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GC-MS Identification of Native Gibberellin-O-Glucosides in Pea Seeds

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Abstract. Mature seeds of *Pisum sativum* cv. "Grapis" were investigated to identify glucosyl conjugates of gibberellins (GAs). Purified and permethylated extracts were analyzed by capillary gas chromatography–mass spectrometry (GC-MS). On the basis of synthetic standard compounds, GA_{20}-13-O-glucoside (4), GA_{29}-2-O-glucoside (7), and GA_{29}-13-O-glucoside (8) were identified by full-scan spectra. This is the first definitive evidence of the native occurrence of gibberellin-O-glucosides in pea.

Seeds of leguminosae have intensively been investigated with respect to the spectrum of free gibberellins (GAs) and their metabolism (see Graebe 1988, MacMillan 1990, Sponsel 1983a,b). From maturing fruits of *Phaseolus coccineus* the first GA-O-glucoside [e.g., GA_8-2-O-glucoside (3)] has been isolated (Schneider 1983, Schreiber et al. 1967). The occurrence of additional GA glucosyl conjugates from legumes (Table 1) has been based on large-scale extractions and classical methods for structural elucidation (Fig. 1). However, from *Pisum sativum* GA-O-glucosyl conjugates have not yet been isolated. By enzyme or acid hydrolysis of aqueous fractions, however, free GAs, especially GA_{20} and GA_{29}, as well as a GA_{29} catabolite, have been identified and this indicates the presence of conjugated forms of GAs in pea (Sponsel et al. 1979, 1983b).

We reexamine pea seeds with respect to the occurrence of GA-O-glucosides using an improved purification method (Schneider et al. 1991b), and we report their identification by gas chromatography–mass spectrometry (GC-MS).

Materials and Methods

Extraction and Purification

Mature seeds of field grown *Pisum sativum* cv. "Grapis" plants (96 g) were macerated and extracted with 150 ml of methanol containing 20% water. The extraction was repeated twice. The combined methanol extracts were evaporated (6.0 g), loaded onto a DEAE-Sephadex A 25 column (250 ml), and eluted with an acetic acid gradient in methanol (Schneider et al. 1991b). The polar acidic fraction (168 mg) was methylated by etheral diazomethane.

Permethylation and HPLC

The methylated polar acidic fraction was permethylated with methyl iodide/sodium hydride (Schneider 1987), and the product was purified on a silica column with a methanol gradient in toluene. The pooled fractions of the permethylation products were separated on Lichrosorb RP18 (10 μm, 10 × 250 mm) with methanol:water (75:25; 2.5 ml min^{-1}). The fraction, V_R = 25.0–44.0 ml, containing the permethylated gibberellin-O-glucosides (PG-1) was evaporated and subjected to GC-MS. For methodological details see Schneider et al. (1991b) and Sembdner et al. (1987).

GC-MS Conditions

A Hewlett Packard quadrupole mass spectrometer (HP 5970B) with a HP 5890 GC equipped with a cross-linked methylsilicone-fused silica column was used (Chrompack CP-Sil5 C8 25 m × 0.32 mm, film thickness 0.25 μm, phase ratio 319, helium 2.5 ml/min, splitless injection, direct inlet interface 230°C, ion source 220°C, injection 275°C, electron impact energy 70 eV). The temperature program was as follows: 60°C (1 min) to 270°C (30°C min^{-1}). The Kovats retention indices (KRIs) were calculated on the basis of the retention times of standard substances and parafilm (compare Hedden 1986, Schmidt et al. 1988). The slight shifts obtained for sample KRIs may be due to overloading effects.

Results and Discussion

Mature seeds of *Pisum sativum* cv. "Grapis" were extracted with methanol, and the extracts were purified on DEAE-Sephadex. After permethylation of the acidic fraction and purification on silica chro-

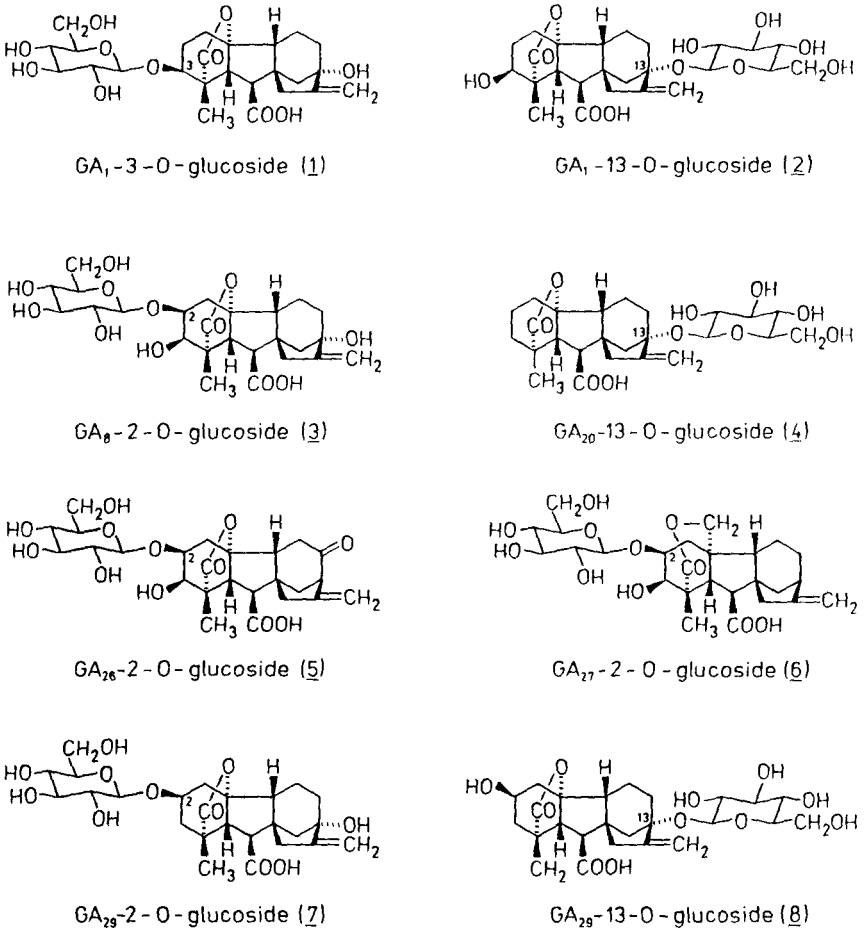

Fig. 1. See text.

Table 1. Gibberellin-O-glucosides (GA-O-G) (Fig. 1) and gibberellin glucosyl esters (GA-GE) isolated from leguminosae and convulvulaceae.

Phaseolus coccineus	GA_1-3-O-G (1) (Schliemann and Schneider 1989) GA_8-2-O-G (3) (Schreiber et al. 1967)
Phaseolus vulgaris	GA_8-2-O-G (3); GA_1-GE; GA_4-GE; GA_{37}-GE; GA_{38}-GE (Hiraga et al. 1974)
Pharbitis nil	GA_3-3-O-G 2; GA_8-2-O-G (3); GA_{26}-2-O-G (5); GA_{27}-2-O-G (6); GA_{29}-2-O-G (7) (Yokota et al. 1971)
Pharbitis purpurea	GA_5-GE; GA_{44}-GE (Yamaguchi et al. 1980)
Dolichos lablab	GA_1-3-O-G (1) (Yokota et al. 1978)
Cytisus scoparius	GA_{35}-11-O-G (Yamane et al. 1974)
Pisum sativum	GA_{20}-13-O-G (4); GA_{29}-2-O-G (7); GA_{29}-13-O-G (8)

matography, the sample was fractionated by preparative reverse-phase high-performance liquid chromatography (RP-HPLC). The fraction PG-1 (V_R = 25–44 ml), which contains presumptive permethylated GA-O-glucosides, was subjected to capillary GC-MS.

By monitoring of characteristic ions (Schmidt et al. 1988) (selected ion monitoring = SIM), evidence was obtained for the presence of GA_{20}-13-O-glucoside (4), GA_{29}-2-O-glucoside (7), and GA_{29}-13-O-glucoside (8) at the appropriate KRIs (see Table 2). Using full-scan detection with a repeated injection, we succeeded in obtaining complete spectra of GA_{20}-13-O-glucoside (4), GA_{29}-2-O-glucoside (7), and GA_{29}-13-O-glucoside (8) (Table 2). There were indications of the presence of GA_1-3-O-glucoside (1) based on SIM (Table 2). Full-scan detection, however, failed to confirm the presence of GA_1-3-O-glucoside (1). Also, using SIM and full-scan mode, we attempted without success to find GA_8-2-O-glucoside (3), which in *Phaseolus* accounts for the major GA-conjugate (Schneider 1983).

The present identification of GA-glucosides in pea by definitive methods is confirmatory of earlier observations that hydrolysis of polar fractions from pea yields in free GA_{20} and GA_{29} (Sponsel et al. 1983b). Surprisingly, both isomers, the 2-O-glucoside (7) and the 13-O-glucoside (8) of GA_{29},

Table 2. Chromatographic retention (KRI = Kovats retention index), as well as characteristic ions (m/z) and their abundancies (%) of permethylated gibberellin-O-glucosides isolated from *Pisum sativum* and of permethylated standard compounds.

Compound	KRI	Characteristic ions							
GA_{20}-13-O-G (4)		88	101	187	269	297	329	389	m/z
Standard	3418	72	36	8	10	14	100	35	(%)
PG-1 SIM	3424	52	28	—	25	20	100	39	(%)
PG-1 full scan	3426	58	30	4	16	18	100	50	(%)
GA_{29}-2-O-G (7)		88	101	315	327	359	419	594	m/z
Standard	3590	100	79	61	12	59	21	7	(%)
PG-1 SIM	3595	100	100	63	12	51	18	4	(%)
PG-1 full scan	3598	100	90	68	12	60	21	10	(%)
GA_{29}-13-O-G (8)		88	101	187	283	327	359	419	m/z
Standard	3521	74	35	8	8	14	100	32	(%)
PG-1 SIM	3530	69	20	—	6	16	100	24	(%)
PG-1 full scan	3531	48	22	6	6	15	100	34	(%)
GA_1-3-O-G (1)		88	101	315	327	359	375	594	m/z
Standard	3573	80	100	50	3	29	20	13	(%)
PG-1 SIM	3579	90	100	35	10	25	30	—	(%)

were found. This is the first evidence for the endogenous occurrence of GA_{29}-13-O-glucoside (8) in plants. Its origin could be from free GA_{29}, but 2β-hydroxylation of intact GA_{20}-13-O-glucoside (4) cannot be excluded.

With respect to the physiological role of GA conjugation, the occurrence of GA_{20}-13-O-glucoside (4) is highly relevant. It could be a permanent inactivation form or, possibly, a temporary repository for GA_{20}, from which aglucone could be liberated by enzymatic hydrolysis during seed germination. If hydrolyzed, GA_{20}-glucosyl conjugates could serve as sources of GA_1, GA_5, and GA_3 via GA_{20} (MacMillan 1990). These would be independent from de novo biosynthesis during seed germination. GA_{20}-13-O-glucoside (4) could also be identified in *Zea mays* and *Hordeum vulgare* (Schneider et al. 1991a,c).

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