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Photoperiod Modification of [^{14}C]Gibberellin A_{12} Aldehyde Metabolism in Shoots of Pea, Line G2[1]

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ABSTRACT

In G2 peas (*Pisum sativum* L.) apical senescence occurs only in long days (LD), and indeterminate growth is associated with elevated gibberellin (GA) levels in the shoot in short days (SD). Metabolism of GA_{12} aldehyde was investigated by feeding shoots grown in SD or LD with [^{14}C]GA_{12} aldehyde through the cut end of the stem for 0.5 to 6 hours in the light and analyzing the tissue extract by high performance liquid chromatography. More radioactive products were detected than can be accounted for by the two GA metabolic pathways previously known to be present in peas. Three of the major products appear to be GA conjugates, but an additional pathway(s) of GA metabolism may be present. The levels of putative C_{20} GAs, [^{14}C]GA_{53}, [^{14}C]GA_{44}, [^{14}C]GA_{19}, and/or [^{14}C]GA_{17}, were all elevated in SD as compared to LD. Putative [^{14}C]GA_9 was slightly higher in LD than in SD. Putative [^{14}C]GA_{53} was a major metabolite after 30 minutes of treatment in SD but had declined after longer treatment times to be replaced by elevated levels of putative [^{14}C]GA_{44} and [^{14}C]$GA_{19/17}$. Metabolism of GA_{20} was slow in both photoperiods. Although GA_{20} and GA_{19} are the major endogenous GAs as determined by gas chromatography-mass spectrometry, putative [^{14}C]GA_{20} and [^{14}C]GA_{19} were never major products of [^{14}C]GA_{12} aldehyde metabolism. Thus, photoperiod acts in G2 peas to change the rate of GA_{53} production from GA_{12} aldehyde, with the levels of the subsequent GAs on the 13-OH pathway being determined by the amount of GA_{53} being produced.

In the G2 genetic line of peas (*Pisum sativum* L.) senescence of the apical bud, which is a prelude to the senescence of the whole plant, takes place only in long photoperiods (17). In short photoperiods growth is indeterminate. The prevention of senescence by SD is associated with the presence of an elevated level of GA[3] bioactivity (18) due to GA_{19} and GA_{20} (2). A higher content of biologically active GAs could be caused by either an enhanced synthesis or decreased rate of metabolism to inactive products.

Several GAs are now known in both seeds and shoots of peas (2 and references therein; 7, 13). These GAs are all derived from GA_{12} aldehyde, the first GA compound on the GA synthetic pathway (20). The feeding of [^{14}C]GA_{12} to a cell free extract of pea seeds *in vitro* led to the formation of most of the GAs previously detected in pea seed plus some additional GAs, such as GA_{53} (8), which had been hypothesized to be present but not previously detected presumably because of their low concentration in the tissue. (GA_{53} has now been shown to be present in G2 pea seed [13].) The GA pathways in peas elucidated from the above, and from theoretical considerations (5), are

$$GA_{12}\text{ aldehyde} \rightarrow (GA_{12}\ ?) \rightarrow GA_{53}$$

$$\rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_{29} \rightarrow GA_{29}\text{ catabolite and}$$

$$\searrow\ GA_{17}$$

$$GA_{12}\text{ aldehyde} \rightarrow GA_{12} \rightarrow GA_{15} \rightarrow GA_{24} \rightarrow GA_9 \rightarrow GA_{51} \quad (8).$$

To determine the effect of photoperiod on GA metabolism, G2 pea shoots which had been grown under SD or LD were supplied with [^{14}C]GA_{12} aldehyde and the metabolism of the [^{14}C]GA_{12} aldehyde investigated.

MATERIALS AND METHODS

Plant Material. Pea (*Pisum sativum* L.) plants of genetic line G2 (14) were grown individually in a peat-vermiculite mixture in 4-L plastic pots. The seed was sown in the greenhouse and the plants transferred 1 month later to growth chambers with photoperiods of 9 (SD) or 18 (LD) h, and 20° d and 17° night temperatures. After 16 d, when the plants had leaves at nodes 18 or 19 (cotyledons = 0) in SD or LD, respectively, the stem was cut 10 nodes below the apical bud, 2 h after the start of the photoperiod, and the upper shoot placed in water. A second cut was made just above the second node below the apical bud to leave the upper shoot, with one expanded and one-half expanded leaf, and the apical bud enclosed in the ensheathing stipules. At the time of harvest the SD plants had a mean of 2 flowers while the LD plants had 2 flowers plus 3 young fruit (early flowers abort in SD). The apical buds of the shoots from both photoperiods were large and vigorous.

Chemicals. [^{14}C]GA_{12} aldehyde (approximately 200 mCi mmol^{-1}) was synthesized by Birnberg *et al.* (1). [2,3-^{3}H]GA_{20} (2.1 Ci mmol^{-1}) was a gift from R. P. Pharis and [^{3}H]GA_9 (36 Ci mmol^{-1}) was purchased from A. Crozier.

Treatment Conditions. The shoots prepared above were placed individually in 0.5 ml water containing 0.1 μCi of [^{14}C]GA_{12} aldehyde, or 0.17 μCi of [^{3}H]GA_{20}, in a small plastic vial (1.5 × 2.5 cm i.d.). Two shoots were used per treatment. The vials were placed 40 cm below a bank of cool-white fluorescent lights (230 μE m^{-2} s^{-1}) at 20°C. The treating solution was taken up via the cut end of the stem in about 15 min. Immediately after the solution was absorbed the vial was filled (about 4 ml) with distilled H_2O and the water was replenished at about 30 min intervals. At 0.5, 1, 3, or 6 h after the start of treatment the shoots were removed from the vials, the flowers or flower buds cut off for separate analysis, and the shoots weighed, frozen in

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[3] Abbreviation: GA, gibberellin.

liquid N_2, and stored at −20°C until extraction 10 d later.

Extraction. Each frozen shoot (mean weight 3 g) was cut into about 1 cm pieces with a razor blade, the pieces placed in 20 ml cold 80% aqueous methanol overnight at 4°C, and then ground with a 1 cm head Polytron (Brinkman Instruments) with an added rinse of 10 ml 80% methanol. The sample was centrifuged at 10,000*g* for 5 min, and the supernatant removed and vacuum filtered through filter paper. The precipitate was stirred with a further 10 ml 80% methanol incubated overnight at 4°C and, after filtration, the filtrates combined and passed through a C_{18} Sep Pak (Waters, Milford, MA) (to remove Chl and lipids) followed by a 10 ml rinse of 80% methanol. The material eluted from the Sep Pak was then evaporated to 0.5 ml or less at 33°C *in vacuo*. The samples were transferred to centrifugal filters (Rainin, Woburn, MA) followed by a 0.1 N acetic acid rinse of the evaporator flask (final volume <0.9 ml), and centrifuged through a glass fiber prefilter and a nylon 66 membrane filter (pore size 0.45 μm) prior to HPLC. The insoluble residue was dried at room temperature, combusted in a Packard sample oxidizer, and the resulting $^{14}CO_2$ collected and counted in order to determine the efficiency of extraction.

HPLC. Samples (0.8 ml) were loaded into individual 1 ml syringes and injected onto an analytical C_{18} HPLC column (5 μm Nucleosil 4.6 × 150 mm) using an automatic injector. The column was eluted with a 0.1 N acetic acid to acetonitrile (containing 0.1 N acetic acid) gradient as follows: 0 to 20% over 2 min; 20 to 35% over 15 min; 35 to 75% over 15 min; 75 to 100 over 2 min and holding at 100% for 5 min before returning, reequilibration with 0.1 N acetic acid and rerunning. (Acetonitrile was used instead of methanol in order to hasten the elution of GA_{12} aldehyde). The column eluate was passed through an on-line radioactivity monitor (model 7140, Packard Instrument) and the data recorded as counts per each 12 s interval. The proportion of each compound was calculated from the area of each peak.

Reapplication of [^{14}C]GA_{12} Aldehyde Metabolite. A principal early metabolite of [^{14}C]GA_{12} aldehyde (peak G) in SD was collected from two HPLC runs and evaporated to dryness *in vacuo*. It was then taken up in water and reapplied to the base of cut tips of SD-grown shoots for 3 h, as for [^{14}C]GA_{12} aldehyde. The solution proved toxic, apparently due to the presence of other compounds in the plant extract. During the HPLC analysis of this experiment the retention times were later than for previous experiments (because of pump problems). The identification of the peaks was made by normalizing the retention times using pre- and post-HPLC runs of total [^{14}C]GA_{12} aldehyde metabolites. The experiment was performed twice with similar result.

Testing for Conjugates. Tentative identification of conjugates was made according to the method of Koshioka *et al.* (11) which relies on their being retained on a short SiO_2 partition column that is designed to elute free GAs in hexane:ethyl acetate. Individual peaks were isolated from a sample HPLC run, rerun alone on HPLC (same system) for further purification, and then dissolved or dispersed in 95% ethyl acetate:5% hexane saturated with 0.5 N formic acid and loaded onto a column of silicic acid, hydrated with 0.5 N HCOOH, in a Pasteur pipette (4.2 g SiO_2 hydrated with 2.7 ml of HCOOH for about 10 columns). The column was eluted with 10 ml of ethyl acetate:hexane solution as above, and an aliquot counted. The column was then eluted with 10 ml of methanol. Free GAs are eluted in the ethyl acetate/hexane while GA conjugates are retained on the SiO_2 partition column, and subsequently eluted with methanol.

RESULTS

Approximately 93% of the radioactivity in the tissue was injected onto the HPLC column, with about 7% remaining in the insoluble pellet, after extraction. The chromatographic patterns from pairs of shoots were extremely similar, in some cases almost identical, but varied with time and photoperiod under which the shoots had grown. Several peaks of radioactivity were evident even after 30 min of treatment and about 15 major [^{14}C] metabolites were present by 3 h. The [^{14}C]GA_{12} aldehyde was almost completely metabolized by 1 h. Some early metabolites were only seen at short times (0.5–1 h) and had disappeared, or were much decreased at longer treatment times (Figs. 1 and 2).

GA metabolism, in general, produces products of increasing polarity. GA_{12} aldehyde was the last radiolabeled compound to elute from the column, and thus, as metabolism occurred, the primary, secondary, tertiary, etc. products were eluted from the column progressively earlier. For convenience, the major metabolites have been designated by letter in order of increasing polarity (Fig. 1). In shorter treatment times (0.5–1 h) an early metabolite (peak D) running just before putative GA_9 predominated, though this was virtually absent after 6 h of treatment. As peak D lessened in amount it was replaced by a close, but later running, peak (C) that ran coincident with [^{3}H]GA_9, and this compound (putative [^{14}C]GA_9) was the principle metabolite at 3 and 6 h. In both photoperiods two other metabolites (peaks H and M) were always prominent and increased with time of treatment. The metabolites M to Q appeared slowly, and steadily built up with time, in order of increasing polarity. M was evident by 30 min but more polar peaks did not appear until longer times of treatment.

LD *versus* SD-Grown Shoots. There was no difference in the rate of metabolism of the GA_{12} aldehyde between SD and LD. While many of the metabolites produced from GA_{12} aldehyde were similar in LD- and SD-grown shoots, there were some quantitative differences (Fig. 2). In SD, after 0.5 to 1 h of treatment, peak G was the second most prominent (after peak D) with peak I being the next most prominent peak. The levels of metabolites in peaks G and I were much less in LD. By 6 h metabolite G in SD had largely disappeared, being scarcely more prominent than in LD at that time of treatment. However, by 6 h peak K, though present in both LD and SD, was more prominent in SD than LD. (It is possible that peak J is also enhanced in SD. It is part of the IJK overlapping peak complex. Estimates of the position of peaks and shoulders indicated the differences in area to be predominantly in peaks I and K, but the exact area distribution has still to be accurately determined using further separations.)

The level of peak C (putative [^{14}C]GA_9) was greater in LD than SD at all treatment times, and a small peak (B) running just after peak C was only clearly evident in LD (between 0.5 and 3 h).

Isolation and Metabolism of Peak G. As metabolite G appears predominantly in SD only at short treatment times and is followed by the later enhancement of metabolites I, K (and possibly J), peak G was isolated and re-fed to SD shoots as described for [^{14}C]GA_{12} aldehyde. This led to the appearance of radiolabeled metabolites of G coincident with peaks H, I, J, K and, to a much lesser extent, P (Fig. 3). In agreement with the time course studies, and the clustering of metabolites G, I, J, and K in pea cotyledons (13), metabolite G appears to be the precurser of metabolites I, J, K, and possibly P. It is possible that peak H is not a natural metabolite of G as peak H is high in LD-grown shoots even though peak G is present in very small amounts, and peak H is absent from cotyledons (13) in the presence of peak G, I, J, and K, while it is present in seed coats in the absence of peaks G, I, J, and K (data not shown).

Peak Identification. Peak C co-chromatographs with [^{3}H]GA_9 and J with [^{3}H]GA_{20} on the same acetic acid to acetonitrile gradient used for the other runs. Using GC-MS following purification of metabolites from G2 pea seed cotyledons (13), peak G has been shown to contain GA_{53}; I contains GA_{44}; J contains

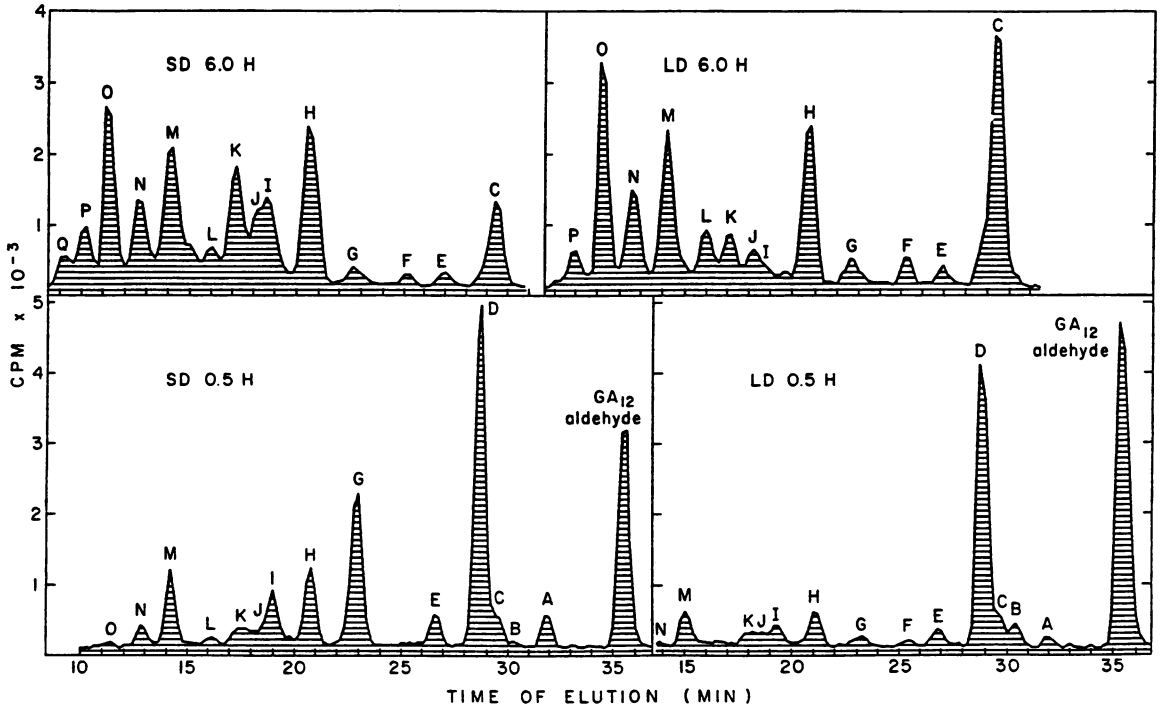


FIG. 1. Representative elution profiles of [^{14}C]GA_{12} aldehyde metabolites from a C_{18} HPLC column with a water to acetonitrile gradient. The peaks are lettered in the order of increasing polarity. The metabolites were extracted from G2 pea shoots grown in SD (left) or LD (right) and allowed to take up [^{14}C]GA_{12} aldehyde in water (with further water added as needed—see text) for 0.5 h (lower) or 6 h (upper) through the cut base of the stem in the light.

GA_{20}; and K contains a mixture of GA_{19} and GA_{17}. Thus, putative [^{14}C]GA_{53} and putative [^{14}C]GA_{44} are enhanced by SD at early times of treatment while production of putative [^{14}C]GA_{44}, [^{14}C]GA_{19}, and [^{14}C]GA_{17} is elevated following longer treatment times. The levels of putative [^{14}C]GA_{53} declines after a maximum at 1 h of treatment.

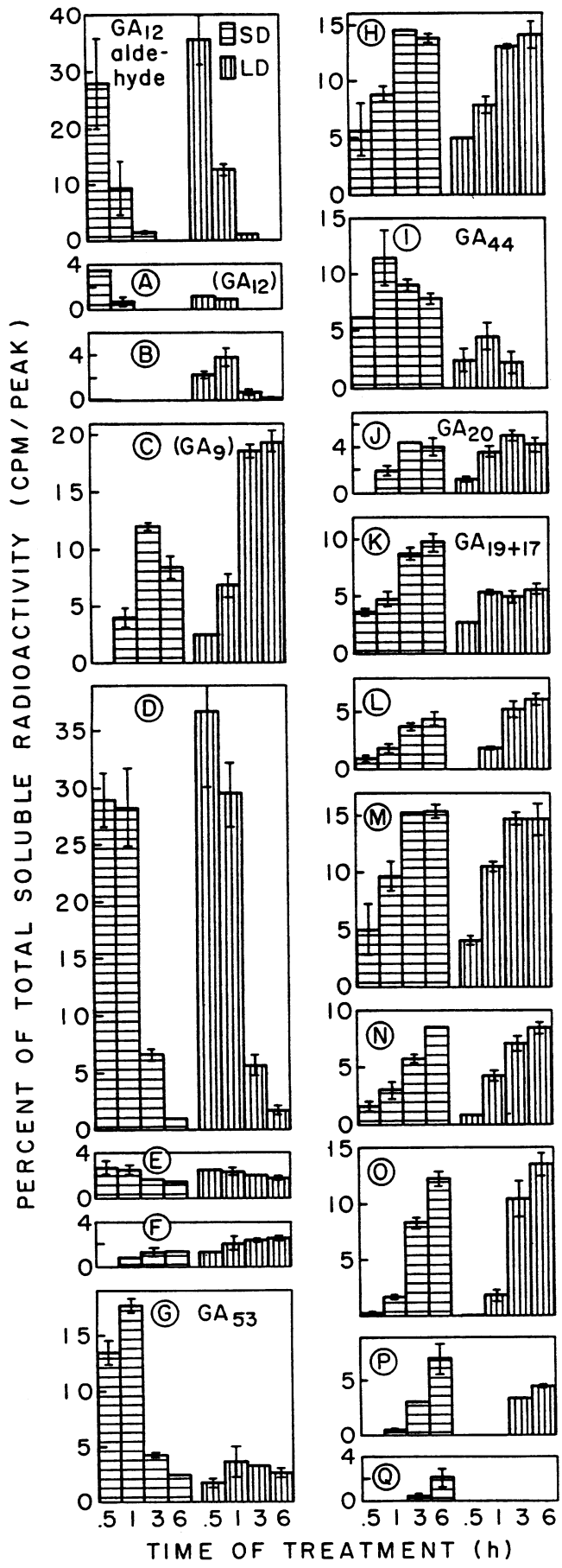
Testing for Conjugates. Using the method described above (where free GAs elute from a SiO_2 partition column in 95% ethyl acetate:5% hexane while GA conjugates remain on the column [11]) the metabolites present in peaks C, D, G, and H all appear to be free GAs, while those in peaks M, N, and O behave like conjugated GAs (Table I). Peaks P and Q eluted mainly, but not entirely, with the ethyl acetate:hexane. Given the polar nature of these peaks, as determined by their early elution from the C_{18}HPLC column, they are probably free GAs.

Treatment with [^{3}H]GA_{20}. Pea shoots were treated with [^{3}H]GA_{20}, as for [^{14}C]GA_{12} aldehyde, for 1, 3, or 5 h. Over the course of the experiment only a single polar metabolite, coincident with peak P, was produced (Table II). From the known metabolism of GA_{20} in (dwarf) peas (3, 7), and the polarity of the product, it is likely that P is GA_{29} although this identity has not yet been confirmed. The product was just evident after 1 h, and by 5 h accounted for about 16% of the total radioactivity (present as [^{3}H]GA_{20} or product) in the shoots. There was no difference between LD and SD. Thus, GA_{20} metabolism is slow compared to the metabolism of GA_{12} aldehyde. There is, however, a primary isotope effect in the metabolism of [^{3}H]GA_{20}. [2($\alpha\beta$), 3-^{3}H]GA_{20} would be metabolized to [2(α), 3-^{3}H]GA_{29} slower than [^{14}C]GA_{20} would be metabolized to [^{14}C]GA_{29}, and the [^{3}H]GA_{20} probably also loses an unknown percentage of the [^{3}H] in the process. Thus, the rate of GA_{20} metabolism may be underestimated by several-fold.

DISCUSSION

GA_{12} aldehyde is rapidly metabolized in pea shoots to a wide range of products, including some on the pathway GA_{12} aldehyde $\rightarrow GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_{29}$ with GA_{17} as a side branch of the pathway from GA_{19}. This pathway was originally proposed on theoretical grounds by Hedden *et al.* (5) and these GAs have been previously found as a group in spinach (15), maize (6) and *Agrostemma* (12). GA_{44} to GA_{29} have been identified as endogenous components of G2 pea seed while GA_{19} to GA_{29} have been identified as endogenous compounds in pea shoots (2). The remaining compounds are probably present in pea shoots but in amounts too small to be detected by the past methods used.

All the compounds on the above pathway have been shown to be produced from [^{14}C]GA_{12} *in vitro* by extracts of pea seeds (8). The compounds present in greatest quantity in G2 pea shoots are GA_{20} and GA_{29} with lesser amounts of GA_{19} (2), yet putative [^{14}C]GA_{20}, [^{14}C]GA_{19}, and [^{14}C]GA_{29} represent minor metabolites from [^{14}C]GA_{12} aldehyde after a 6 h treatment period. This is possibly because the production of [^{14}C]GA_{20} is relatively slow so that little is present after 6 h. The metabolism of (radioactive) GA_{20} is, however, even slower so that over long periods of time GA_{20} would build up and thus be the GA present in the largest detectable amounts. Metabolite H, which appears to be a free GA although is currently unidentified, builds up quite rapidly to become one of the major metabolites by 6 h, and tends to remain relatively high beyond 24 h, though its endogenous level is unknown. Based on its polarity and speed of production, it does not appear to be on the above pathway. Metabolites M, N, and O (which appear to be GA conjugates) build up progressively with time. Their nature is under investigation. Conjugates would not have been detected in our previous examination of the endogenous GAs in G2 pea shoots (2). It is of interest, however, that, while these [^{14}C]GA_{12} aldehyde metabolites are low in seeds (over a 6 h period of treatment) (13) and conjugates appear to be virtually absent from pea seed (20), [^{14}C] conjugate-like substances form a major sink for ^{14}C from [^{14}C]GA_{12} aldehyde in shoots over the 6 h period of treatment. Metabolite O further



increases in amount at 24 or 30 h (data not shown). Extracts of pea seed have been shown to rapidly convert [^{14}C]GA_{12} aldehyde to a glucosyl conjugate of GA_{12} aldehyde (8). While such a conjugate could be formed in intact pea shoots, it is not one of the rapidly formed products and is unlikely, based on retention time on HPLC, to be M, N, or O.

Another group of [^{14}C] compounds present almost certainly represent those on the pathway of GA_9 synthesis, as putative [^{14}C]GA_9 appears to be a major metabolite of [^{14}C]GA_{12} aldehyde at 6 h, particularly in LD shoots. This pathway is GA_{12} aldehyde $\rightarrow GA_{12} \rightarrow GA_{15} \rightarrow GA_{24} \rightarrow GA_9$ (8) with further metabolism of GA_9 to GA_{51} (20). As peak D is the major metabolite at short treatment times, and declines by 3 h concomitant with the build-up of peak C (putative [^{14}C]GA_9), it is possible that peak D represents a precursor of GA_9, such as GA_{15} or GA_{24}. It is also possible that peak B may be on the GA_9 synthetic pathway. This is based on its appearance in LD shoots (which contain more peak C at longer treatment times), its position of elution, and its early appearance followed by a decline in amount.

Gibberellin A_1 is a metabolite of GA_{20} in tall peas carrying the dominant allele for the gene *Le* (7). GA_1 is, however, an unlikely metabolite in light-grown G2 peas as it was not found by GC-MS, even though specifically looked for (2), and G2 is a dwarf pea (*le*) lacking the dominant *Le* allele which has specific control over the conversion of GA_{20} to GA_1.

Prolonged metabolism of GA_{20} leads to the production of GA_{29} catabolite via GA_{29} (21, 22), and GA_{29} catabolite has been detected as an endogenous component of G2 pea shoots (2). It is unlikely, however, that much GA_{29} catabolite is present as a metabolite of GA_{12} aldehyde because the metabolism of applied [^{3}H]GA_{20} is comparatively slow with only a small percentage conversion to putative [^{3}H]GA_{29} over the period of treatment. The metabolism of applied [^{3}H]GA_9 is somewhat faster (data not shown), so it is possible that GA_{51} and GA_{51} catabolite (20) are present as metabolites.

Even when one adds up all the GA intermediates and metabolites on the GA_9 and GA_{20} pathways, plus conjugates, it is evident that there are still some other GA_{12} aldehyde metabolites present that cannot be accounted for by the above pathways. It therefore seems possible that at least one other pathway of GA metabolism may exist in these G2 pea shoots. Nash (16) has previously noted at least 50 radioactive compounds produced in *Phaseolus coccineus* seedlings injected with [^{3}H]GA_{12} aldehyde, which exceeded the number of GAs identified in *P. coccineus* by approximately 3-fold.

Although every radiolabeled metabolite was found in shoots from both SD and LD photoperiods, and most metabolites were similar in amount, three radiolabeled peaks were clearly greater in SD than LD. At 0.5 to 1 h putative [^{14}C]GA_{53} was present almost exclusively in SD, while at 3 and 6 h it had decreased to a low level, presumably through further metabolism, while putative [^{14}C]GA_{44}, and a combined peak of putative [^{14}C]GA_{19} + putative [^{14}C]GA_{17} had increased. It is possible that putative [^{14}C]GA_{20} had also increased, as our HPLC did not yield a complete separation of GA_{20} and GA_{44}. There was no evidence for any photoperiodic difference in the (slow) metabolism of [^{3}H]GA_{20}, thus probably allowing putative [^{14}C]GA_{19} (and possibly putative [^{14}C]GA_{20}) to build up under SD. This indicates that the pathway

FIG. 2. The kinetics of metabolism of [^{14}C]GA_{12} aldehyde by G2 pea shoots grown in SD or LD. Peak designation is described in Figure 1. The scale for all metabolites is identical except for GA_{12} aldehyde where the scale is half. Metabolite peaks which yielded an identification by GC-MS have a GA number designation. Those which co-elute with authentic GA standards have the designation in parentheses. The nature of metabolites with no GA designation is not yet known. The error bars represent ±SE. Where no bar is shown the SE is less than the thickness of the line.

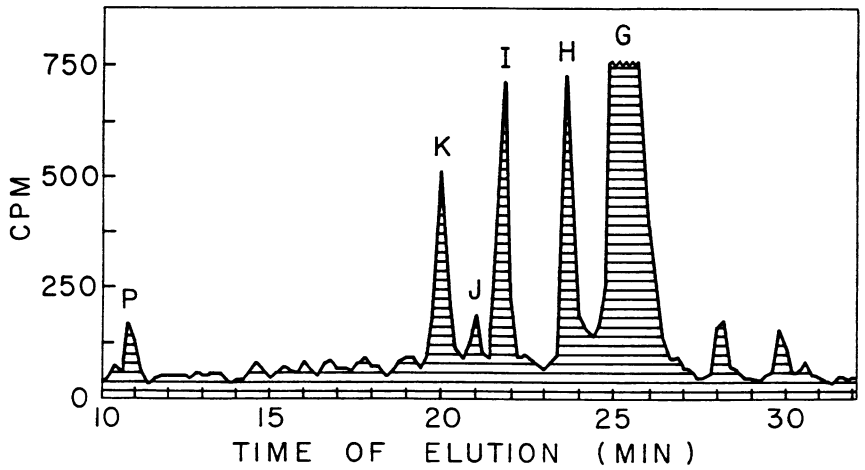


FIG. 3. HPLC separation of a shoot extract following further metabolism of the peak G [^{14}C]GA_{12} aldehyde metabolite which was extracted and reapplied to shoots of SD-grown G2 peas. The HPLC conditions are slightly different from those in Figure 1 as explained in "Material and Methods."

Table I. *Percentage of Radioactivity in Nine Different [^{14}C] HPLC Peaks, and Their Tentative Designation as a Free GA or GA Conjugate Based on Their Elution from a Silicic Acid Partition Column by 95% Ethyl Acetate/5% Hexanes, or Subsequently by 100% Methanol*

HPLC Peak Designation	Percentage Eluted with 95:5 Ethyl Acetate:Hexane	Percentage Eluted Subsequently with Methanol	Likely Nature: Free or Conjugate
C	95	5	Free
D	99	1	Free
G	90	10	Free
H	88	12	Free
M	9	91	Conjugate
N	8	92	Conjugate
O	5	95	Conjugate
P	65	35	Free or mixed peak
Q	63	37	Free or mixed peak

Table II. *Radioactivity Present in HPLC Peak P, as a Percentage of the Total Radioactivity Eluted from HPLC following the Metabolism of [2,3-^{3}H]GA_{20} by Shoots of G2 Peas Grown under SD or LD.*

Treatment Time	Shoots Grown Under	
	SD ± SE	LD ± SE
h	%	
1	2.7 ± 1.9	3.7 ± 5.3
3	9.6 ± 4.7	12.7 ± 2.0
5	15.4 ± 2.6	17.7 ± 0.7

GA_{12} aldehyde → GA_{53} → GA_{44} → $GA_{17/19}$ → GA_{20} is under photoperiodic control with photoperiod altering the extent to which GA_{12} aldehyde is metabolized to putative GA_{53}. Since [^{14}C]GA_{12} incubations with pea embryos did not produce the same metabolites as incubations with [^{14}C]GA_{12} aldehyde (data not shown) GA_{53} aldehyde (rather than GA_{12}) is a possible intermediate between GA_{12} aldehyde and GA_{53}.

While metabolism of [^{14}C]GA_{12} aldehyde to components of the 13 hydroxy GA pathway appears to be enhanced by SD, the amount of putative [^{14}C]GA_9 is enhanced by LD. It is not known if this is indeed a specific effect of LD or simply a response to a higher lever of [^{14}C]GA_{12} aldehyde available to the nonhydroxyl pathway in LD shoots as a result of the decreased metabolism in the 13-OH pathway. As [^{14}C]GA_{12} aldehyde was fed at greater than trace amounts it is possible that the metabolic pathways in general may have been slightly perturbed by the added [^{14}C]GA_{12} aldehyde. Nonetheless, there appeared to be no significant difference between SD and LD in the rate of metabolism of the applied [^{14}C]GA_{12} aldehyde.

The photoperiodic control of senescence in G2 is mediated by phytochrome (19) but not in the same fashion as the photoperiodic control of flowering in many species. Photoperiod is largely quantitative and additive such that the effect of SD cannot be reversed by a light break in the dark period (17). Nonetheless, the effect is photoperiodic rather than photosynthetic as 18 h of 50% light intensity still induces senescence as found in LD and not SD (10).The effect of LD can be reversed by reimposition of SD at any time prior to senescence (17).

The presence of enhanced metabolism of [^{14}C]GA_{12} aldehyde to form increased amounts of putative [^{14}C]GA_{19} in SD agrees with the finding of increased endogenous GA-bioactivity in SD (18), which was subsequently identified as GA_{20} and GA_{19} (2). This is thus another instance of photoperiodic regulation of the GA_{53} → GA_{20} pathway. In spinach (4, 15) the step GA_{19} → GA_{20} is under photoperiodic control, occurring only in LD. By contrast, in *Agrostemma* (12) LD appear to generally speed up GA metabolism increasing all GAs after GA_{53}, while GA_{53} remained unchanged. In these systems the LD conversion of GA_{19} to GA_{20} has been proposed to be causal in stem elongation prior to flowering. In G2, however, the photoperiod control operates at a very early step in this pathway. The net result would be to increase the content of biologically active GAs (*e.g.* GA_{19} and GA_{20}) in the shoot in SD (18). We suggest that this enhanced GA level produced in the bud in SD maintains the active growth and sink strength of the vegetative structures of the apical bud (9; MO Kelly, PJ Davies, in preparation), and so prevents the shift in nutrient partitioning from the developing vegetative structures to the developing reproductive structures in the apical bud, which ultimately brings about apical senescence.

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