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CONVERSION OF GIBBERELLIN A_{14} TO OTHER GIBBERELLINS IN SEEDLINGS OF DWARF *PISUM SATIVUM*

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Key Word Index—*Pisum sativum*; Leguminosae; dwarf pea; gibberellins; gibberellin A_{14}; interconversion; gas-liquid radiochromatography.

Abstract—Gibberellin A_{14}-[17-^{3}H] applied to seedlings of dark grown dwarf pea (*Pisum sativum* L. cv. Meteor) was converted to GA_1, GA_8, GA_{18}, GA_{23}, GA_{28} and GA_{38}. The sequence of interconversion of $GA_{14} \rightarrow GA_{18} \rightarrow GA_{38} \rightarrow GA_{23} \rightarrow GA_1 \rightarrow GA_8$ is indicated. Identifications were made by gas-liquid radiochromatography using three liquid stationary phases.

INTRODUCTION

EXTRACTION of gibberellins (GAs) from seedlings of dwarf pea (*Pisum sativum* L.) gives two main fractions containing GA-like activity, one chromatographically similar to GA_1 **(1)** and the other similar to GA_5 **(4)** or GA_{20} **(2)**.[1,2] Using TLC-bioassay, zones other than those of GA_1 or GA_5/GA_{20} have been indicated to have GA-like activity.[3,4] The only GA from dwarf pea that has been characterized is GA_{20} **(2)**, isolated from pods[5] and identified in fruit.[6] The metabolism of GA_1-[^{3}H][7,8] and GA_5-[^{3}H][9] in seedlings of dwarf pea, cv. Progress No. 9, has been investigated. GA_1-[^{3}H] was converted to a biologically active compound of lower R_f on TLC, whilst GA_5-[^{3}H] was converted to a biologically active compound chromatographically similar to GA_1. In seedlings of dwarf pea cv. Meteor, GA_5-[^{3}H] was converted to GA_3 **(5)** and an unknown compound chromatographically similar to GA_3.[10]

GA_{14} **(6)** has been shown[11] to be a precursor of GA_3 **(5)** in the fungus *Gibberella fujikuroi*, although GA_{14}-aldehyde **(7)** gives improved incorporations to GA_3.[12] Although dwarf pea only gives a small response to applied GA_{14},[13] it is of interest to determine whether this GA is also a precursor of gibberellins in this species. In this paper we report the conversion of gibberellin A_{14}-[17-^{3}H] (hereafter abbreviated to GA_{14}-[^{3}H]) to other GAs in seedlings of dwarf pea cv. Meteor.

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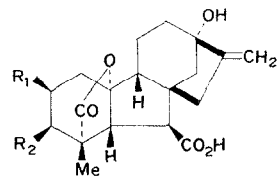
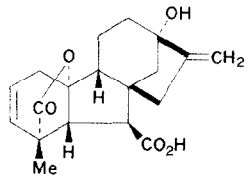
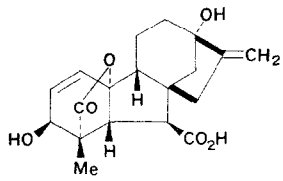


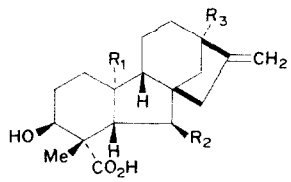
(1) R_1 = H; R_2 = OH
(2) R_1 = H; R_2 = H
(3) R_1 = OH; R_2 = OH



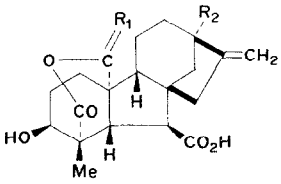
(4)



(5)



(6) R_1 = Me; R_2 = CO_2H; R_3 = H
(7) R_1 = Me; R_2 = CHO; R_3 = H
(8) R_1 = Me; R_2 = CO_2H; R_3 = OH
(9) R_1 = CHO; R_2 = CO_2H; R_3 = OH
(10) R_1 = CO_2H; R_2 = CO_2H; R_3 = OH
(11) R_1 = CHO; R_2 = CO_2H; R_3 = H
(12) R_1 = CO_2H; R_2 = CO_2H; R_3 = H



(13) R_1 = H,H; R_2 = OH
(14) R_1 = O; R_2 = H
(15) R_1 = O; R_2 = OH

RESULTS

GA_{14}-[^{3}H] (70·1 μCi in each of two experiments) was applied to dark grown dwarf pea plants. After 20 hr the treated plants did not show significant increase in growth over controls, whereas at 40 hr the treated plants showed a significant internode elongation response (see Experimental). The shoots were harvested at 20 and 40 hr, and for each harvest three extracts were obtained, a neutral ether extract (20 hr: 0·26 × 10^6 dpm; 40 hr: 0·69 × 10^6 dpm), an acidic, ethyl acetate extract (20 hr: 36·6 × 10^6 dpm; 40 hr: 26·1 × 10^6 dpm) and an acidic butanol extract (20 hr: 4·6 × 10^6 dpm; 40 hr: 5·2 × 10^6 dpm). The acidic ethyl acetate extracts were partially purified by silica-gel partition chromatography[14,15] and the eluted fractions were combined (Table 1) according to counts obtained from direct liquid scintillation spectrometry. The trimethylsilyl ether derivatives of the methyl esters (TMSMe derivatives) of the combined fractions were examined by gas-liquid radiochromatography (GLRC) using three column liquid phases, 2% QF1, 2% SE30 and 1% XE60 and the retention times of the radioactive peaks compared to those of standards. The results are summarized in Table 1. From the 20-hr experiment, fractions 3–6 contained GA_{14} **(6)**, fractions 7–11 contained a residual amount of GA_{14}, fractions 17–19 contained

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GA_{18}[16] **(8)** and GA_{38}[17] **(13)** and fractions 19–21 contained GA_{23}[18] **(9)**. Fractions 23–24 contained trace quantities of GA_8 **(3)** and GA_{28}[19] **(10)**. From the 40-hr experiment, fractions 3–6 contained GA_{14} **(6)**, fractions 7–11 contained a small quantity of residual GA_{14}, fractions 14–16 contained GA_1 **(1)**, fractions 17–19 contained GA_{18} **(8)** and GA_{38} **(3)**, fractions 20–22 contained GA_{23} **(9)** and fractions 23–24 contained GA_8 **(3)** and GA_{28} **(10)**. The presence of GA_1 **(1)** could not be confirmed in the 20 hr extract. No radioactive peaks other than those assigned were observed. The percentage conversions from applied GA_{14}-[^{3}H] were estimated from the radiochromatograms and are given in Table 1.

TABLE 1. GLRC RETENTION TIMES OF TMSMe DERIVATIVES OF SILICA-GEL PARTITION COLUMN FRACTIONS, WITH COMPARISON STANDARDS

Silica-gel partition column fractions	Retention time (min) on 3 columns 2% QF1 (206°)	2% SE30 (203°)	1% XE60 (209°)	Identity	% incorporation from GA_{14}-[^{3}H]* 20 hr	40 hr
3–6	4·4	8·4	6·1	A_{14}		
7–11	4·6	8·4	6·1	A_{14}		
14–16	14·1	15·3	15·3	A_1		0·57
17–19	6·3	13·8	7·3	A_{18}	4·8	1·74
	40·0	33·4	54·4	A_{38}	1·43	0·80
20–22	11·4	18·8	14·8	A_{23}	0·36	0·96
23–25	7·1	17·7	9·6	A_{28}	0·01	0·14
	17·5	25·7	17·5	A_8	0·02	0·50
Standard GAs						
A_1	14·0	15·3	15·3			
A_3	16·4	16·8	18·5			
A_8	17·4	25·5	17·5			
A_{14}	4·4	8·5	6·2			
A_{18}	6·2	13·8	7·4			
A_{23}	11·4	19·1	14·9			
A_{28}	7·0	17·9	9·6			
A_{36}	8·4	12·2	12·4			
A_{38}	40·0	33·2	54·1			

* Calculated on precursor utilized (GA_{14}-[^{3}H] applied GA_{14}-[^{3}H] washed from plant prior to extraction).

In the 20 hr experiment GA_{18} **(8)** was formed in highest yield (indicating a direct conversion from GA_{14}), followed by GA_{38} **(13)**, then GA_{23} **(9)**. The other GAs were only formed in trace quantities. In the 40 hr experiment the amounts of GA_{18} **(8)** and GA_{38} **(13)** had decreased, whereas the yield of GA_{23} **(9)** had increased moderately. GA_1 **(1)** was now present, and the yields of GA_8 **(3)** and GA_{28} **(10)** were considerably increased. In conjunction with the structural relationship between the GAs, these data indicate that applied GA_{14} is most probably metabolized in dwarf pea seedlings in the sequence GA_{14} **(6)** → GA_{18} **(8)** → GA_{38} **(13)** → GA_{23} **(9)** → A_1 **(1)** → GA_8 **(3)**. Intermediate steps may occur, but are not indicated.

The conversion of C_{20}-GAs to C_{19}-GAs in a higher plant has thus been confirmed. It has been suggested[20,21] that this could occur via a Baeyer–Villiger type oxidation of a C-

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20 carbonyl function. In the fungus *Gibberella fujikuroi* the C-20 aldehyde, GA_{36} **(11)**, may be a direct precursor of C_{19}-GAs.[22] In the same species GA_{13} **(12)** is not a precursor of GA_3 **(5)**, GA_4 or GA_7,[11,23] but GA_{13} anhydride **(14)** gives low incorporations into these GAs.[23] Comparing this with the present work, GA_{23} **(9)** or GA_{28} anhydride **(15)** could be the direct precursors of GA_1 **(1)**, and GA_{28} **(10)** would appear to be less likely as a precursor. Furthermore, the conversion of GA_{14} to GA_{28} was lower than those of the other GAs and this may indicate that GA_{28} is not a precursor of GA_1, but rather a side product from GA_{23}.

The above biosynthetic sequence is supported by the reported biological activities of GA_{18}, GA_{23}, GA_{28} and GA_{38}.[18] Results from six bioassays indicated that the degree of biological activity is related to the oxidation of carbon atom 20.[18] Oxidation of the methyl group of GA_{18} **(8)** to hydroxy-methyl (GA_{38}, **13**) or formyl (GA_{23}, **9**) increased the biological activity, but further oxidation to the carboxyl (GA_{28}, **10**) eliminated the activity. Hence, if the activity of the C_{20}-GAs is related to their ease of conversion in the higher plant to C_{19}-GAs, then GA_{18} **(8)**, GA_{38} **(13)** and GA_{23} **(9)** would be the precursors of GAs such as GA_1 **(1)**, whereas GA_{28} **(10)** would be a sideproduct of this pathway. However, other interpretations of the inactivity of GA_{28} could be given.[18] It is noteworthy that dwarf pea gave little or no response to applied GA_{14}-[^{3}H] during the first 20 hr, whereas after 40 hr a statistically significant internode elongation response was observed. During the first time period there was no detectable conversion to GA_1 **(1)** and a small conversion to GA_8 **(3)**, whereas during the second time period conversion to these C_{19}-GAs was considerably increased.

The ease of conversion of GA_{14} to other GAs in dwarf pea cv. Meteor may indicate that this GA or a similar compound is a natural precursor of GAs in this plant. GA_{18}, GA_{23} and GA_{28} have been identified as endogenous constituents of seeds of yellow lupin (*Lupinus luteus*),[16,18,19] and GA_{18} and GA_{23} characterized from seeds of another leguminous plant, *Wistaria floribunda*.[24] In the light of the present results, such gibberellins may also be native to dwarf pea seedlings and the biosynthetic sequence reported herein may be common to leguminous plants.

EXPERIMENTAL

GLRC. Preparation of TMSMe derivatives[16] and GLRC conditions[10] were the same as those previously described.

Preparation of GA_{14}-[^3H]. Using MeI-[^{3}H] the method of Cross *et al.*[11] was employed to prepare GA_{14}-[17-^{3}H] from 16-oxo-17-nor-gibberellin A_{14}. The product was crystallized 2× from acetone–Et_2O to give GA_{14}-[17-^{3}H] (sp. act. 61 mCi/mM). The product chromatographed as one spot on TLC. As the methyl ester on GLC and the TMSMe derivative on GLRC, it chromatographed as a single peak.

Application to dwarf pea and extraction. Dwarf peas (*Pisum sativum* L. cv. Meteor) were grown in darkness for 5 days at 25°. GA_{14}-[^{3}H] (310×10^6 dpm, 800 μg) was dissolved in 95% EtOH (400 μl) and 5 μl droplets of this solution were applied to the plumular hook of each of 80 plants (10 μg per plant). After 20 hr the mean length of the 2nd internode of the treated plants was 13·9 mm (controls 7·2 mm). After 40 hr the mean length of the 2nd internode of the treated plants was 26·0 mm (controls 10·3 mm). Plants were harvested after 20 and 40 hr (40 plants each). The shoots were separated from the rest of the plant, washed with MeOH and extracted with MeOH–H_2O (4:1). After evaporation of the MeOH *in vacuo* at 35°, the aq. soln was adjusted to pH 9·0 with 0·5 M phosphate buffer washed 6× with an equal vol. Et_2O then adjusted to pH 3·0 and extracted with EtOAc (6×) and *n*-BuOH (3×). The counts (corrected to dpm) present in each fraction were as follows. Extraction after 20 hr: MeOH wash of shoots, $77{\cdot}1 \times 10^6$ dpm (representing 49·7% of the applied radioactivity); Et_2O, $0{\cdot}26 \times 10^6$ dpm; EtOAc, $36{\cdot}6 \times 10^6$ dpm; *n*-BuOH, $4{\cdot}6 \times 10^6$ dpm; and residual buffer soln, $0{\cdot}078 \times 10^6$

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dpm. Extraction after 40 hr: MeOH wash of shoots, 68·6 × 10^6 dpm (representing 44·3% of the applied radioactivity); Et_2O, 0·69 × 10^6 dpm; EtOAc, 26·1 × 10^6 dpm; η-BuOH, 5·2 × 10^6 dpm; and residual buffer soln, 0·093 × 10^6 dpm. All radioactivity in MeOH washings of the shoots resided in GA_{14}-[^{3}H]. The residue from the EtOAc solns was chromatographed on a silica-gel partition column.[14,15] 25 fractions were collected and these were combined according to counts obtained by direct liquid scintillation spectrometry as follows: fractions 3–6, 7–11, 14–15, 17–19, 20–22, 23–24. Derivatives were prepared and examined by GLRC.

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