The Ring Contraction Step in Gibberellin Biosynthesis

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Summary The conversion of *ent*-7α-hydroxykaurenoic acid (**1**) into gibberellin A_{12}-aldehyde (**2**) and *ent*-6α,7α-dihydroxykaurenoic acid (**3**) involves the loss of one hydrogen atom at C-6.

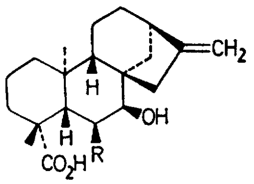
WE have previously demonstrated the conversion of *ent*-7α-hydroxykaurenoic acid (**1**) by ring contraction into gibberellin A_{12}(GA_{12})-aldehyde (**2**) and by hydroxylation to *ent*-6α,7α-dihydroxykaurenoic acid (**3**) in a cell-free system from the endosperm of *Cucurbita maxima* seed.[1,2] The formation of GA_{12}-aldehyde (**2**) is part of the pathway by which the cell-free system converts mevalonate into several gibberellins including gibberellin A_4.[2,3] In the presence of Mn^{2+}, however, GA_{12}-aldehyde (**2**) is converted only into GA_{12} (**4**), further steps being inhibited. The *ent*-6α-hydroxylation is an alternative reaction which takes place simultaneously with the ring contraction both in the presence and absence of Mn^{2+}. The incubation of *ent*-7α-hydroxykaurenoic acid (**1**) in the presence of Mn^{2+} leads to the formation of only four major products: GA_{12}-aldehyde (**2**), GA_{12} (**4**), *ent*-6α,7α-dihydroxykaurenoic acid (**3**), and an oxidation product of the latter, an unidentified *ent*-kaurenoic acid derivative. We now present results on the mechanism of the ring contraction and *ent*-6α-hydroxylation using this system and ^{3}H : ^{14}C double labelled substrate.

Table. Radio-activity (d.p.m.) of *ent*-7α-hydroxykaurenoic acid and products

Compound	^{3}H	^{14}C	^{3}H : ^{14}C
ent-7α-hydroxykaurenoic acid (**1**)			
Initial (1/10th aliquot)	6792	3335	2·04
Recovered	28555	12654	2·26
GA$_{12}$-aldehyde (**2**)	6993	7126	0·98
GA$_{12}$ (**4**)	4279	4748	0·90
ent-6α,7α-Dihydroxykaurenoic acid (**3**)	6496	5841	1·11
" " " " metabolite ..	4506	3502	1·29

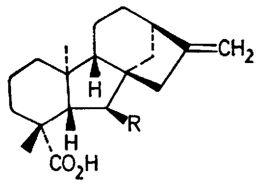
ent-6,6-[^{3}H$_{2}$]-7α-Hydroxykaurenoic acid was prepared from *ent*-7-oxokaurenoic acid by exchange with MeONa–MeO^{3}H followed by reduction with AlPr$^{i}_{3}$–Pr^{i}OH and was mixed with ^{14}C-labelled *ent*-7α-hydroxykaurenoic acid prepared from 2-[^{14}C]mevalonate in the cell-free system. The specific activity of the ^{3}H-label in the mixture was 1·9μCi/μmol; the amount of ^{14}C was varied to produce ^{3}H : ^{14}C ratios from 1·39 to 2·04. The doubly labelled substrate was incubated with endosperm preparation in the presence of Mn^{2+} and the usual co-factors.[1] The products were isolated by t.l.c. and their radioactivity measured by liquid scintillation counting using internal standardisation. In several instances the identity of the products was confirmed by g.l.c. Their identification by combined g.l.c.–m.s. has been described previously.[1,2]

The results presented in the Table show that the ^{3}H : ^{14}C ratios in the products are only half of the ratio in the substrate. Thus the formation of GA$_{12}$-aldehyde (**2**) and *ent*-6α,7α-dihydroxykaurenoic acid (**3**) involves the loss of one hydrogen atom from the 6-position in *ent*-7α-hydroxykaurenoic acid (**1**). As would be expected from this mechanism an isotope effect was observed. Since the ^{3}H and ^{14}C labels are present in different molecules in the mixed substrate, the isotope effect leads to a slower conversion of ^{3}H-molecules than ^{14}C-molecules and the ^{3}H : ^{14}C ratio increases in unchanged substrate as the reaction proceeds. Up to a 7-fold increase in this ratio was observed in experiments with the reaction nearly complete with respect to the ^{14}C-substrate.



(1) R = H

(3) R = OH



(2) R = CHO

(4) R = CO$_{2}$H

Based upon a ^{3}H : ^{14}C ratio of 2 : 1 in the substrate, the average ^{3}H : ^{14}C ratios for GA$_{12}$-aldehyde (**2**) and *ent*-6α,7α-dihydroxykaurenoic acid (**3**) in seven experiments were 0·98 and 1·12 respectively. A lower ratio 0·82 was obtained for GA$_{12}$ (**4**) and is probably due to partial exchange during the oxidation step. Our results conflict with those of Hanson *et al.*,[4] who observed no loss of ^{3}H in GA$_{12}$-aldehyde (**2**) isolated from the culture of the fungus *Gibberella fujikuroi* after incubation with [1,1-^{3}H$_{2}$,1-^{14}C]geranyl pyrophosphate. They suggested on the basis of a low (0·005%) incorporation that the *ent*-6α-hydrogen atom migrates to C-7 during ring contraction. Our results are, however, in accord with the proposition by the same group that the ring contraction is initiated by abstraction of the *ent*-6α-hydrogen itself.[5]

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