDL medium for 11 days the strain REC-193A of *Gibberella fujikuroi* produces the usual range of gibberellins and *ent*-kaurenoid metabolites. After 3–5 days under the same conditions of culture, this slow growing strain produces virtually none of these metabolites. These short term cultures were found to convert gibberellin A_{12}-aldehyde into gibberellins A_{12} (8·3%), A_{14} (45%), A_4 (*ca.* 17%) and A_7 (*ca.* 6%). Under identical conditions of culture gibberellin A_{12} was largely unmetabolised. These results show that 3-hydroxylation is the first step in the conversion of gibberellin A_{12}-aldehyde into gibberellins A_{14}, A_4 and A_7.

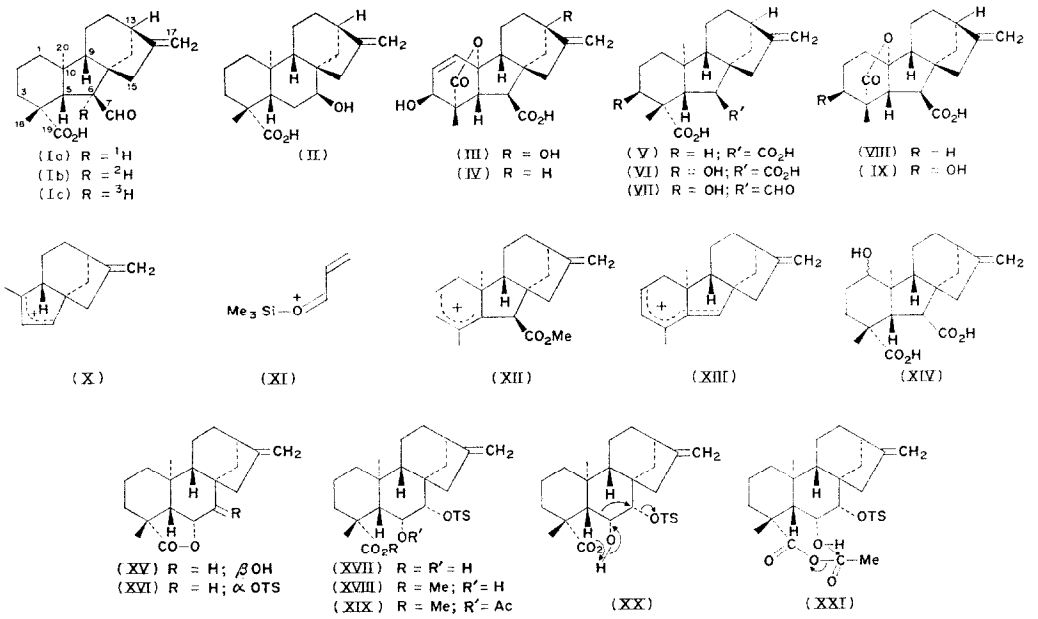
MOST of the published work on the biosynthesis of the gibberellins (GAs) in *Gibberella fujikuroi* has been conducted with high yielding strains such as ACC 917. This paper concerns the 3-hydroxylation of GA_{12}-aldehyde (Ia) in the slow growing strain REC-193A which is wild-type for GA production and a mutant for colonial growth and amylase activity.[1] This strain was obtained[1] as a random ascospore from the perithecium of a strain of *G. fujikuroi* originally isolated from rice plants in Japan. Subsequent papers will deal with mutants that block specific steps in the GA biosynthetic pathway.

GA_{12}-aldehyde (Ia) is probably the initial *ent*-gibberellane from the *in vivo* contraction of *ent*-7α-hydroxykaur-16-en-19-oic acid (II). It occurs in cultures[2] of *G. fujikuroi* ACC 917 which convert it efficiently and specifically[3] into GA_3 (III). This aldehyde (Ia) is also formed[4] from mevalonic acid lactone in a cell-free preparation from the seed of *Cucurbita pepo*. In cultures of *G. fujikuroi* ACC 917 Cross *et al.*[3] found that the incorporation of [17-^{14}C]-GA_{12}-aldehyde into [17-^{14}C]-GA_3 was much higher (15·4%) than that (0·7%) of the corresponding acid, GA_{12} (V). From this observation and the non-incorporation of GA_9 (VIII) into GA_3 (III) Cross *et al.*[3] concluded that 3-hydroxylation in the biosynthesis of GA_3 (III) occurred before oxidation to GA_{12} (V) and before the formation of the 19,10-lactone. Direct evidence is now presented in support of this view.

When strain REC-193A was grown in shake-flask culture on a potato-dextrose liquid (PDL) medium for 11 days, the usual range of GAs and related *ent*-kaurenoids was produced

* Part VI in the series "Fungal Products". For Part V see (1973) *J. Chem. Soc. Perkin I* in press.

[1] PHINNEY, B. O. and FUKUYAMA, M. (1973) in preparation.
[2] HANSON, J. R., HAWKER, J. and WHITE, A. F. (1972) *J. Chem. Soc. Perkin I* 1892.
[3] CROSS, B. E., NORTON, K. and STEWART, J. C. (1968) *J. Chem. Soc.* C, 1054.
[4] GRAEBE, J. E., BOWEN, D. H. and MACMILLAN, J. (1972) *Planta* **102**, 261.



These metabolites were conveniently identified by combined GC–MS of the derivatized total extract from the culture filtrates; those identified by GC–MS of the methylated extract and of the methylated–trimethylsilylated extract are listed in Table 1. When REC-193A was grown on PDL medium for 5 days or less, little or no GAs were produced as shown by the GC trace of the methylated extract (Fig. 1a). When grown on PDL containing GA_{12}-aldehyde (Ia) this substrate was rapidly metabolised (Fig. 1b) mainly to GA_{14} (VI) and slightly to GA_{12} (V); these products from GA_{12}-aldehyde were identified by GC–MS of the methylated extract from 5-day cultures. In contrast GA_{12} (V) was largely unmetabolized (Fig. 1c) by REC-193A, grown under identical conditions of culture. These cultures were able to metabolize at least 5·5 mg GA_{12}-aldehyde (Ia) per 100 ml culture medium in 5 days. A time course study of this metabolism from 1 to 5 days showed that GA_{12} (V) and GA_{14} (VI) were initially formed in equal amount. However, after 3 days GA_{12} (V) had reached a maximum level and thereafter only the GA_{14} concentration increased until the GA_{12}-aldehyde (Ia) had been completely metabolized.

TABLE 1. METABOLITES OF *Gibberella fujikuroi* REC-193A IDENTIFIED BY GC–MS OF THE METHYLATED AND METHYLATED TRIMETHYLSILYLATED EXTRACT FROM 11-DAY CULTURES

GA_1		
GA_4	GA_{13}	Fusaric acid
GA_7	GA_{14}	4-Hydroxyphenyl acetic acid
GA_9	GA_{25}	*ent*-6,7-*seco*kaur-16-ene-6,7,19-trioic acid
GA_{12}		*ent*-7-oxo-6,7-*seco*kaur-16-ene-6,19-dioic acid

The specific conversion of GA_{12}-aldehyde into GA_{14} (VI) was confirmed by using [6-^{2}H]-GA_{12}-aldehyde (Ib); GC–MS of the methylated extract from a 5-day culture (see Fig. 1b) established that GA_{12} (V) and GA_{14} (VI) were formed without dilution of the [^{2}H]-label. The percentage conversion of GA_{12}-aldehyde was determined from a feed of

[6-^{3}H]-GA_{12}-aldehyde (Ic). After 3 days' culture, unlabelled GA_{12} (V) and unlabelled GA_{14} (VI) were added to the total extract which was then fractionated by partition chromatography on Sephadex G25 with the solvent system C_6H_6–AcOH–H_2O (8:5:3) (see Ref. 5).

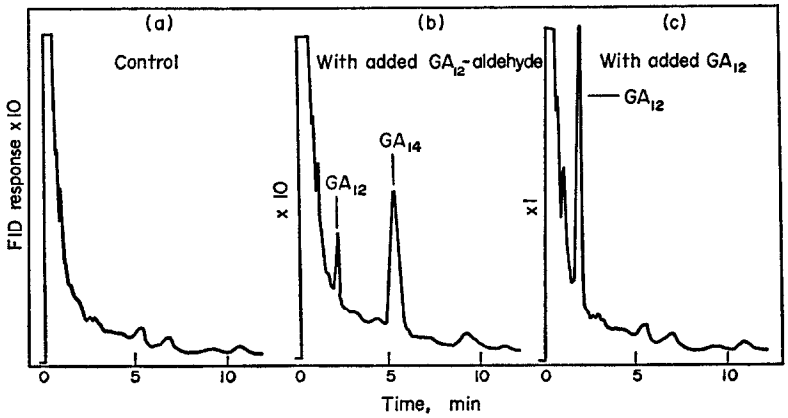


FIG. 1. GC OF METHYLATED 10^{-3}-ALIQUOTS OF UNFRACTIONATED EXTRACTS FROM 5-DAY CULTURES OF *Giberella fujikuroi* REC-193A.

The fractions were monitored by scintillation radio-counting. The [6-^{3}H]-GA_{12}, recovered from fractions 19–25, was crystallized to constant activity which showed 8·3% incorporation of the [^{3}H]-label from the substrate (Ic). The [6-^{3}H]-GA_{14}, recovered from fractions 35–42 contained 45% of the total label from the substrate (Ic). Fractions 48–55 which contained 17·3% of the label were methylated and shown by GC–MS to contain mainly GA_4 (IX) and two unidentified minor products. Similar GC–MS analysis of fractions 60–70 which contained 6·6% of the label revealed the presence of only GA_7 (IV). Fractions 31–39 contained 16% of the label; GC–MS of a methylated aliquot showed the presence of GA_{14} (VI) and a major component with the same MW as GA_{14}; several minor but unidentified components were also detected. Combined gas chromatography-radio counting (GC–RC) of this fraction, essentially by the method of Belham and Neal,[6] showed that the major component and at least two of the incompletely resolved minor components were radioactive. GC–MS of the methylated–trimethylsilylated fractions 31–39 showed that the major component formed a mono-TMSi ether. The MS of this derivative was similar to that of $MeGA_{14}$ TMSi and indicated that this major component was isomeric with GA_{14}. The presence of the hydroxy group in ring A was indicated by the base peak at *m/e* 231 which corresponds to the ion (X) obtained from cleavage of the 1, 10- and 4,5-bonds; the related $MeGA_{14}$ and $MeGA_{18}$ TMSi ethers show[7] significant ions at *m/e* 231 and 319 respectively which correspond to analogous fragmentations. An intense ion at *m/e* 129 (XI) is present which is characteristic[7] of 3-TMSi ethers of GAs, but which could also be formed from 1-TMSi ethers by a similar fragmentation. The absence of a 3-hydroxyl group is suggested by the absence of significant peaks at *m/e* 298 and 239 which are present in the MS of $MeGA_{14}$ and $MeGA_{14}$ TMSi ether and which can be ascribed to the conjugated ions (XII) and

[5] PITEL, D. W., VINING, L. C. and ARSENAULT, G. P. (1971) *Can. J. Biochem.* **49**, 183.
[6] BELHAM, J. E. and NEAL, G. E. (1972) *Anal. Biochem.* **45**, 6.
[7] BINKS, R., MACMILLAN, J. and PRYCE, R. J. (1969) *Phytochemistry* **8**, 271.

(XIII). Furthermore, the formation of a 3α-hydroxy GA is biogenetically improbable while 1-hydroxylation is known, for example, in the fungal GA_{16}. It is therefore suggested that the major component in fractions 31–39 is a 1-hydroxy GA (XIV).

The results from the feed of [6-^{2}H]-GA_{12}-aldehyde show that this aldehyde is converted into GA_{12} (V), GA_{14} (VI), GA_4 (IX) and GA_7 (IV) in short term cultures of *G. fujikuroi* REC-193A. The non-metabolism of GA_{12} (V) under identical culture conditions could be due to the inability of the diacid to penetrate. However, this is unlikely since GA_{12} is rapidly metabolised by another mutant of *G. fujikuroi* (unpublished results). It is concluded therefore that GA_{12} is probably not on the direct pathway between GA_{12}-aldehyde (Ia) and GA_3 (III). Thus 3-hydroxylation occurs before oxidation of GA_{12}-aldehyde (Ia) to GA_{12} (V) and before formation of the 19,10-lactone bridge. The inferred intermediacy of GA_{14}-aldehyde (VII) will be substantiated in a forthcoming publication. Our results also provide the first demonstration that the enzymes required for GA synthesis are present in the trophophase[8] of *G. fujikuroi* cultures; all previous biosynthetic studies relate to the idiophase.

Gibberellin A_{12}-aldehyde (Ia), required for the above studies, was prepared from *ent*-6β,7α-dihydroxykaur-16-en-19-oic acid 19,6-lactone (XV), essentially as described by Cross *et al.*[3] The overall yield was significantly increased in two ways. First the efficiency of the ring contraction of the intermediate tosylate (XVI) to GA_{12}-aldehyde by *t*-BuOK in *t*-BuOH was increased by 5–10% by the addition of 10% H_2O.[9] Secondly the by-product (XVII) from this ring contraction was converted in fair yield into GA_{12}-aldehyde (Ia) by refluxing collidine or by sodium hydride in tetrahydrofuran at room temperature. The mechanism of the ring contraction of the hydroxy-acid (XVII) is of interest since Cross *et al.*[3] observed that the corresponding Me ester (XVIII) underwent ring contraction with difficulty and in minute (4%) yield with NaOMe in refluxing MeOH in contrast to the 35% yield from the 6-acetate (XIX). We have found that the Me ester (XVIII) is also stable to NaH in tetrahydrofuran. The depicted mechanism (XX) for the ring contraction of the acid (XVII) may account for these results. Steric hindrance to the abstraction of the 6-hydroxy proton in the Me ester (XVIII) can be circumvented in the acid (XVII) by internal attack of the carboxyl anion. The sterically less demanding attack by base at the carbonyl carbon of the 6-acetate (XIX) would account for the observed rearrangement of the latter. These steric arguments are supported by the ease of acetylation of the acid (XVII), observed in the present studies and explicable by intramolecular acetylation via the mixed anhydride (XXI), compared to the difficult acetylation of the Me ester (XVIII).[3,10]

[6-^{2}H]- and [6-^{3}H]-gibberellin A_{12}-aldehydes (Ib) and (Ic) were prepared from GA_{12}-aldehyde (Ia) in two ways: (a) with NaOMe in MeO^2H or MeO^3H, and (b) more conveniently in boiling tetrahydrofuran containing NaOMe and ^2H_2O or ^3H_2O. [6-^{3}H]-Gibberellin A_{12}-aldehyde (Ic) was purified by chromatography on Sephadex LH20 using the bi-phasic solvent system, PE–EtOAc–AcOH–MeOH–H_2O (50:15:10:10:2), a method developed in this laboratory by C. M. Wels (unpublished results). The [6-^{3}H]-aldehyde (Ic) had a specific activity of 0·89 μCi μmol^{-1}. [6-^{2}H]-Gibberellin A_{12}-aldehyde (Ib) was shown by MS to contain *ca.* 90%-[$^2H_1$] which was located at the 6-position by NMR. The high field doublet, present in the unlabelled aldehyde (Ia) at τ 6·78 and part of the AM system of the 5,6-protons, was absent from the spectrum. The GA_{12}-aldehydes (Ia–c) were stable as crystalline solids but were oxidized to GA_{12} (V), slowly as gums and more rapidly in

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[9] HANSON, J. R. personal communication.
[10] HANSON, J. R. (1966) *Tetrahedron* **22**, 2877.

solution. [6-^{2}H]-Gibberellin A_{12}-aldehyde (Ib) was preparatively oxidized by Jones reagent to GA_{12} (V) without loss of label providing another example[11] that oxidation of aldehydes with chromium trioxide does not proceed via the enol.

EXPERIMENTAL

Provenance of G. fujikuroi *REC*-193A. Strain REC-193A was obtained[1] from a wild-type, N-481, of *G. fujikuroi*, originally isolated from rice seedlings on Honshu Island, Japan. During sub-culturing of N-481, perithecia appeared fortuitously. REC-193A was one of the strains that came from the germination of 500 ascospores which were collected randomly from these perithecia. REC-193A is thus homocaryotic in origin.

Culture conditions. Sub-master cultures of *G. fujikuroi* REC-193A, grown on slopes of potato-dextrose agar, were used to innoculate potato-dextrose liquid[12] medium (100 ml) in 500 ml conical flasks. These cultures, grown at 25° for 3 days on a reciprocal shaker, were successively sub-cultured, to ensure homogeneity, by transferring innocula (1 ml) to fresh medium (100 ml) in 500 ml conical flasks. After 3 such transfers, the cultures were grown at 25° for 3–11 days with, or without, the following substrates (added in Me_2CO to the hot autoclaved media before the third transfer): (a) GA_{12}-aldehyde (Ia; 0·5–5·5 mg); (b) [6-^{2}H]-GA_{12}-aldehyde (Ib; 0·5–5·5 mg); (c) [6-^{3}H]-GA_{12}-aldehyde (Ic; *ca.* 100 μg); and (d) GA_{12} (V; 0·5–5·5 mg).

Analysis of metabolites. (a) *From* 11*-day cultures without added substrate.* A portion (10^{-3}) of the unfractionated EtOAc extract from the culture filtrate (100 ml) was methylated (CH_2N_2) then trimethyl silylated with hexamethyldisilazane–trimethylsilylchloride (1:1) in pyridine. The derivatized metabolites were examined by GC–MS on a Varian MAT CH7 fitted with a Biemann–Watson molecular separator. The GC conditions were: 2% QF-1 on Gaschrome Q (100–120 mesh) in a glass column (213 cm × 1·6 mm i.d.) with an He flow-rate of 7 ml min^{-1} and temp. programmed from 210° at 2° min^{-1}. The compounds were identified by direct comparison of the MS with those of authentic compounds.[7]

(b) *From 5-day cultures without added substrate.* A portion (10^{-3}) of the total extract was methylated and examined by GC on 2% QF-1 on Gaschrome Q (80–100 mesh) in a glass column (150 × 0·47 cm i.d.) isothermally at 200° with N_2 flow-rate of 75 ml min^{-1}. The FID trace is shown in Fig. 1.

(c) *From 5-day cultures with added* [6-^{1}H]- and [6-^{2}H]-GA_{12}*-aldehydes.* The crude extracts from each substrate were separately examined by GC as in (a) after methylation and by GC–MS after methylation and after methylation–trimethylsilylation as in (b) except that a 2% SE33 column was used. The GA_{12} and GA_{14} from the unlabelled substrate were identified from the published[7] MS of these derivatives. The MS of the derivatives of [6-^{2}H]-GA_{12} and -GA_{14} showed the same [^{2}H]-content as the [6-^{2}H]-GA_{12}-aldehyde.

(d) *From a 3-day culture with added* [6-^{3}H]-*GA_{12}-aldehyde.* The filtrate from a shake-flask (20 ml) culture grown in the presence of [6-^{3}H]-GA_{12}-aldehyde (*ca.* 101 μg, 628 000 decomp. min^{-1}) was extracted with EtOAc. Unlabelled GA_{12} (9·93 mg) and GA_{14} (11·54 mg) were added to the extract which was dissolved in the minimum vol. of the aqueous phase of the two phase system C_6H_6–AcOH–H_2O (8:5:3) and absorbed in Sephadex G25 (fine, 200 mg). The latter was then added to a column (136 × 1·5 cm) of Sephadex G25 (fine) which had been swollen with the aqueous phase and equilibrated with the organic phase of the above solvent system. The column was eluted with the organic phase (flow-rate 1·25 ml min^{-1}) and fractions (5 ml) were collected. Aliquots were evaporated to dryness and counted with 32% efficiency in a Tracerlab Corumatic 200 Scintillation Counter in 5% Butyl-PBD in toluene-methoxyethanol (3:2). Fractions 19–25 were pooled and the purple pigment was removed from the recovered material by TLC on silica gel with EtOAc–PE–AcOH (50:50:1). The band at R_f 0·3 was eluted with EtOAc and the recovered material was crystallized from Me_2CO–PE to constant activity to give GA_{12} m.p. 245–248°; 5250 decomp. mg^{-1} min^{-1} (8·3% incorporation); one peak by GC of a methylated aliquot. Fractions 40–47 were pooled and treated as above. The material recovered from TLC [silica gel; EtOAc–PE–AcOH (60:40:1)] at R_f 0·25 crystallized from MeCOEt–PE to constant activity to yield GA_{14} m.p. 221–223°, 24 537 decomp. mg^{-1} min^{-1} (45% incorporation); one peak by GC of Me ester. Fractions 48–55 (108 603 decomp. min^{-1}; 17·3% incorporation) were bulked. GC of a methylated aliquot showed a major peak, identified as GA_4 Me ester by GC–MS under the conditions specified in (b). Fractions 60–70 (41 500 decomp. min^{-1}; 6·6%) were bulked. GC of a methylated aliquot showed only one peak identified as GA_7 Me ester by GC–MS under the conditions specified in (b). Fractions 31–39 (99 800 decomp. min^{-1}; 16% incorporation) were bulked and an aliquot was examined by GC–MS as described in (b) as the Me and MeTMSi derivatives. The major peak which was shown to be radioactive by the method of Belham and Neal[6] had the following MS. Me ester *m/e* (%) 376 (M^+, 4), 344(100), 330(10), 329(23), 269(9), 227(6), and 164(15); Me ester TMSi ether *m/e* (%) 448 (M^+,11), 433(10), 416(85), 401(10), 287(59), 259(26), 231(100) and 129(57).

Gibberellin A_{12}-aldehyde (Ia). (a) *From* ent-6*β-hydroxy-*7*β-tosyloxykaur*-16-*en*-19-*oic acid* 19,6-*lactone* (XVI). The tosylate (180 mg) in a mixture of KOH (1·8 g), *t*-BuOH (15ml) and H_2O (1·5 ml) was refluxed for

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[12] Spector, C. and Phinney, B. O. (1908) *Physiol. Plant.* **21**, 127.

6 hr under N_2. The reaction mixture was concentrated *in vacuo*, diluted with H_2O, acidified to pH 2·5 and then extracted with EtOAc. The gum, recovered from the EtOAc, was purified by TLC on silica gel HF with EtOAc–PE–AcOH (50:50:1). Elution of the band at R_f 0·5 (bright green colour with 5% H_2SO_4 in EtOH at 120°) with EtOAc gave GA_{12}-aldehyde (Ia), crystallized from Me_2CO–PE in needles (66·5 mg), m.p. 159–162° (lit.[3] m.p. 159–163°) identified from the published IR;[3] τ 9·26 (3H, *s*, 20-H), 8·86 (3H, *s*, 18-H), 6·78 (1H, *J* 5·5, 13·0 Hz, 6-H), 5·26 (1H, *br*, 17-H), 5·14 (1H, *br*, 17-H), and 0·32 (1H, *J* 5·5 Hz, 7-H); *m/e* (% base peak) 316 (M^+, 3), 298(15), 270(67), 241(100) and 239(22). The Me ester of GA_{12} aldehyde, prepared with CH_2H_2 had *m/e* (%) 330(M^+, 7), 287(12), 270(43), 255(15), 241(100), and 239(19). In some experiments, substantial quantities of a second product, R_f 0·35, were obtained. Elution of this band with EtOAc and crystallization of the recovered gum from EtOAc–PE gave ent-6β-*hydroxy*-7β-*tosyloxykaur*-16-*en*-19-*oic acid* (XVII), m.p. 161·5–162° (with decomp.) (Found: C, 66·3; H, 7·6; S, 6·3, $C_{27}H_{34}O_6S$ requires C, 66·4; H, 7·4; S, 6·5%); ν_{max} 3550, 1745, 1655, 1599 and 898 cm^{-1}; τ 8·93 (3H, *s*, 20-H), 8·90 (3H, *s*, 18-H), 7·57 (3H, *s*, Ar-$\underline{C}H_3$), 5·60 (1H, *br*, 6-H), 5·47 (1H, *d*, *J* 3Hz, 7-H), 5·21 (2H, *br*, 17-H_2), 2·74 (2H, *d*, *J* 8 Hz, Ar$\underline{H}$) and 2·21 (2H, *d*, *J* 8 Hz, Ar$\underline{H}$). The methyl ester (XVIII) crystallized from EtOAc–PE with m.p. 179·5–180° (lit.[3,13] m.p. 190–192° and 186–188°); ν_{max} 3365, 3040, 1691, 1658, 1598 and 887 cm^{-1}; τ 8·95 (6H, *s*, 18-H and 20-H), 7·59 (3H, *s*, ArMe), 6·28 (3H, *s*, OMe), 5·72 (1H, *br d*, *J* 2 Hz, 6-H), 5·55 (1H, *br d*, *J* 2 Hz, 7-H), 5·22 (2H, *br*, 17-H_2), 4·57 (1H, *s*, 6-OH, exchanged with D_2O), 2·69 (2H, *d*, *J* 8 Hz, Ar$\underline{H}$) and 2·14 (2H, *d*, *J* 8 Hz, Ar$\underline{H}$). The acetate Me ester (XIX, 30 mg), prepared from the acid (34 mg), pyridine (10 ml) and Ac_2O (3 ml) at 20° for 24 hr, followed by methylation with CH_2N_2 had m.p. 177·5–178° (with decomp.) (lit.[3] m.p. 177–178°) with IR and NMR spectra identical to the published spectra.

(b) *From* ent-6β-*hydroxy*-7β-*tosyloxykaur*-16-*en*-19-*oic acid* (XVII) *and* NaH. The tosylate (50 mg), NaH (125 mg; 50% dispersion in oil) and THF (25 ml) were stirred at 20° for 24 hr; H_2O was added cautiously and the mixture was concentrated *in vacuo*. After the addition of 2 N HCl the product was recovered in EtOAc and purified as in (a) to give GA_{12}-aldehyde (16·6 mg), identified by TLC and by GC–MS of the methyl ester.

(c) *From* ent-6β-*hydroxy*-7β-*tosyloxykaur*-16-*en*-19-*oic acid* (XVII) *and collidine*. The tosylate (900 mg) in dry collidine (70 ml) was refluxed for 24 hr under N_2. After the addition of H_2O and 2 N HCl, the product was recovered in EtOAc and chromatographed twice on a column of silica. Gibberellin A_{12}-aldehyde (297 mg) was eluted with 15–20% EtOAc in PE and identified by TLC and by GLC of the methyl ester.

[6-^{2}H]-*Gibberellin* A_{12}-*aldehyde* (Ib). (a) Gibberellin A_{12}-aldehyde (7 mg), MeO^2H (0·5 ml) and NaOMe (60 mg) were heated at 110° for 18 hr in a sealed tube. After the addition of ^2H_2O the solution was concentrated and acidified to pH 2·5. The product was recovered in EtOAc and purified by TLC on silica gel HF with Me_2CO–PE–AcOH (30:70:1). The band at R_f 0·45 was eluted with EtOAc to give [6-^{2}H]-GA_{12}-aldehyde (5 mg) containing *ca.* 90%-[^{2}H] from the MS of the acid and Me ester; the NMR showed a singlet at 0·32τ for 7-H, no 6-H signal at 6·78τ. (b) Gibberellin A_{12}-aldehyde (4 mg), NaOMe (1·3 g), THF (2 ml), distilled from CaH_2), and ^2H_2O (1 ml) were boiled for 5 hr. After the addition of saturated aqueous KH_2PO_4, the product was recovered in EtOAc and was purified as in (a) to give [6-^{2}H]-GA_{12}-aldehyde (2 mg) containing *ca.* 90%-[^{2}H] by MS.

[6-^{3}H]-*Gibberellin* A_{12}-*aldehyde* (Ic). (a) GA_{12}-aldehyde (80 mg), MeO^3H (0·5 ml, *ca.* 25 mCi) and NaOMe (60 mg) were heated at 100° for 24 hr in a sealed tube. Tritiated water (0·25 ml) was added to the solution which was reduced in volume *in vacuo* and acidified. The product, recovered in EtOAc, was purified by TLC on silica gel with Me_2CO–PE–HOAc (30:70:1); recovery of the material at R_f 0·6 in EtOAc gave crude [6-^{3}H]-GA_{12}-aldehyde (56 mg, *ca.* 6100 decomp. min^{-1} μg^{-1}) which was purified by liquid-chromatography as described in (c). (b) GA_{12}-aldehyde (230 mg), THF (20 ml, distilled from CaH_2, NaOMe (2 g) and ^3H_2O (1 ml, *ca.* mCi) were refluxed under N_2 for 7·5 hr. The reaction mixture was added to satd. aq. KH_2PO_4 which was extracted with EtOAc. The product was subjected to TLC as in (a) to give crude [6-^{3}H]-GA_{12}-aldehyde (163 mg, *ca.* 6000 decomp. min^{-1} μg^{-1}) which was further purified as in (c). (c) *Purification by Sephadex chromatography*. To a column (100 × 1·5 cm) of Sephadex LH20 swollen in the aqueous phase of the bi-phasic system PE–EtOAc–AcOH–MeOH–H_2O (50:15:10:10:2) was added [6-^{3}H]-GA_{12}-aldehyde (30·6 mg, 202 × 10^6 decomp. min^{-1}) in the minimum vol. of the aqueous phase. The column was eluted with the organic phase and 10 ml fractions were collected. [6-^{3}H]-Gibberellin A_{12}-aldehyde, obtained from fractions 16–23, was crystallized from Me_2CO–PE to constant radioactivity (5 mg, 6210 decomp. min^{-1} μg^{-1}).

Gibberellin A_{12} (V). Gibberellin A_{12}-aldehyde (Ia, 33·3 mg) in acetone (5 ml) was stirred with Jones reagent (0·06 ml) at −10° for 2 hr. MeOH was then added to the solution which was concentrated *in vacuo*, diluted with H_2O and acidified to pH 2·5. The product recovered in EtOAc was purified by TLC on silica gel HF with EtOAc–PE–AcOH (50:50:1). Elution of the band at R_f 0·3 gave GA_{12} (16 mg) m.p. 245–247·5° (from Me_2CO–PE) (lit. m.p. 244–247°), identified by MS of Me ester.[7]

[6-^{2}H]-*Gibberellin* A_{12}. [6-^{2}H]-GA_{12}-aldehyde (6·8 mg) in acetone (1 ml) was treated with Jones reagent (0·012 ml) as above to give [6-^{2}H]-GA_{12} (6·8 mg). GC–MS of the Me ester showed *ca.* 90% [$^2H_1$]-content.

[13] GALT, R. H. B. and HANSON, J. R. (1965) *J. Chem. Soc.* 1565.

[6-^{3}H]-*Gibberellin* A_{12}. [6-^{3}H]-Ga_{12} was obtained from a tritiation of GA_{12}-aldehyde in which the sealed tube exploded during heating. A portion of the gummy product recovered from the portective metal tube was chromatographed on a column (100 × 1·5 cm) of Sephadex LH20 as described above for [6-^{3}H]-GA_{12}-aldehyde. In addition to [6-^{3}H] GA_{12}-aldehyde in fractions 16–23, [6-^{3}H]-GaA_{12} was recovered from fractions 30–40 and crystallized from Me_2CO–PE to constant activity (4900 decomp. min^{-1} μg^{-1}).

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