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INTERCONVERSION OF GIBBERELLIN A_{20} TO GIBBERELLIN A_{29} BY ETIOLATED SEEDLINGS AND GERMINATING SEEDS OF DWARF *PISUM SATIVUM*

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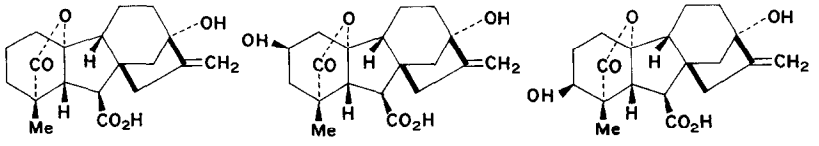
Abstract—Tritium labelled gibberellin A_{20} ([^{3}H]-GA_{20}) applied to etiolated shoots and germinating seeds of dwarf pea (*Pisum sativum* L. cv. Meteor) was converted to gibberellin A_{29}. Identifications were made by GLRC and GC–MS.

INTRODUCTION

SHOOTS of dwarf pea seedlings have been shown to contain two main gibberellin-like (GA-like) substances.[1,2] One of these has similar chromatographic properties to those of GA_5 and/or its dihydro derivative GA_{20}, and the other, chromatographic properties similar to those of GA_3 and/or its dihydro derivative GA_1. More recently Jones[3] detected the presence of 2 additional GA-like substances in shoots of a tall variety of pea. Recent work[4,5] has shown the presence of at least 6 GA-like substances in shoots of light grown, tall "Alaska" pea seedlings and in chloroplasts isolated from the same variety. At the present time GA_{20} (**1**) is the only gibberellin characterized from peas, identified in both pod[6] and seed[7] of tall cultivars.



(1) GA_{20} (2) GA_{29} (3) GA_1

Extracts of etiolated dwarf pea seedlings var. "Meteor" contained 4 main zones of GA-like activity when extracts were separated by TLC.[8] Two zones had similar chromatographic properties to those of GA_1/GA_3 and GA_5/GA_{20}, whilst the third zone was simi-

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[8] RAILTON, I. D., DURLEY, R. C. and PHARIS, R. P., unpublished data.

lar to GA_9. [^{3}H]-GA_9 applied to etiolated shoots of dwarf pea var. Meteor was converted into GA_{20}.[9] [^{3}H]-GA_{20} has therefore been synthesized in an effort to follow the metabolism of this compound in etiolated dwarf pea seedlings and in germinating seeds.

RESULTS

Twenty hours following application of [^{3}H]-GA_{20}, etiolated shoots of dwarf pea (see Experimental) were extracted and fractionated by partition. Three fractions were obtained, a neutral ether fraction ($1{\cdot}6 \times 10^6$ dpm), an acidic ethyl acetate fraction (131×10^6 dpm) and an acidic butanol fraction ($1{\cdot}93 \times 10^6$ dpm). The acidic ethyl acetate fraction was separated by TLC on silica gel H in EtOAc–$CHCl_3$–HCO_2H (50:50:1). Two zones of radioactivity (R_f 0·1–0·2; R_f 0·5–0·7) were eluted with water saturated-ethyl acetate, reduced to dryness, derivatized and examined as the trimethylsilylethers of the methyl esters (TMSMe derivatives) by gas liquid radiochromatography (GLRC) on three liquid stationary phases, 2% QF1, 2% SE30 and 1% XE60. The results are summarized in Table 1.

TABLE 1. GLRC RETENTION TIMES OF TMSMe DERIVATIVES OF SILICA GEL PARTITION COLUMN (SEEDS) AND TLC (SHOOTS) FRACTIONS, WITH COMPARISON STANDARDS

Silica gel partition column fractions	Retention time (min) on 3 columns 2% QF1 (206°)	2% SE30 (203°)	1% XE60 (207°)	dpm of peak (× 10^{-6})
4–8	9·1	8·6	11·9	45·0
17–19	13·5	16·9	15·4	68·2
TLC zones (R_f)				
0·1–0·2	13·5	16·9	15·4	8·2
0·5–0·7	9·1	8·6	11·9	74·8
Standard GAs				
A_1	13·6	15·0	14·9	
A_4	9·6	8·7	11·3	
A_{20}	9·1	8·6	11·9	
A_{29}	13·5	16·9	15·3	

In a similar experiment mature seeds of dwarf pea were imbibed in an aqueous solution of [^{3}H]-GA_{20} and extracted 45 hr later following radicle emergence. Three fractions were again obtained; a neutral ether fraction ($0{\cdot}79 \times 10^6$ dpm) an acidic ethyl acetate fraction (121×10^6 dpm) and an acidic butanol fraction ($2{\cdot}8 \times 10^6$ dpm). The acidic ethyl acetate fraction was purified on a PVP column and then chromatographed on a silica gel partition column. Two main zones of radioactivity in fractions 4–8 and 17–19, detected by direct scintillation counting, were each reduced to dryness, converted to their TMSMe derivatives and examined by GLRC. The results are summarized in Table 1.

TLC zone (R_f 0·5–0·7), from extracts of shoots, separated into one radioactive component on GLRC, with identical retention times to those of the TMSMe derivative of GA_{20}. The other zone (R_f 0·1–0·2), was also composed of a single radioactive compound with identical retention times on GLRC to those of the TMSMe derivative of GA_{29} (**2**). Silica gel column fractions 4–8 from extracts of mature seeds, contained a single radioactive compound with identical retention times on GLRC to those of the TMSMe derivative

[9] RAILTON, I. D., DURLEY, R. C. and PHARIS, R. P. (1974) *Plant Physiol.* in press.

of GA_{20}. Fractions 17–19 also contained a single radioactive compound with identical retention times on GLRC to those of TMSMe GA_{29}. The percentage conversion of GA_{20} to GA_{29} was 3·8% in shoots and 34·0% in seeds.

Unequivocal identification of GA_{29} as the major metabolite of [^{3}H]-GA_{20} in etiolated shoots, was obtained by GC–MS. Two mg of GA_{20} and 10·6 μCi [^{3}H]-GA_{20} were applied to 200 dwarf pea seedlings and these were extracted, fractionated and purified as described in the Experimental. Direct scintillation counting of fractions from a silica gel partition column revealed a single radioactive zone associated with fractions 14–17. These fractions were pooled, derivatized and examined by GC–MS. A compound was obtained with an identical mass spectrum to that of authentic TMSMe GA_{29}.

The fact that GA_{20} is an endogenous GA in pods and seeds of tall pea[6,7] and that a compound with identical chromatographic properties to those of GA_{20} occurs in both dwarf "Meteor"[8] and tall "Alaska" seedlings,[4,5] suggests that GA_{20} is native to seedlings and seeds of dwarf pea. GA_{20} was converted into a single metabolite, GA_{29}. GA_{29} and not GA_1 (**2**)[10] is therefore the major metabolite of GA_{20} in dwarf pea. GA_{29} has similar chromatographic properties to those of the other major gibberellin-like substance in peas, identified on circumstantial evidence, as GA_1.[1] GA_{29} and not GA_1 may therefore be endogenous to dwarf pea.*

Both GA_{20} and GA_{29} have been characterized from immature seeds of Japanese morning glory, *Pharbitis nil*[11,12] and GA_{20} could therefore be the immediate precursor of GA_{29} in this plant, as in dwarf pea. Interestingly, GA_{29} was isolated from seeds of *P. nil* as its 2-*O*-β-D-glucopyranosyl ether[12] and the presence of high levels of radioactivity in butanol fractions from seeds and seedlings of dwarf pea suggests that GA_{29} may be readily converted into its glucoside in this species.

EXPERIMENTAL

GLRC. Sample preparation and analysis were as described previously.[10,13,14]

GC–MS. This was performed using a Varian 1200 GLC connected by a double stage Biemann–Watson type molecular separator to a Varian Mat CH5 mass spectrometer. The 1·83 m × 2 mm i.d. GLC column contained 2% QF1 on gaschrom Q (80–100 mesh) at a temp. of 198° with He carrier gas flowing at 18 ml/min.

Preparation of [^{3}H]-GA_{20}. This was prepared by a novel method devised by N. Murofushi and will be described in detail elsewhere (Murofushi, Durley and Pharis, in preparation).

Application to dwarf pea and extraction. (a) *Shoots.* Dwarf peas were grown in darkness for 5 days at 25°. [^{3}H]-GA_{20} (100 μCi, 6·3 μg) was applied in 5 μl droplets of 95% EtOH to the plumular hook of each of 30 seedlings (*ca.* 0·2 μg per plant). After 20 hr the shoots were separated from the seeds, surface washed in absolute MeOH and ground in a prechilled mortar with acid washed sand and ice-cold 80% MeOH. After removing the MeOH *in vacuo* at 35°, the aqueous phase was adjusted to pH 9·0 by the addition of an equal vol. of 0·5 M phosphate buffer (pH 9·0) and partitioned 6 × against equal volumes of Et_2O. The aqueous phase was then adjusted to pH 3·0 with 1 N HCl and partitioned 6 × against EtOAc and then 4 × against *n*-BuOH. The radioactivity (liquid scintillation) present in each fraction was as follows: Et_2O, 1·6 × 10^6 dpm; EtOAc, 131 × 10^6 dpm; *n*-BuOH, 1·93 × 10^6 dpm; residual buffer, 0·06 × 10^6 dpm. The EtOAc fraction was reduced *in vacuo* and chromatographed on TLC (Silica gel H) using EtOAc–$CHCl_3$–HCOOH (50:50:1). The TLC plate was divided into 10 equal strips and radioactivity eluted from each strip with H_2O saturated–EtOAc. After reducing each fraction to dryness *in vacuo* they were derivatized and examined by GLRC. (b) *Seeds.* Dry, mature seeds of dwarf pea (*ca.* 50 seeds) were imbibed in dist. H_2O (20 ml) containing 98 μCi (6·1 μg) [^{3}H]-GA_{20} under continuous illumination at 25°.

* Added in proof: GA_{20} and GA_{29} have recently been characterized in seeds of dwarf pea *cv.* Progress No. 9: Frydman, V. M. and MacMillan, J. (1973) *Planta* **115,** 11.

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Once all water had been taken up, it was replaced with fresh soln and seeds left for a total of 45 hr until radicle emergence. Seeds were ground in a mortar and extracted as described for shoots. The radioactivity (liquid scintillation) associated with each fraction was as follows: Et_2O, $0{\cdot}79 \times 10^6$ dpm; EtOAc, $1{\cdot}21 \times 10^6$ dpm; *n*-BuOH, $2{\cdot}8 \times 10^6$ dpm; residual buffer, $0{\cdot}013 \times 10^6$ dpm. The EtOAc fraction from seeds was reduced *in vacuo* and the residue taken up in a minimum vol. of 0·1 M phosphate buffer (pH 8·0) and purified on a column of poly-*N*-vinylpyrrolidone (PVP).[15] After re-extracting the effluent from the PVP column with EtOAc at pH 3·0, the residue from the EtOAc fraction was chromatographed on a silica gel partition column.[16,17] Fractions from the silica gel column were pooled according to counts obtained by direct scintillation spectrometry, and were reduced to dryness *in vacuo* and derivatized for GLRC.

Sample preparation for GC–MS. 2 mg GA_{20} and 10·6 μCi [^{3}H]-GA_{20} were applied to a total of 200 etiolated seedlings of dwarf pea. 20 hr following GA_{20} application, the shoots were extracted as described above. The EtOAc fraction was purified on a column of PVP and then on TLC using EtOAc–$CHCl_3$–HCOOH (50:50:1). A single zone of radioactivity (R_f 0·1–0·2) was eluted with H_2O saturated EtOAc and rechromatographed on a silica gel partition column. Fractions 14–17 were pooled and examined by GC–MS.

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