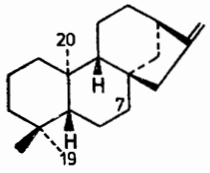
Removal of C-20 in Gibberellin Biosynthesis

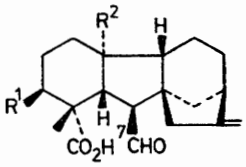
By BRIAN DOCKERILL, ROGER EVANS, and JAMES R. HANSON*
(*The School of Molecular Sciences, University of Sussex, Brighton, Sussex* BN1 9QJ)

Summary Gibberellin A_{13} and A_{14} 7-aldehydes have been tentatively identified as metabolites of gibberellin A_{12} aldehyde in a cell-free system derived from *Gibberella fujikuroi*; gibberellin A_{13} 7-aldehyde is incorporated into gibberellic acid, and the C-20 carbon atom which is removed at this stage has been isolated as carbon dioxide.

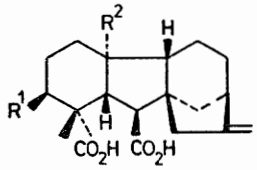
THERE are four stages in the biosynthesis of the C_{19} gibberellin plant growth hormones such as gibberellic acid (**11**).[1] These are: first, the cyclization of geranylgeranyl pyrophosphate to form ent-kaur-16-ene (**1**); second, the oxidative modification of this to afford ent-7α-hydroxykaur-16-en-19-oic acid; third, the ring contraction of this to form gibberellin A_{12} aldehyde (**2**); and fourth, the relationship between the gibberellins including the loss of the angular C-20 carbon atom to form the C_{19} gibberellins. We report some experiments concerning this stage in the biosynthesis.



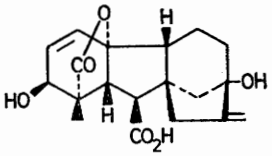
(1)



(2) R^1 = H, R^2 = Me
(3) R^1 = OH, R^2 = Me
(4) R^1 = OH, R^2 = CO_2H



(5) R^1 = OH, R^2 = Me
(6) R^1 = OH, R^2 = CH_2–O–(19,20 lactone)
(7) R^1 = OH, R^2 = CHO
(8) R^1 = OH, R^2 = CO_2H
(9) R^1 = H, R^2 = Me
(10) R^1 = O, R^2 = Me (7,19-dimethylester)



(11)

There are several groups of C_{20} gibberellins in which each member differs from the other in the oxidation level of C-20, *e.g.* gibberellins A_{14} (**5**), A_{37} (**6**), A_{36} (**7**), and A_{13} (**8**), and it has been tempting to link these in a biosynthetic sequence. However gibberellins A_{13} (**8**)[2,3] and A_{14} (**5**)[4] are not converted into the C_{19} gibberellins by *Gibberella fujikuroi* (wild-type strain ACC 917) over a 24 h period whilst an earlier precursor, ent-kaur-16-ene (**1**) is efficiently incorporated in this time. Gibberellin A_{14} (**5**) is slowly metabolized[5] by the mutant B1-41a in which the normal biosynthesis is blocked, and by the 'wild-type' fungus in a longer incubation.[2] Gibberellin A_{12} 7-aldehyde (**2**)[2] is the first detectable[6] compound possessing the gibberellin skeleton to be formed by *G. fujikuroi*. There is then a divergence in the biosynthetic pathways.[4,5] Whereas metabolism of the aldehyde (**2**) by *G. fujikuroi* (ACC 917) affords the characteristic (based on mevalonate incorporation) distribution of 3-hydroxy- and 3-deoxy-gibberellins, the 7-carboxylic acid, gibberellin A_{12} (**9**), affords only 3-deoxygibberellins. With longer incubation periods[2] or with the blocked B1-41a mutant,[5] some (induced ?) transformation of the acid to 3-hydroxylated gibberellins has been observed in which the acid may act as a substrate for an aberrant path through a metabolic grid. It has been suggested[2] that the 7-carboxylic acids are at too high an oxidation level and that the aldehydes may be the preferred intermediates.

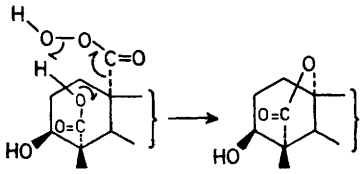
[7-^{3}H,17-^{14}C]Gibberellin A_{12} aldehyde (**2**) was prepared by reducing the untritiated aldehyde[2] with sodium [^{3}H]borohydride followed by reoxidation to the aldehyde with pyridinium chlorochromate. It was incubated with a 10,000 g cell-free system, supplemented with NAD–NADH and NADP–NADPH, and derived as described previously[7] from *G. fujikuroi*. Two unstable metabolites, both containing ^{3}H and ^{14}C, have been separated by radio-t.l.c. One was identified as gibberellin A_{14} aldehyde (**3**)[8] by monomethylation (mass spectroscopy), oxidation (CrO_3, loss of ^{3}H), and further methylation to afford 3-oxo-gibberellin A_{14} dimethyl ester (**10**) (identical on g.l.c. and t.l.c. with authentic material prepared from gibberellin A_{14}). This aldehyde was incorporated into gibberellic acid in 7% yield. The other, more polar, metabolite was homogeneous by t.l.c. and was tentatively identified as gibberellin A_{13} 7-aldehyde (**4**). It was readily autoxidized with the loss of tritium, to form gibberellin A_{13} (**8**), identified by co-chromatography and by the mass spectrum of its trimethyl ester. The aldehyde was incorporated into gibberellic acid (**11**) (12·9%) and gibberellin A_4/A_7 (2·9%) by intact cultures of *G. fujikuroi*.

The final oxidation level of the C-20 carbon atom which is lost was determined as follows. ent-Kaur-16-ene (**1**), which is the parent hydrocarbon of gibberellic acid,[9] was prepared labelled, *inter alia*, at C-20 by biosynthesis from [2-^{14}C]acetate. It was incubated with a culture of *G. fujikuroi* for 24 h whilst the normal biosynthesis of endogenous ent-kaur-16-ene was inhibited with AMO 1618.[10] The carbon dioxide produced by the fermentation was collected in ethanolamine[11] and counted. The efficiency of collection (98·2%) was standardized against sodium [^{14}C]carbonate. Radio-t.l.c. scanning showed that gibberellic acid and gibberellins A_4 and A_7 were the radioactive C_{19} gibberellins which had been produced. These were isolated and counted (see Table). The fermentations were also examined by dilution analysis for formaldehyde (as its dimedone derivative) and formic acid (as its *p*-bromophenacyl derivative). Whilst the carbon dioxide was active at the level (*ca.* 1/11th) to be expected on the basis of the activity of the C_{19} gibberellins, both the formaldehyde and formic acid derivatives were inactive. Furthermore no radioactive carbon dioxide was detected when gibberellic acid, labelled in the γ-lactone ring, was incubated with *G. fujikuroi* over this period. Hence the final fate of the C-20 carbon atom is CO_2.

A mechanism for this decarboxylation must account for five observations. First, the retention of mevalonoid hydrogen at C-1, C-5, and C-9 in the formation of the C_{19} gibberellins excluding unsaturated intermediates at these centres[12] (unlike sterol demethylation); second, the in-

TABLE. Radio-activity (d.p.m. × 10^{-5}) in carbon dioxide

Kaurene fed	Gibberellic acid	Gibberellins A_4/A_7	Total C_{19} gibberellins	Expected CO_2	Found CO_2	% Recovery
15·13	1·696	1·319	3·015	0·2740	0·2182	79·6
15·13	1·558	1·290	2·848	0·2589	0·1972	76·2



SCHEME

corporation of both 19-oxygen atoms from gibberellin A_{12} (alcohol) in the lactone ring;[13] third, the intervention of gibberellin A_{13} 7-aldehyde; fourth, the formation of carbon dioxide;[14] and fifth, the formation of the lactone ring on the same face of the molecule as the departing C-20 atom. A possible mechanism might involve a C-20 per-acid (see Scheme).

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