Plant Physiol. (1979) 64, 214–219
0032-0889/79/64/0214/06/$00.50/0

Metabolism of Tritiated Gibberellin A_{20} in Immature Seeds of Dwarf Pea, cv. Meteor[1]

Received for publication October 16, 1978 and in revised form January 22, 1979

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ABSTRACT

Tritium-labeled gibberellin A_{20} ([^{3}H]GA_{20}) was applied via the pedicel to immature pods and seeds of dwarf peas and three harvests were made at days 5, 10, and 23 (mature) after application. Of the five metabolites of [^{3}H]GA_{20}, the three in highest yield were GA_{29}, an α,β-unsaturated ketone, and a compound (B), whose structure was only tentatively assigned. The metabolic sequence $GA_{20} \rightarrow GA_{29} \rightarrow$ compound B $\rightarrow$ the ketone was indicated. The amount of [^{3}H]GA_{29} in both seeds and pods was highest at day 5 and declined to its lowest level at maturity. The amount of the [^{3}H]ketone in the seed increased with time to its highest level at maturity. It is suggested that compound B and the ketone represent the major pathway of catabolism of GA_{29}, a 2β-hydroxylated GA of low biological activity, and that the ketone is not metabolized, or only slowly metabolized, during seed maturation.

The roles of gibberellins (GAs) in the growth of seeds and fruit have yet to be adequately resolved. Many GAs are present in substantially higher levels in immature seeds (4, 7) than in other parts of the plant. These high levels of GAs may be causally correlated with seed and fruit growth (1, 10) or may ensure that adequate levels are stored in the seed for use in growth processes during germination (1). Evidence for the latter role has been cast in doubt by recent work with ^{3}H-GAs (cited in ref. 1).

The major biologically active GA in immature pod and seed of pea is GA_{20} (7, 8). In seeds of dwarf pea high levels of the less biologically active GA_{29} are present together with much smaller amounts of GA_9, GA_{17}, GA_{38}, GA_{44}, and GA_{51} (18).

The metabolism of [^{3}H]GA_9, [^{3}H]GA_{20}, and [^{3}H]GA_{29} has been investigated in dwarf pea. In seedlings (cv. Meteor) GA_9 was converted to GA_{20} and 2,3-dihydro-GA_{31} (16), whereas *in vitro* cultured seed (cv. Progress No. 9) converted GA_9 to GA_{20}, GA_{51}, 2,3-dihydro GA_{31} and, in high yield, to a conjugate of 2,3-dihydro-GA_{31} (18). Since 2,3-dihydro-GA_{31} and its conjugate have not yet been isolated from pea, it may be an artifact, although this inference (18) from negative data may be premature. GA_{20} is converted to GA_{29} in seedlings, germinating seed, and *in vitro* cultured immature seed (17, 18). GA_{29} is metabolized in low yield to other unidentified products (15) in seedlings and germinating seed (cv. Meteor). In *in vitro* cultured immature seed (cv. Progress No. 9) GA_{29} was apparently not metabolized further, except perhaps very slowly to a GA_{29} conjugate, inferred to be a glucosyl ether (18). The pathways leading to GA catabolism or GA storage are not known, although GAs could be stored as conjugates (*e.g.* glucosyl ethers or glucosyl esters [references cited in 1 and 10]).

In this communication we report the metabolism of [^{3}H]GA_{20} of high specific activity in pods and immature seeds of dwarf pea (cv. Meteor) *in vivo*, noting that [^{3}H]GA_{20} is not only an efficient precursor of GA_{29}, but is further metabolized to a more stable unconjugated product which, unlike GA_{29}, accumulates in the seed with maturity. This latter compound, an α,β-unsaturated ketone (Fig. 1), has been known to be endogenous to pea seedlings for some time (J. MacMillan, personal communication) but has only recently been characterized structurally (19; and Gaskin, Kirkwood and MacMillan, unpublished work). Although earlier studies (18) failed to observe the ketone as a product of [^{3}H]GA_{20} or [^{3}H]GA_{29} metabolism, after discussion with ourselves and a reexamination (19) of this work, Sponsel and MacMillan have found some evidence which is consistent with our conclusion, namely that the ketone is a product of the metabolism of [^{3}H]-GA_{20}. The present communication also notes the kinetics of metabolism of [^{3}H]GA_{20} to GA_{29}, thence to the ketone (Fig. 1) via a possible intermediate, the structure of which is tentatively assigned (compound B, Fig. 1).

MATERIALS AND METHODS

Preparation of [2,3-^{3}H]GA_{20}. The labeled compound (specific activity 3.3 Ci/mmol) was prepared by hydrogenation of GA_5 methyl ester 16,17-epoxide with hydrogen enriched with tritium, followed by removal of the epoxide oxygen and hydrolysis (11). As the methyl ester, [^{3}H]GA_{20} chromatographed as a single peak on GLC (QF-1 and SE30). Using GC-RC[5] the MeTMSi derivative of [^{3}H]GA_{20} chromatographed as a single radioactive and mass peak using three GLC columns (Table I).

Application to Pods and Harvesting. All experiments were performed on intact plants of dwarf pea (*Pisum sativum* cv. Meteor). Sixteen days after anthesis, when the seeds were just visible through the pod, [^{3}H]GA_{20} (150×10^6 dpm, 6.8 μg) was injected into the pedicel of 15 pods. Five pods were harvested 5 days later (day 5), the seeds were separated from the pods and each homogenized at 0 C with methanol-water (80:20). The mixture was stirred for 3 h at 0 C, filtered, this procedure repeated, the filtrates then being combined. The sections of pedicel where the injection was made and the rest of the plant were extracted separately. By scintillation counting of an aliquot from each extract, the distribution of radioactivity throughout the plant was determined. Subsequent harvests of five pods each were made at 10 and 23 days after feeding (days 10 and 23) and the pods and

[1] This work was supported by National Research Council of Canada Grant A-2585 to R. P. P.

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[5] Abbreviations: FID: flame ionization detector; GC-RC: gas chromatography-radioactivity counting; MeTMSi: methyl ester trimethylsilyl ether derivative; PVPP: polyvinylpolypyrrolidone.

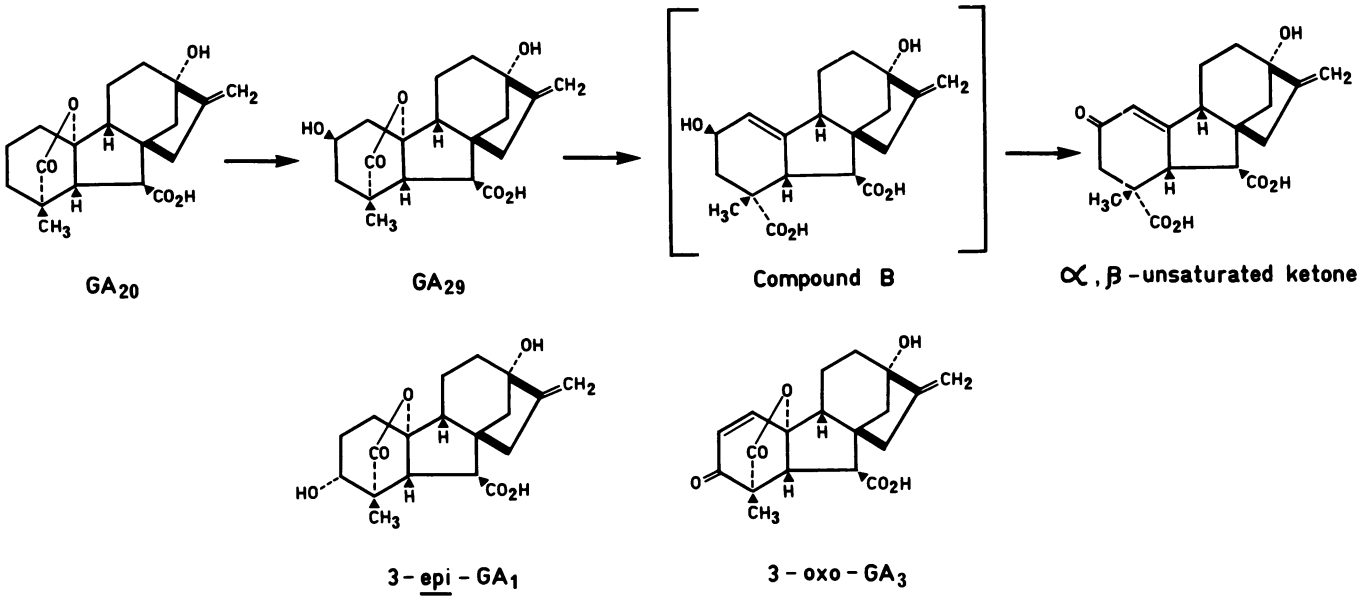

FIG. 1. Structure of certain GAs and GA-derived products. A possible metabolic pathway from GA_{20} to the ketone is indicated. The structure of compound B is bracketed since it is only tentatively assigned.

Table I. GC-RC of MeTMSi derivatives of products of 2,3-[^{3}H]-GA_{20} in maturing pods and seeds of dwarf pea cv. Meteor

Silica gel partition column fraction groupings	Retention time (min)			Compound
	2%QF1	2%SE30	1%XE60	
6-7	9.6	8.5	11.9	GA_{20}
17-19	13.7	16.6	16.0	GA_{29}
	13.1	10.6	18.0	C
19-21	24.0	15.0	27.8	A (the ketone)
	6.0	13.5	8.0	B (GA catabolite)
	14.8	15.6	19.2	D (present only in pods)
GA standards				
GA_1	14.0	15.4	15.3	
GA_{20}	9.5	8.4	11.9	
GA_{29}	13.6	16.7	16.1	
GA_{34}	12.6	15.2	13.4	
3-epi-GA_1	25.4	22.8	32.2	
C/D reag'd GA_{20}	25.4	8.3	28.1	
C/D reag'd GA_{29}	33.6			
3-oxo-GA_3	22.4	12.4	29.6	
GA_{13}	5.6	12.0	8.6	
GA_{18}	6.2	13.8	7.6	

seeds extracted separately as described above.

For each seed or pod extract, after the methanol was evaporated *in vacuo* at 35 C the aqueous solution was adjusted to pH 9.0 with 0.5 M K-phosphate buffer and KOH, washed with ½ volume diethyl ether three times, then adjusted to pH 3.0 and extracted with ⅔ volume ethyl acetate four times and butanol three times. The ethyl acetate and butanol fractions were subjected to −70 C to freeze the water (ice removed by filtering), then taken to dryness *in vacuo* at 35 C. The residues from the ethyl acetate extracts were purified on a column of insoluble PVPP (30 × 1.5 cm) eluted with 0.1 M K-phosphate buffer (pH 8.0) (6). The first 300 ml of eluate from the column was collected, adjusted to pH 3.0, and extracted as above with ethyl acetate. The residue from this ethyl acetate solution was chromatographed on a silica gel partition column (3), 27 fractions collected, these being combined after liquid scintillation spectrometry of aliquots as follows: fractions 6 and 7, 17 and

18, 19 to 21, and 25 to 27. Derivatives were prepared and examined by GC-RC.

GC-RC. Samples were converted to MeTMSi derivatives and a known quantity was chromatographed using a Hewlett Packard F and M 402 GLC connected via the auxiliary outlet to a modified Nuclear-Chicago 4998 gas flow proportional counter. The tubing from the auxiliary outlet and the counting chamber were both coated with SE30 liquid phase. Three GLC columns (1.83 × 3.2 mm *i.d.*) contained 2% QFI (206 C), 2% SE30 (203 C), and 1% XE60 (209 C) on Gas-chrom Q (80–100 mesh) with helium carrier gas flowing at 55 ml min^{-1}. The effluent gas was split between the FID (250 C) and the gas flow proportional detector (250 C) in the ratio 1:10. Butane-counting gas at 25 ml min^{-1} was added to the effluent gas prior to passing through the proportional detector chamber. Using the three columns we were able to separate GA_1-GA_{42}, which were available to us.

GC-MS. A Varian 1200 GLC was connected by a double stage Biemann-Watson type molecular separator to a Varian Mat CH5 mass spectrometer. GLC columns 2% QF1 or 2% SE30 on Gas-chrom Q (80-100 mesh) were 1.8 m × 2 mm *i. d.* and were maintained at 198 C using helium as the carrier gas at a flow of 18 ml min^{-1}.

Sample Preparation for GC-MS. [^{3}H]GA_{20} (50 × 10^6 dpm, 3μg) was injected into the pedicel of 15 pods as described above. A single harvest was made at day 17, the seeds separated, extracted, and the extracts purified and chromatographed as described above. Silica gel partition column fractions 17 and 18, and 19 to 21, containing the majority of the radioactivity, were derivatized and examined by GC-RC and GC-MS.

RESULTS

Five days after [^{3}H]GA_{20} was injected into the pedicel, 97% of the recovered radioactivity was found in the developing pods and seeds. Of the remainder, 1% was at the site of injection and 2% was transported to other parts of the plant.

Most of the radioactivity from pods and seeds at each of 5-, 10-, and 23-day harvests partitioned into the ethyl acetate-soluble fraction. This fraction contains most of the acidic GAs and may contain conjugates of some less polar GAs. The dpm from the butanol soluble fraction were small, hence this fraction was not examined further.

Three major zones of radioactivity eluting from the silica gel partition column were observed (Fig. 2). Fractions 6 and 7 corresponded to the elution time of GA_{20}, fractions 17 to 21 to GAs more polar than GA_3 or GA_1 (*e.g.* GA_{29}), and fractions 25 to 27 to very polar GAs. At day 5 most of the radioactivity (the sum of the 27 fractions) was associated with the pods, but this declined from day 10 to 23 (maturity), when only a fraction of the initial dpm remained. In contrast, the amount of radioactivity in the seed was small at day 5, increased at day 10, and was relatively large at maturity.

Analysis by GC-RC allowed the mass and radioactivity to be detected simultaneously. Retention times of the labeled metabolites as MeTMSi derivatives, together with standards, are given in Table I. Silica gel column fractions 6 and 7 contained one peak, which co-chromatographed with GA_{20} on all GC-RC columns. Fractions 17 and 18 contained a peak which co-chromatographed with GA_{29} on all GC-RC columns. In addition to this expected metabolite, there were four other labeled metabolites, designated A, B, C, and D. None of these had retention times corresponding to known GAs. Compound D, present in small amounts and only found in pods, was a highly polar compound. Although it eluted from the silica gel column in fractions 25 to 27 it did not have long GLC retention times. It may have been a conjugate which hydrolyzed on recovery from the silica gel column. Compound C, eluting in fractions 17 and 18, was in both pod and seed but only in trace quantities. Compound B, also eluting in fractions 17 and 18, was present in larger amounts and had GC-RC retention times consistent with a di- or tricarboxylic GA (*e.g.* compare with GA_{13} or GA_{18}, Table I). This metabolite may be a catabolite of GA_{20} or GA_{29}.

Compound A was present in both seeds and pods, and like GA_{29} it was a major product of GA_{20} metabolism. Its GLC retention times (Table I) were similar to (but not the same as) a dihydroxylated GA such as 3-*epi*-GA_1 or an oxidized GA such as 3-oxo-GA_3 (Fig. 1). A product of incomplete derivatization of GA_{29} Me, the mono-TMSi of GA_{29} Me, eluted from the 2% QF1 solution at 23.5 min (retention times on other columns unknown), which is similar to compound A (Table I). However, it could not be compound A since GA_{29} and compound A were separable on the silica gel partition column. Nor could compound A be the C/D-ring rearranged GA_{29} since the retention time of the derivatives do not match on 2% QF1 (Table I).

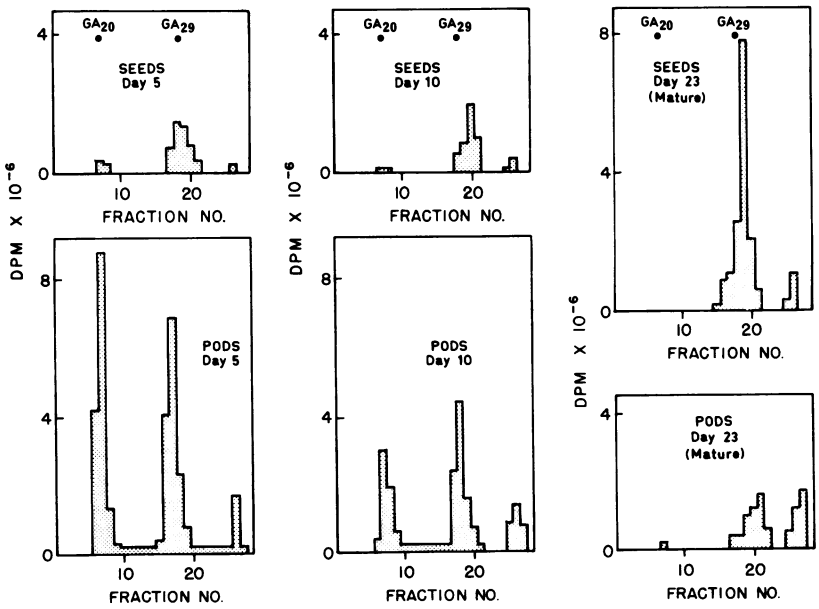

FIG. 2. Distribution of radioactivity in maturing seeds and pods of dwarf pea after application of [^{3}H]GA_{20} (50 × 10^6 dpm) on day 16 after anthesis. Ethyl acetate-soluble fractions from harvests 5, 10, and 23 days (mature) chromatographed on silica gel partition columns.

It was expected that if metabolite A was not an artifact of [^{3}H]GA_{20} metabolism it would also be present in the seed. Therefore, GC-MS was used to obtain the mass spectrum of an endogenous compound which co-chromatographed with the radioactivity of compound A. Thus, from the second experiment (*e.g.* where 50 μCi [2.3 μg] of [^{3}H]GA_{20} was injected, the seeds being harvested at day 17), residues of silica gel fractions 17 and 18 corresponding to the elution time of GA_{29}, and 19 to 21 corresponding to the elution time of compound A, were converted to MeTMSi derivatives for GC-RC. A large mass peak from fractions 17 and 18 was detected by FID and QF1 and SE30 columns corresponding to the radioactive peak assigned to GA_{29} MeTMSi (Fig. 3a). Using similar columns the sample was examined by GC-MS and the scan of the mass peak was found to be identical to the mass spectrum of authentic GA_{29} MeTMSi.

Similarly, a large mass peak detected by FID was observed on QF1 and SE30 columns in fractions 19 to 21, corresponding to the radioactive peak assigned to compound A (Fig. 3B). Examination by GC-MS of the mass peak on similar columns gave a mass spectrum as shown in Figure 4. Ions from an adjacent peak were subtracted, these being determined by taking several scans immediately before and after the elution of MeTMSi compound A. No peak was observed at m/e 506, indicating that the compound was not a dihydroxylated C_{19}-GA. The molecular ion and base peak (excluding the ion at m/e 73) at m/e 446 was 60 atomic mass units less than GA_{29} MeTMSi. Fragment ions at M-15, M-29, M-41, M-72, M-89, and M-119 were typical of a GA MeTMSi derivative.

The fragment ions at M-15 (loss of methyl), M-89 (loss of $(Me)_3SiO$), M-90 (loss of $(Me)_3SiOH$) indicated that the derivative was a trimethylsilyl ether. The fragment ions at M-32 and M-59 were typical of those from a methyl ester of a carboxylic acid. The fragment ion at M-119 indicated the presence of two methoxycarbonyl groups. The strong molecular ion indicated the presence of a C-13 hydroxyl group although ions at m/e 207/208 were not abundant.

After this work was completed, correspondence with Dr. J. MacMillan revealed that his research group had observed a substance in pea seed (cv. Progress No. 9) which, as the MeTMSi derivative, had similar GLC retention times and mass spectrum to

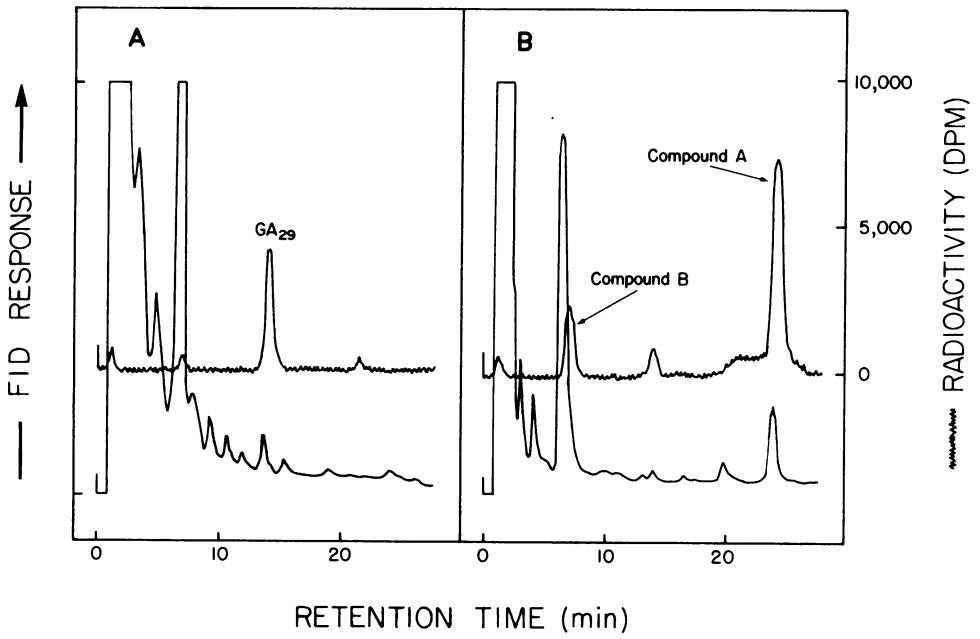

FIG. 3. GC-RC traces of MeTMSi derivatives of compounds from silica gel partition chromatography of purified extracts of maturing seeds of dwarf pea cv. Meteor after application of [^{3}H]GA_{20}. The 2% QF1 column at 206 C was eluted with He at 55 ml min^{-1}. The effluent was split between the radioactivity counter and the FID in the ratio 4:1. A: MeTMSi-derivatized residues from partition column fractions 17 and 18 containing radioactive and mass peaks which co-chromatographed with GA_{29}. B: MeTMSi-derivatized residues from partition column fractions 19 to 21 containing radioactive peaks of compounds A and B, the former of which co-chromatographed with a mass peak (the ketone) at 24.0 min. Note that the radioactive peaks were recorded 0.3 min later than the FID peaks. This was due to time averaging of the counts, necessary to smooth out the signal from the counter.

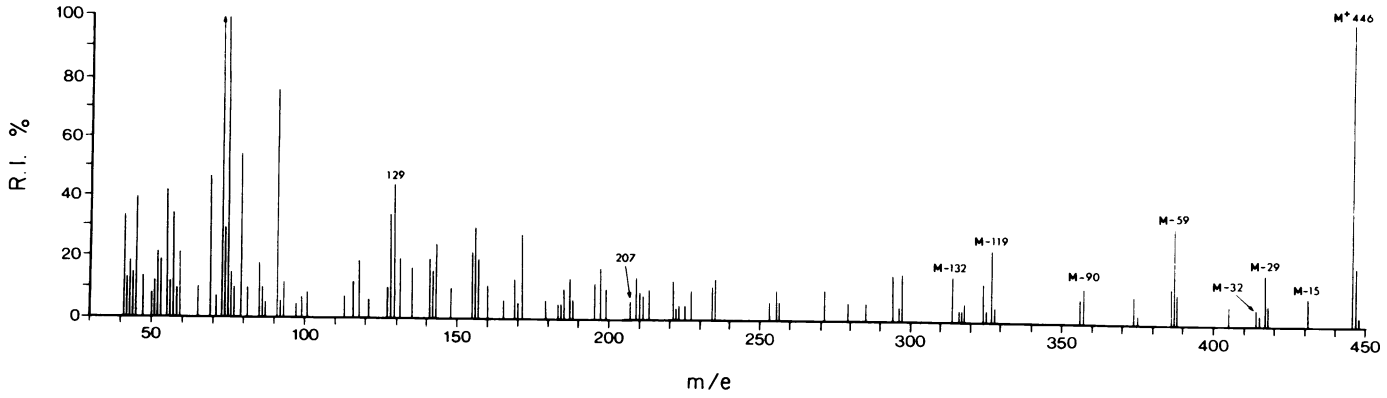

FIG. 4. Mass spectrum of MeTMSi derivative of ketone.

compound A MeTMSi. Recently the structure of this compound has been determined by synthesis and comparison of mass spectra (19; and Gaskin, Kirkwood and MacMillan, unpublished work) as the α,β-unsaturated ketone (Fig. 1). Therefore, the ketone is a metabolic product of GA_{20}. Although [^{3}H]GA_{29} has not been applied to pea seed to confirm its intermediacy between GA_{20} and the ketone, the sequence $GA_{20} \rightarrow GA_{29} \rightarrow$ the ketone is nevertheless indicated from the structural considerations, and from the observation that as the radioactive yield of GA_{29} decreased as the yield of the ketone increased (Fig. 5).

The possibility that compound B was a metabolic intermediate between GA_{29} and the ketone was also examined. If this is the case, a possible structure is that indicated in brackets in Figure 1. Indirect support for such a structure is shown by the similarity in GLC retention times of the MeTMSi derivatives of compound B and GA_{18} (Table I). An alternative intermediate, a keto-lactone, would not fit the GC-RC retention times of any of the ^{3}H products (Table I). A possible metabolic sequence from GA_{20} to the α,β-unsaturated ketone in dwarf pea is given in Figure 1. Nevertheless, it should be pointed out that no direct proof of the structure of compound B has yet been found.

[^{3}H]GA_{29} was the major labeled metabolite of [^{3}H]GA_{20} in the pods (Fig. 5). The concentration was maximal at day 5 and thereafter declined. A very low level of the [^{3}H]ketone was found at each harvest in the pods, indicating that this metabolite of GA_{29} was either transported to the seeds (where it was metabolized) or else catabolically metabolized in the pods. A low level of all ^{3}H-metabolites was found in the pods at maturity (day 23). The level of [^{3}H]GA_{29} in the seeds was much lower than in the pods, but like the pods, was maximal at day 5 and slowly decreased as the seed matured.

In the seeds the labeled ketone was present in small amounts at day 5 (Fig. 5). By day 10 the level of the [^{3}H]ketone had increased and the possible intermediate, compound B, was now present in detectable quantities. At maturity the ketone was present in large amounts and compound B in moderate amounts. The major increase in ^{3}H-catabolites in the seed was in marked contrast to that noted for the pods.

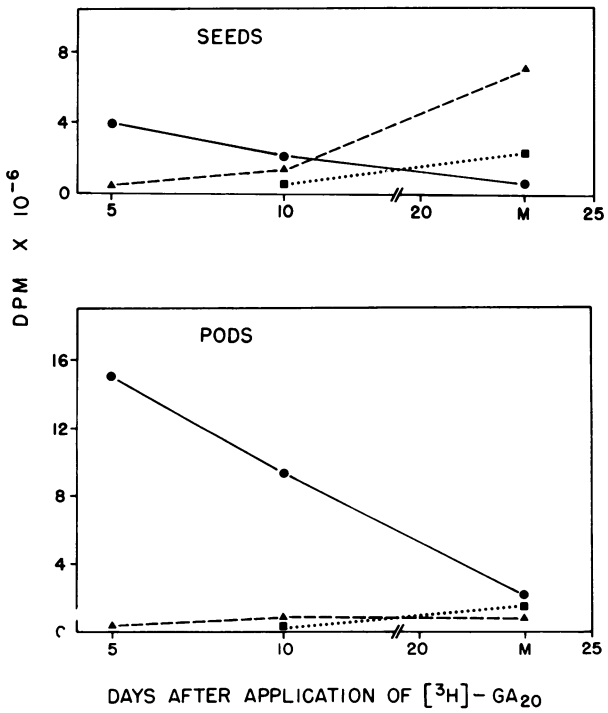

FIG. 5. Levels of radioactive metabolites in maturing seeds and pods of dwarf peas 5, 10 and 23 days (M=mature) after a single pulse application (16 days after anthesis) of [^{3}H]GA_{20} (50 × 10^6 dpm). (●——●): [^{3}H]GA_{29}; (▲-----▲): [^{3}H]ketone; (■·····■): ^{3}H-metabolite B.

DISCUSSION

The two major products of [^{3}H]GA_{20} metabolism in maturing pea seeds were [^{3}H]GA_{29} and the ^{3}H-α,β-unsaturated ketone (Fig. 1). [^{3}H]GA_{29} was an expected metabolite since this conversion has been shown previously in peas (18, 19). However, conversion to the ketone has not been previously demonstrated. Another labeled metabolite, compound B, tentatively assigned the structure indicated in Figure 1, may be the intermediate between GA_{29} and the ketone.

[^{3}H]GA_{29} was formed rapidly, and was present in both seed and pod (Fig. 5) at the first harvest (day 5; 21 days after anthesis). However, the level of GA_{29} declined thereafter, presumably due to conversion to the ketone. The level of the [^{3}H]ketone in the pod did not increase significantly during maturation. On the other hand, the amount of [^{3}H]ketone in the seed was small at the first harvest, but increased during maturation, finally becoming the major ^{3}H-metabolite at maturity. Compound B was also present in the seed in moderate amounts at maturity (Fig. 5).

The labeled ketone and compound B appear to be stored in the seed, which may act as a sink for biologically inactive catabolites of highly biologically active GAs. These catabolic compounds appear to be metabolized very slowly during seed development and maturation. However, another possibility is that the compounds are stored in the mature seed for subsequent use in growth during germination. We do not favor this possibility since other GAs which are keto-derivatives (*e.g.* GA_{26} and GA_{33}) are almost inactive in a number of bioassays (2, 21) and conversion back to GA_{29}, itself a GA of low activity, or to another biologically active GA does not seem likely.

The ketone has not yet been observed as a metabolic product of [^{3}H]GA_{20} or [^{3}H]GA_{29} in dwarf pea seedlings or germinating seed (15, 17), although GA_{20} was efficiently converted to GA_{29}, especially in germinating seed (17). The metabolism of GA_{29} in both seedlings and germinating seeds has been reported to be slow (15) and the identity of the products not determined. Either the duration of the experiments (20-48 h) was insufficient to observe the product, or alternatively, these products were metabolized faster in the seedlings and germinating seed, and may not accumulate as in the mature seed.

The results herein and recent results by Sponsel and MacMillan (19), also using an *in vivo* method, contrast to their earlier results (18) which were consistent with the conclusions from their *in vitro* method. In this culture system [14,15,17-^{3}H]GA_{29} was apparently not metabolized, except perhaps very slowly. This stability of the substrate may have been due to lack of resolution of the labeled products (19) or to the *in vitro* system used. We have observed that *in vitro* systems can alter GA metabolic pathways and rates of metabolism (Harada, Pharis and Sassa, unpublished results). Although, as far as we are aware, GA levels in cultured pea seeds have not been compared to those grown *in vivo*, it is known that the levels of hormones such as cytokinins in immature dwarf pea seeds are markedly different in seed grown in culture compared to seed grown on the whole plant (9). In our case the plants not only remained intact throughout the course of the experiment, but care was taken not to damage the seeds during application of [^{3}H]GA_{20} and tests were subsequently conducted to demonstrate the viability of the seed. The labeled GA was not applied in doses which exceeded the endogenous levels of the seed (18) since we used GA_{20} of high specific activity, thus enabling the exogenous carrier GA_{20} to be applied in amounts (< 0.5 μg/pod—about 5 seeds p/pod) much lower than the endogenous level of GA_{20} in the seed [6 μg/seed].

Since seeds contain high levels of endogenous GAs, GC-MS

verification of pathways is difficult and there is a need for accurate techniques to separate each of the many labeled products of GA metabolism. In the present study a direct flow GC-RC system was used, the radioactive peaks being matched with mass peaks to within 0.1 min. Utilization of three columns (5) ensured separation of products on at least one. This approach allowed detection of a metabolite (the ketone) which had previously been missed (18).

An interesting point concerning the work is the probable loss of tritium label during [^{3}H]GA_{20} metabolism. Since the majority of the tritium should be at the C-2 and C-3 positions (configuration or stereochemistry unknown), it is possible that some tritium loss could occur during conversion of GA_{20} to GA_{29}. Complete loss of the tritium at C-2 would occur during oxidation to the ketone and some loss of tritium would be expected to occur by exchange at C-3. One of the reasons that Sponsel and MacMillan (19) failed to observe conversion of GA_{29} to the ketone using MS was apparently due to complete loss of the deuterium label at C-1 and C-3 by exchange. However, in the preparation of [^{3}H]GA_{20} precursor for the present study (11) other positions of the molecule were undoubtedly labeled by catalytic exchange of H_2 by tritium during reduction (a high percentage of tritium in the H_2 gas was used). For example, Nadeau and Rappaport (13) have demonstrated that over a quarter of the tritium label was randomly incorporated by catalytic exchange of H_2 during the hydrogenation of GA_3 to GA_1 using carrier-free tritium. Hence, during the metabolism of [^{3}H]-GA_{20} to the ketone in our pea seeds, even though some label was lost at C-2 and C-3, the product would still retain a portion of its radioactivity. What proportion of radioactivity remained was not determined, so that yield of the ketone was probably much higher than that depicted in Figure 5.

It has been assumed that a major pathway of GA deactivation is by C-2 hydroxylation and conjugation to molecules such as glucose (4). The formation of GA_8 and GA_8 glucoside from GA_1 has been demonstrated in barley aleurone layers (14) and immature (20) and germinating (12) bean seed. Although GA_{20} was converted to GA_{29} (as well as GA_1) in immature bean seeds, the conversion to GA_{29} glucoside was not reported (20). Thus, oxidation of the C-2 hydroxyl group and opening of the lactone ring in the postulated sequence of GA_{29} → compound B → the ketone demonstrates an alternative pathway of GA catabolism to biologically inactive compounds.

The reasons for the accumulation of the ketone in the seed remain unclear. Its breakdown is either very slow, or possibly enzymes responsible for its further metabolism are not present during seed development and maturation. The low counts obtained in the butanol-soluble fraction would indicate that direct conjugation of GA_{29}, giving GA_{29} glucoside, is not a major pathway in dwarf pea seed or pods. During germination of the seed, the radioactivity associated with the ^{3}H-metabolite B and the [^{3}H]ketone rapidly disappear, only minor amounts remaining in acidic, ethyl acetate-soluble and butanol-soluble substances.

Acknowledgments—The authors acknowledge useful correspondence and discussion with Drs. J. MacMillan and V. Sponsel (University of Bristol) and examination of their manuscript (19) when in press. We are grateful for the gift of GA_{29} from Prof. N. Takahashi (University of Tokyo). Mr. W. Morf is thanked for expert technical assistance.

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