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INTERCONVERSION OF GIBBERELLIN A_5 TO GIBBERELLIN A_3 IN SEEDLINGS OF DWARF *PISUM SATIVUM*

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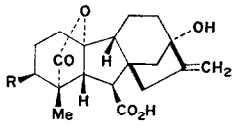
Key Word Index—*Pisum sativum;* Leguminosae; gibberellin interconversions; biosynthesis.

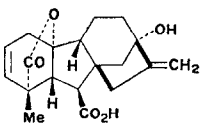
Abstract—[^{3}H]-Gibberellin A_5 ([^{3}H]-GA_5) applied to seedlings of dark-grown dwarf pea (*Pisum sativum* L. cv. Meteor), was converted to two acidic compounds, GA_3 and a chromatographically similar unknown. Identification of GA_3 was made by gas–liquid radiochromatography using three stationary phases.

INTRODUCTION

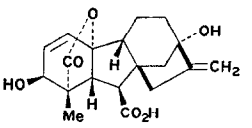
EXTRACTION of gibberellins (GAs) from seedlings of dwarf pea (*Pisum sativum* L.) gives two main fractions containing GA-like activity, one chromatographically similar to GA_1 (I) and the other similar to GA_5 (III) or GA_{20} (II).[1,2] However, the GAs in these two fractions have apparently not been chemically characterized. The only GA from dwarf pea that has been characterized is GA_{20}, isolated from pods[3] and identified in fruit.[4] The metabolism of [^{3}H]-GA_1[5,6] and [^{3}H]-GA_5[7] in seedlings of dwarf pea, cv. Progress No. 9, has been investigated. [^{3}H]-GA_5 was converted to an acidic compound chromatographically similar to GA_1.[7] Similar feeding experiments in pea pods indicated the formation of two other GA-like metabolites from [^{3}H]-GA_5.[7]



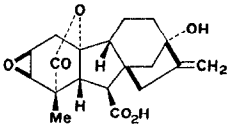
GA_1 (I) R = OH
GA_{20} (II) R = H



GA_5 (III)



GA_3 (IV)



GA_6 (V)

In order to further investigate the metabolism of the GA_5/GA_{20} fraction in dwarf pea, [^{3}H]-GA_5 was applied to seedlings and the trimethylsilyl ether derivatives of the methyl esters (TMSMe) of the acidic products were analyzed by gas–liquid radiochromatography (GLRC). [^{3}H]-GA_5 was used since it is readily produced[7,8] from [^{3}H]-GA_1, whereas lack of a convenient method has thus far prevented the labelling of GA_{20}. [^{3}H]-GA_1 was itself prepared by selective catalytic reduction of the 1,2-double bond of GA_3.

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[8] MACMILLAN, J. and PRYCE, R. J. (1967) *J. Chem. Soc.* C, 550.

RESULTS

The results obtained by Musgrave and Kende[7] using light- or dark-grown seedlings were essentially similar except that in the dark a larger incorporation of [^{3}H]-GA_5 to the more polar metabolite was obtained. Therefore to ensure a reasonable yield of the metabolic products for analysis, dark-grown seedlings were used in our experiments.

Shoots of dark-grown dwarf pea plants (see Experimental) were extracted after 24 hr. Three extracts were obtained, a neutral ether extract ($1{\cdot}48 \times 10^6$ cpm) an acidic ethyl acetate extract and an acidic butanol extract ($10{\cdot}6 \times 10^6$ cpm). The acidic ethyl acetate extract was partially purified by silica gel partition chromatography[9,10] and the eluted fractions were combined according to counts obtained from direct liquid scintillation counting. The TMSMe derivatives of the combined fractions were examined by GLRC on 2% QF1, 2% SE30 and 1% XE60 and the results are summarized in Table 1. Combined fractions 5–8 contained one significant peak, combined fractions 16–19 contained two significant peaks. The other fractions contained no significant peaks. GLRC clearly demonstrated the presence of radioactive peaks corresponding to GA_5 (III) in fractions 5–8 and GA_3 (IV) in fractions 16–19. The other metabolite present in fractions 16–19 had GLC properties very similar on all 3 columns to that of GA_3 TMSMe, indicating a structural similarity to GA_3. However, when chromatographed at 201° (rather than at 206°) on the QF1 column it became apparent that it was not GA_3 (see Table 1) and probably not *iso*-GA_3 (4 → 2 lactone).[11] The incorporation of GA_5 to GA_3 was 0·8% and to the other metabolite was 1·0% of the applied [^{3}H]-GA_5.

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°)	Counts/min of peak ($\times 10^{-5}$)
5–8	10·1	8·5	13·5	145
16–19	16·0 (broad)	15·6	19·7	6·72
		16·8	18·6	5·28
16–19*	19·3			
	20·2			
Standard GAs				
A_1	14·0	15·4	15·3	
A_3	16·4	16·8	18·6	
A_3*	20·3			
A_4	10·0	9·1	11·7	
A_5	10·1	8·6	13·4	
A_6	16·4	11·3	18·5	

* Column temp 201°.

The high count in the butanol extract may indicate that GA_5 and/or its metabolic products are further converted to glucosides[12] or other 'water soluble' GAs (i.e. such as GA_{32})

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and hence do not accumulate in the rapidly growing seedling. Given this dynamic system it is difficult to establish whether GA_5 is biologically active *per se* or whether its activity is derived from interconversion products. Of interest here, however, were the results from an earlier experiment in which diseased plants did not respond well in terms of height growth to applied [^{3}H]-GA_5, and produced only the other unknown metabolite from [^{3}H]-GA_5. It is possible that GA_5 is converted to GA_3 via GA_6 (V). However, we were unable to demonstrate the presence of this possible intermediate. Surprisingly, an expected metabolic product, GA_1, a GA believed to be present in dwarf pea,[1] was not formed in detectable quantities. If GA_1 is indeed endogenous to pea, it is possible that GA_{20} is its precursor.

The specific activity of the GA_5 (129 mCi/mM) was similar to that of precursor GA_1 (138 mCi/mM), indicating little or no loss of tritium during the elimination of *p*-toluenesulfonic acid with collidine. Since this elimination would be expected to occur stereospecifically *trans*, the 2 α-hydrogen atom would be lost. Therefore it would appear that during the selective hydrogenation of GA_3 to GA_1 using the partially poisoned catalyst 5% Pd–$CaCO_3$,[14] the addition of hydrogen or tritium is mainly β to the ring. This hydrogenation therefore exhibits a greater degree of stereospecificity than that of a similar experiment reported earlier.[7] If GA_5 was biosynthetically converted to GA_3 via GA_6 and if an analogy can be made to *in vitro* reactions, loss of tritium would not be expected during $GA_6 \rightarrow GA_3$ since again this should involve *trans*-elimination. Therefore no loss of tritium has been assumed when calculating the percentage conversion of GA_5 to GA_3.

EXPERIMENTAL

GLRC. Samples were converted to the TMSMe derivatives[13] and a known quantity of each sample was chromatographed using a Hewlett Packard F & M 402 GLC connected directly to a modified Nuclear Chicago 4998 gas-flow proportional radioactivity monitoring system. Three 1·83 m × 3·2 mm i.d. GLC columns containing 2% QF 1 (temp. 206°), 2% SE30 (203°) and 1% XE60 (209°) on gaschrom Q (80–100 mesh) with helium carrier gas flowing at 55 ml/min were routinely employed. The effluent gas was split between the gas-flow proportional detector (temp. 250°) and F.I.D. (250°) in the ratio 10:1. Using the 3 columns we were able to separate the gibberellins A_1–A_{35}.

Preparation of [^{3}*H*]-GA_5. [^{3}H]-GA_1 was obtained from and purified by Vining[14,15] and diluted with GA_1 to a specific activity of 138 mCi/mM. This compound was used to prepare [^{3}H]-GA_5 using methods similar to those reported earlier.[7,8] The crude product was purified by TLC giving a mixture of [^{3}H]-GA_5 (III) and [^{3}H]-3α-chloro-gibberellin A_{20}.[8] The mixture was separated by chromatography on charcoal–celite (5:1, 1 × 19 cm). Elution with acetone (25 ml) gave an oil (0·1 mg); further elution with acetone (30 ml) gave a gum which was crystallized 2× from EtOAc–petrol. (b.p. 55–75°) to give [H^3]-GA_5 (5·6 mg, 2·18 mCi, and recrystallized to a constant specific activity of 129 mCi/mM). Further elution with acetone (59 ml) gave an oil which crystallized from EtOAc–petrol. (b.p. 55–75°) to give [^{3}H]-3α-chlorogibberellin A_{20} (2·1 mg).

As the methyl ester and the TMSMe derivative, [^{3}H]-GA_5 chromatographed as a single peak on GLC and GLRC using three stationary phases.

Application to dwarf pea and extraction. Dwarf peas (*Pisum sativum* cv. Meteor) were grown in darkness for 5 days at 25°. [^{3}H]-GA_5 (9·25 × 10^7 cpm, 108 μg) was dissolved in 95% EtOH (200 μl) and 5 μl droplets of this solution were applied to the plumular hook of each of 40 plants (*ca.* 2·7 μg per plant). After 24 hr the mean length of the treated shoots was 8·6 cm (controls 5·7 cm) and the mean length of the 2nd internode of the treated plants was 3·0 cm (controls 1·4 cm). 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 aqueous solution was adjusted to pH 9·0, washed 6× with an equal vol Et_2O, then adjusted to pH 3·0 and extracted (EtOAc × 6, *n*-BuOH × 3). The counts (corrected for quenching) present in each fraction were as follows: MeOH wash of shoots, 3·52 × 10^7 cpm (representing 38% of the applied radioactivity); Et_2O, 1·48 × 10^6 cpm; acidic *n*-BuOH, 10·6 × 10^6 cpm; and residual buffer solution, 1·4 × 10^6 cpm. The residue from the EtOAc solution was chromatographed on a silica-gel partition column.[9,10] 25 fractions were

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collected and these were combined according to counts obtained from direct liquid scintillation counting as follows: fractions 1–4, 5–8, 9–15, 16–19, 20–22 and 23–25. The fractions were derivatized and examined by GLRC.

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