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ENDOGENOUS GIBBERELLINS IN THE POTATO, *SOLANUM TUBEROSUM*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; identification; gibberellins; gibberellin A_1; gibberellin A_{15}; gibberellin A_{20}.

Abstract—The major gibberellin found by GC-MS in immature berries of potato was GA_{20}; GA_{15} was also identified. A novel gibberellin with a mass spectrum indicative of a monohydroxy, 20-carbon GA with a delta lactone was also detected. Evidence was obtained by selected ion monitoring for the occurrence of GA_{20} and also GA_1 in sprouts on potato tubers.

INTRODUCTION

After harvest, potato tubers experience a rest period when their buds do not sprout, even though environmental conditions may be suitable for growth. During the ensuing winter storage period, sprouts then develop to an extent which largely depends on the time of the end of the rest period and the storage temperature. The degree of sprouting which the tubers have attained by the time of planting in the spring can then have a profound effect on subsequent field performance [1].

It has been known for many years that treatment of freshly harvested tubers with solutions containing gibberellin (GA) can markedly shorten the rest period, and that increasing concentrations of applied GA give correspondingly greater rates of sprout extension subsequently [2, 3]. It is of interest, therefore, to understand the part played by endogenous GAs in termination of the rest period and subsequent sprout development. Several workers have examined whether the level of endogenous GAs is correlated with sprout development [4, 5].

These earlier studies used low resolution chromatographic techniques and bioassays to determine the levels of GA-like substances. They provided no identification of individual GAs. Moreover, they provided no reliable data on GA levels since potato tubers and sprouts contain many acidic growth inhibitors [6] which vary in amount during the rest period and sprout development, and which can interfere with GA bioassays. We therefore used GC-MS with selected ion monitoring (SIM) to identify the endogenous GAs and to establish the feasibility of quantitating them by SIM with heavy isotope-labelled internal standards [7].

RESULTS AND DISCUSSION

As endogenous GAs are usually only trace components of plant tissues, their identification by GC-MS requires substantial purification of the initial extract. The multiplicity of naturally occurring GAs, together with the wide range of polarities which they exhibit, necessitates some means of monitoring fractions from an extract to locate the GAs. Although bioassays cannot provide reliable quantitative data, they do provide the only practical means for screening large numbers of fractions for the presence of endogenous GAs. For this purpose, we found that the *d*-5 dwarf maize assay was least affected by the inhibitory and toxic compounds in potato extracts.

The acidic ethyl acetate-soluble material from an extract of sprouts was chromatographed by reverse phase HPLC and the fractions bioassayed. Three zones of biological activity were detected, with retention times similar to those of dihydroxylated, monohydroxylated and nonhydroxylated GAs, respectively [8]. The amounts of endogenous GAs in these tissues were estimated to be only in the range 1–2 ng per g fresh weight. It was clear from initial work with extracts of tubers and sprouts that, in comparison to the low levels of GAs in these tissues, the levels of interfering compounds were too high to allow identification of the GAs by full scan GC-MS. In immature seeds the levels of GAs are generally much higher than in vegetative tissues. It therefore seemed to be a reasonable strategy to identify the GAs in immature seeds of potatoes by full scan GC-MS and then to investigate the presence of some of those GAs in sprouts by the more sensitive, though less definitive, technique of SIM.

It was not feasible to remove individual seeds from the potato fruits, so 4 kg of entire immature berries were extracted with methanol and batch processed on columns of LiChroprep and cellulose phosphate. The acidic ethyl acetate-soluble fraction was then chromatographed on reverse phase HPLC and each fraction assayed. As was the case with the extracts of sprouts, three zones of GA-like activity were found. The fractions comprising each of these zones were combined and each of the resultant three samples further purified by gradient-eluted silicic acid partition chromatography. In each case, a single zone of biological activity was detected in the eluate. Silicic acid chromatography had no effect on the apparent specific

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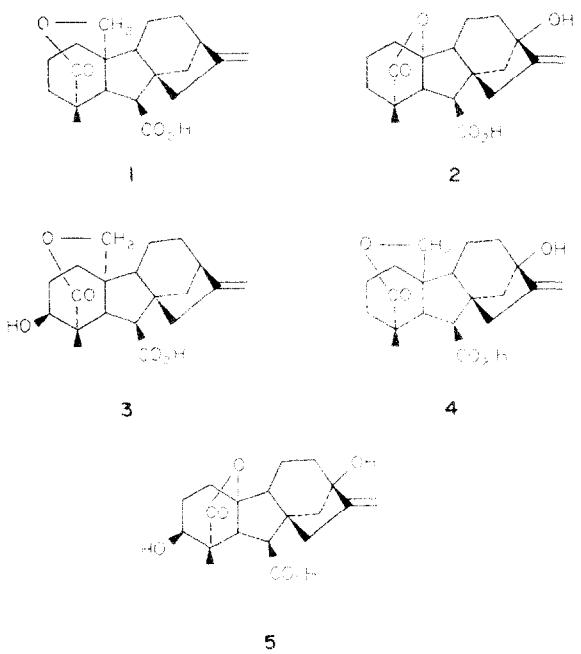
biological activity of sample A (most polar), caused a slight increase in the activity of sample C (least polar), and gave a more than five-fold increase in the activity of sample B (intermediate polarity). These three samples were then methylated, silylated and examined by GC-MS.

Sample C contained a minor component with the same mass spectrum as the methyl ester of GA_{15} (**1**) [*m/z* value (rel. int.): 344 (M^+, 35) 312 (23), 298 (10), 284 (45), 239 (100)]. In the GC profile of sample B, the major component had a mass spectrum identical to that of MeTMSi GA_{20} (**2**) [418 (M^+, 100), 403 (13), 375 (45), 301 (15), 207 (62)]. The only compound in sample A which had a mass spectrum similar to that of a GA was a minor component whose partial mass spectrum is shown in Table 1.

That the ion at *m/z* 432 is the molecular ion is easily deduced from the ions at *m/z* 417 and 342 (losses associated with a TMSi ether), and at 401 and 373 (losses associated with a methyl ester). This M_r is that of the MeTMSi derivative of a monohydroxylated, 20-carbon GA with a delta lactone, such as GA_{3-7} (**3**) or GA_{44} (**4**). Comparison of the spectra of these two compounds with that of the compound in potato sample A (Table 1) however, shows that the potato unknown compound is different from either of these two compounds. The potato GA lacks the ion at *m/z* 207, characteristic of 13-hydroxy GAs such as GA_{44}, and has a base peak of *m/z* 432, whereas GA_{3-7} has a base peak at *m/z* 129. Moreover, on reverse phase HPLC, both GA_{3-7} and GA_{44} elute after GA_{20} [8], while the potato unknown elutes before GA_{20}. The mass spectrum of the potato unknown compound does not match that of any of the known GAs (Gaskin, P. and MacMillan, J., personal communication).

It was next of interest to determine whether GA_{20} occurred in tubers and sprouts, and whether it was possible to quantitate it in these tissues. Reliable quantitation by SIM requires a suitably-labelled internal standard, for which we used [1, 2, 3-$^2H_3$]GA_{20}. The MeTMSi derivative of this compound has M^+ (and base peak) at *m/z* 421, which is easily distinguished from that of the native compound (*m/z* 418) by GC-MS-SIM. Figure 1A shows the SIM trace obtained with this standard, and the



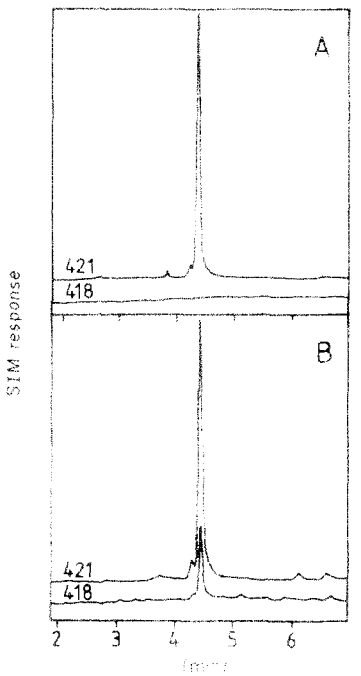


Fig. 1. GC-MS-SIM traces of: (A) deuterated MeTMSi GA_{20} standard; (B) derivatised extract of potato sprouts to which [$^2H_3$] GA_{20} had been added as an internal standard.

Table 1. Relative intensities of significant ions in the mass spectrum of a novel gibberellin from potato berries, compared with those of two known gibberellins

Ion (*m/z*)	GA_{3-7}-MeTMSi	Unknown-MeTMSi	GA_{44}-MeTMSi
432 (M^+)	11	100	68
417	4	13	13
401	2	18	10
373	–	14	19
342	15	31	4
282	18	45	
238			48
237		59	
207	–		100

complete absence of any unlabelled GA_{20} in the preparation.

Known amounts of deuterated GA_{20} were added to methanol extracts of tubers and sprouts. The acidic fractions of these extracts were subjected to batch silica partition chromatography followed by reverse phase HPLC, methylation and normal phase HPLC. Fractions corresponding to the retention time of $MeGA_{20}$ were silylated and analysed by GC-MS-SIM, monitoring for *m/z* 418 and 421. Figure 1B shows the SIM trace for an extract equivalent to 5 g dry wt of sprouts, to which had been added 1 ng [^{2}H]-GA_{20}. Comparison of the area of the *m/z* 421 peak with the areas obtained by the injection of known amounts of [^{2}H]-GA_{20} indicated a recovery of 47% for this extract. The content of endogenous GA_{20} in this extract was calculated to be 52 pg per g dry wt. It is clear from this that it is quite feasible to quantitate the levels of endogenous GA_{20} in potato tubers and sprouts and we are currently engaged in doing this, in relation to storage temperature and physiological age.

Many species which contain GA_{20} also contain GA_1 (5), and metabolic studies have shown that GA_{20} is the precursor of GA_1 [9]. For maize and pea, at least, it has been argued that in fact as far as extension growth is concerned, it is GA_1 which is actually the biologically active GA [10]. Obviously it is important for an understanding of extension growth in potato sprouts to know whether GA_1 occurs in potatoes, along with GA_{20}. The work described above did not detect GA_1 in berries, but it is clear that potato extracts contain sufficient inhibitory compounds to mask small amounts of activity.

To obtain evidence for the presence of GA_1 in tuber sprouts, a purified fraction with the known chromatographic properties of GA_1 was prepared and examined by GC-MS-SIM. Figure 2A shows the SIM trace obtained with a standard of MeTMSi GA_1, monitoring for M^+ (which is also the base peak) at *m/z* 506. Figure 2B shows the trace from the injection of an extract equivalent to 15 g dry weight of sprouts. A peak of *m/z* 506 can be seen at a retention time identical to that of authentic MeTMSi GA_1. On this evidence we conclude that GA_1 is indeed an endogenous GA in potato sprouts, and that study of the role of GAs in sprout development will therefore also require the quantitation of GA_1, as well as GA_{20}.

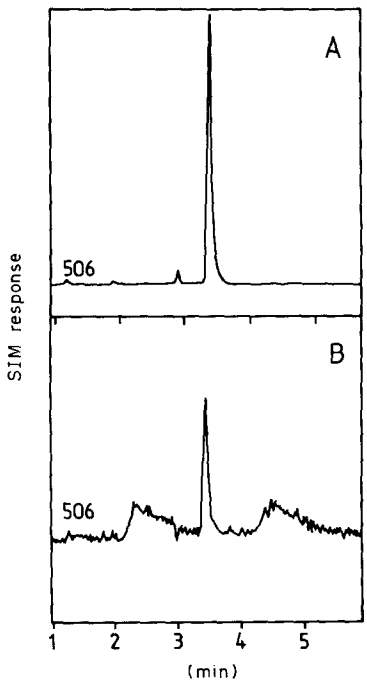


Fig. 2. GC-MS-SIM traces of: (A) standard MeTMSiGA_1; (B) methylated, silylated extract of potato sprouts

EXPERIMENTAL

Plant material. Field-grown tubers of *Solanum tuberosum* cv Homeguard were stored after harvest at 15–20° until they were sprouting. Sprouts 1–2 cm long were removed, frozen in liquid N_2 and freeze-dried. Immature green berries, 1–2.5 cm diameter, were collected from plants of *S. tuberosum* cv Desiree growing in the field. The berries were also frozen in liquid N_2 and lyophilized.

Bioassays. The dwarf maize assay was performed as described previously [11] using *Zea mays* which was homozygous for the *d*-5 mutation.

Extraction. Freeze-dried material was homogenized in 70% MeOH (20 ml/g dry wt), filtered through cheesecloth and two layers of Whatman No. 2 paper and the filtrate stored at 4°. The residue was re-extracted with fresh 70% MeOH at 4° overnight. After filtration of the second extract, the two filtrates were combined and filtered through Whatman GF/F glass fibre paper. The 70% MeOH extact was then pumped over the LiChroprep column.

LiChroprep C18 column. Filtered 70% MeOH extracts were pumped over a column (250 × 22.5 mm, 25–40 μm) of LiChroprep RP-18 reverse phase material at a flow rate of 30 ml/min. The non-retained material (initial volume of extract plus 200 ml 70% MeOH wash) was reduced to an aq. extract by evapn, and the column was regenerated by washing with THF. The capacity of this column to retain material from an extract was gauged from the break-through point for the non-polar pigments in the extract. In addition to reducing the dry wt of extracts by up to 50% (with virtually no loss of added radiolabelled GAs), this batch C18 treatment greatly reduced the tendency of extracts to form emulsions when subsequently partitioned against organic solvents.

Cellulose phosphate. The aq. extract of immature berries was adjusted to pH 3 before being loaded onto a column of 60 g floc cellulose phosphate cation exchanger (NH_4^+ form). The column was washed with 2 l H_2O (pH 3) and all the non-retained material taken as the acidic fraction. This acidic fraction was pumped over the LiChroprep column and the retained acidic materials then eluted with MeOH. H_2O was added to the MeOH and the mixture evapd to give an aq. extract (of greatly reduced vol.) which was adjusted to pH 2.5 and partitioned against EtOAc.

Reverse phase HPLC. When acidic EtOAc-soluble extracts of sprouts were reduced to a small vol., they formed extremely viscous solutions which were impossible to filter. To avoid this problem, H_2O was added to these fractions and the EtOAc removed by evapn. The aq. extract was then pumped onto a column of 10 μm ODS Hypersil (150 × 10 mm) and the column flushed with H_2O (pH 3) at 5 ml/min. The column was then developed with a gradient of MeOH in 0.1 M HOAc, from 0–100% in 30 min at 5 ml/min. Fractions were collected every min and dried by evapn. This procedure was also used for the large extract of immature berries. For later work with sprout extracts (when a batch silicic acid procedure had been adopted), the acidic extract was injected onto the HPLC through an

injection loop (2 ml) in *ca* 1 ml of the starting eluant, and the column developed with a gradient of 10–70% MeOH in 0.1 M acetic acid in 30 min at 5 ml/min.

Silicic acid partition chromatography. After reverse phase HPLC of the extract of berries, fractions which contained biological activity were subjected to gradient-eluted silicic acid partition chromatography. Each sample was dissolved in a small volume of EtOAc and adsorbed by evapn onto 0.5 g silica gel (containing about 20% H_2O as stationary phase). The sample was then loaded onto a column of the same silica gel (180 × 12 mm), packed in hexane. The column was developed with a gradient of EtOAc in hexane (190 ml 40% EtOAc in the first chamber, 190 ml 100% EtOAc in the second chamber) at 20 ml/hr. Solvents were saturated with H_2O adjusted to pH 3 with TFA and were degassed before use. Fractions were collected every 10 ml, dried by evapn and tested for biological activity.

For later work with extracts of sprouts, acidic EtOAc-soluble fractions were cleaned up prior to HPLC by a batch treatment on silica gel [12]. Samples (derived from 50 g dry wt of tissue) were dissolved in a few ml of EtOAc–hexane (9:1) and applied to columns 2 cm dia. containing 10 g silica gel, packed in EtOAc–hexane (9:1). Each column was developed with 100 ml of the same solvent.

Normal phase HPLC. In later work with extracts of sprouts, appropriate fractions from reverse phase HPLC were methylated with ethereal CH_2N_2 and chromatographed on LiChrosorb DIOL (10 μm, 250 × 4.5 mm). Using 5% *iso*-PrOH in hexane at 2 ml/min, $MeGA_{20}$ eluted after 8.5 min. To elute $MeGA_3$ after a similar time, the solvent was changed to *iso*-PrOH–hexane (1:9) and the flow rate to 3 ml/min.

Deuterated GA_{20}. [1, 2, 3-$^2H_3$] GA_{20} was prepared as described in ref. [13]. Isotopic composition by MS: $^2H_4$ 91%, $^2H_3$ 8%, $^2H_1$ 1%. Known amounts of this compound were added as internal standards to initial homogenates of tissue in MeOH.

GC-MS. Methylated samples were dried then silylated (pyridine–hexamethyldisilazane–trimethylchlorosilazane, 9:3:1), before injection onto a Kratos MS 25 GC-MS system. For full scan work conditions were: GC: splitless injection at 270° onto a BP-10 column (12 m × 0.33 mm); temp. programme 200–270° at 8°/min; He 0.6 kg/cm^2; MS (EI): source 190°, jet 250°, inlet 260°, ionizing potential 70 eV. For SIM, GC conditions were the same except that the temp. programme was 230–270° at 8°/min; the MS was set for a dwell of 140 msec, with a settling time of 100 msec.

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