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Communication

Identification of Endogenous Gibberellins from Oilseed Rape[1]

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ABSTRACT

Oilseed rape (*Brassica napus*, canola variety 'Westar') plants were grown in greenhouse conditions and shoots were harvested during the final stages of shoot elongation. Leaves and immature pods were removed and the remaining stem tissue was extracted and purified. The extract was chromatographed on sequential, step-eluted silica gel partition and reverse-phase C_{18} HPLC columns, and gibberellin (GA)-like substances were detected using the 'Tan-ginbozu' dwarf rice microdrop assay. Purified fractions showing GA-like activity were analyzed by capillary gas chromatography-mass spectrometry (GC-MS) and GC-selected ion monitoring (GC-SIM). Gibberellins A_1, A_3, and iso-A_3 were identified by full spectrum GC-MS with GA_1 being the most abundant GA in the stem tissue. Gibberellins A_{19} and A_{20} were identified by GC-SIM and are logical precursors of the GA_1.

The mustard family, Brassicaceae, contains a number of agriculturally and horticulturally important species, including cabbages, cauliflower, broccoli, kale, mustard, and oilseed rape. As well as having considerable economic importance, the genus *Brassica* is also of significant interest to plant physiologists studying bolting, the rapid stem elongation which normally precedes flowering. The involvement of GAs[2] in the regulation of bolting in *Brassica* is suggested by the promotion of stem elongation following the exogenous application of GA_3 (14, 16, 17), and the known involvement of GAs in the regulation of bolting in other cold-requiring or photoperiodic plants (8, 17). However, prior to an evaluation of the possible role of endogenous GAs in the regulation of stem growth in *Brassica*, the specific GAs native to *Brassica* must be identified. Thus, the present study was performed to characterize the principal biologically active GAs of oilseed rape.

MATERIALS AND METHODS

Plant Material. Six oilseed rape (*Brassica napus*, annual Canola variety 'Westar') seeds were planted January 28, 1986 in each 14 × 13 cm pot filled with Metro-mix 200 (W. R. Grace & Co., Ajax, Ontario), a soilless peat and vermiculite medium. Plants were watered to saturation daily, fertilized weekly with 0.25 g 28-14-14 with added micronutrients (Plant Prod 28-14-14, Plant Products Co. Ltd., Bramalea, Ontario), and grown in a glasshouse at the University of Lethbridge (latitude 49.6 N) in which the day/night temperatures were about 25/22°C. Sixteen h supplemental lighting from cool-white fluorescent tubes provided 140 μmol s^{-1} m^{-1} photosynthetically active radiation as determined with Li-Cor quantum sensor (Li-Cor Inc., Lincoln, NE). During bolting, 76 d after planting, shoots from six 110 cm tall plants were harvested, leaves, flowers, and immature pods (siliques) were removed, and the remaining stems were frozen in liquid N_2 and lyophilized.

Extraction and Purification. Gibberellins were extracted and purified from the resultant 44 g of dry stems as previously described (10, 12), prior to step-elution SiO_2 partition chromatography (2, 13). Fractions were bioassayed in serial dilution with the Tan-ginbozu dwarf rice microdrop assay (6, 12).

Biologically active regions from the SiO_2 partition column were subsequently chromatographed on reverse-phase C_{18} HPLC as previously described (3, 11), although the gradient from 10 to 73% MeOH in 1% acetic acid was run over 60 rather than 30 min. For both chromatographic steps (SiO_2 partition and C_{18} HPLC), authentic [^{3}H]GA_1 and [^{3}H]GA_4 (Amersham), [^{14}C]GA_3, [^{3}]GA_8 (7), and [^{3}H]GA_{20} (7) were similarly chromatographed to establish Rts.

GC-MS. Biologically active peaks from C_{18} HPLC were methylated with ethereal diazomethane and silylated with 50:50 pyridine:BSTFA with 1% TMCS (Pierce Chem. Co.). Conditions for GC-SIM analysis were similar to those previously described (12) except that a DB-1-15N column (0.25 μm film thickness, J & W Scientific Inc.) was used. For full spectrum GC-MS, the Hewlett Packard 5970A mass selective detector was operated with the 'Peakfinder' program. Ions from 70 to 600 amu were monitored with a scan speed of 690 amu/s. For GC-MS, the oven temperature was programed from 35°C to 200°C at 15°C/min (11 min), held at 200°C for 1 min, and then programed to rise at 3°C/min to 260°C.

RESULTS AND DISCUSSION

SiO_2 Partition Column Analysis. Substantial peak-tailing was observed for GA-like activity eluting from the SiO_2 column, presumably as a result of overloading with the semipurified extract (Fig. 1). A small region of GA-like activity eluted at the Rt of authentic [^{3}H]GA_{20} (SiO_2 region I). A second region contained considerable levels of GA-like activity; its maximum occurred at the Rt of authentic [^{3}H]GA_1 (SiO_2 region II). Five additional groupings were collected from later SiO_2 fractions although these did not elute as distinct peaks (SiO_2 regions III–

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[2] Abbreviations: GA, gibberellin; EtOAc, ethyl acetate; GC-MS, combined capillary gas chromatrography-mass spectrometry; GC-SIM, GC-selected ion monitoring; MeOH, methanol; MeTMSi, methyl ester-trimethylsilyl ether; Rt, retention time; SiO_2, silica gel; amu, atomic mass units.

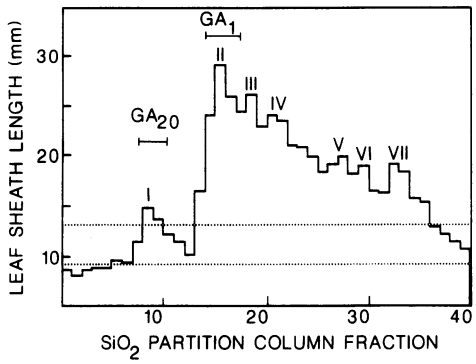

FIG. 1. Elution of GA-like substances as determined with the cv Tanginbozu dwarf rice microdrop assay, from a stepwise-eluted SiO_2 column loaded with semipurified extracts from oilseed rape stems. The lower dashed line represents the leaf sheath length of control seedlings while the upper dashed line represents the response to 10^{-4} μg GA_3 per rice plant.

VII). SiO_2 region II caused dwarf rice leaf sheath elongation equivalent to at least 10^{-1} μg GA_3 per rice plant at all three dilutions (1/100–1/400), the point of saturation in the dwarf rice bioassay. Hence, region II was estimated to contain at least 50 μg GA_3-equivalents purified from the 44 g of stem tissue.

C_{18} HPLC Analysis. SiO_2 region I eluted from reverse-phase C_{18} HPLC as a single peak of GA-like activity at the Rt of [^{3}H]GA_{20} (data not shown). SiO_2 region II was resolved by HPLC into at least four peaks of GA-like activity (Fig. 2). The first (peak IIa, Fig. 2) eluted between [^{3}H]GA_8 and [^{14}C]GA_3. Two closely spaced peaks eluted at the Rts of [^{14}C]GA_3 (peak IIb) and [^{3}H]GA_1 (peak IIc), respectively. The fourth peak eluted just before the Rt of authentic [^{3}H]GA_4, a region in which GA_{19} will elute (10, 12).

Gas Chromatography-Mass Spectrometry. HPLC fractions containing GA-like activity from SiO_2 regions I, II, III, and VII were analyzed by GC-MS and/or GC-SIM. Gibberellin A_{20} was identified by GC-SIM (Table I) in the single peak of biological activity from the C_{18} HPLC of SiO_2 region I.

HPLC peak IIa (Fig. 2) contained iso-GA_3, which was identified from a full spectrum (data not shown) obtained at the GC Rt of authentic iso-GA_3 (Table I) (9). Peaks IIb and IIc were incompletely resolved by HPLC (Fig. 2). Peak IIb contained some GA_1 but principally GA_3, identified from a full mass spectrum (data not shown). Peak IIc contained large amounts of GA_1, identified from a full mass spectrum (data not shown) obtained from a GC peak at the Rt of authentic GA_1 MeTMSi (GC-SIM data for GA_1, GA_3 and iso-GA_3 are shown in Table I). Consistent with the high levels of GA-like activity from both the SiO_2 partition and C_{18} HPLC columns, analysis by GC-MS indicated that GA_1 was present in μg quantities in the purified sample.

GA_{19} was identified from HPLC peak IId, consistent with the chromatographic behavior of biological activity on sequential SiO_2 partition and reversed-phase C_{18} HPLC (Table I). The amount of GA_{19} in the extract was inadequate to obtain a full mass spectrum. Hence, GA_{19} was substantially less abundant than GA_1 or GA_3 in the elongating oilseed rape stems.

SiO_2 regions III to VII were further chromatographed on reverse-phase C_{18} HPLC and, in all cases, GA-like activity eluted at the Rt of GA_1. GC-SIM analysis of peak VII confirmed the presence of GA_1 (data not shown). From peak III, GA-like activity also eluted at the Rt of GA_3 (data not shown). Thus, the GA-like activity in these SiO_2 regions resulted principally from peak tailing of GA_1, rather than from the presence of additional GAs.

Although GA_3 has been identified from purified tissue from a number of plants (15), it is generally used as a standard for

Table I. *Capillary GC-SIM of MeTMSi Derivatives of Authentic GAs and Putative GAs Purified from Oilseed Rape Shoots*

	SiO_2 Fraction	HPLC Rt	GC Rt	Constituent Ions		
			min	*percentage abundance*		
Authentic GA_1	16	34	12.27	506 (100)[a]	491 (13)	448 (20)
				377 (12)	313 (17)	
Putative GA_1	16–17	34–35	12.29	506 (100)	491 (11)	448 (21)
				377 (23)	313 (7)	
Authentic GA_3	16–17	31–33	12.59	504 (100)[b]	489 (10)	473 (2)
				445 (5)	431 (5)	414 (3)
Putative GA_3	16–17	30–31	12.57	504 (100)	489 (7)	473 (3)
				445 (4)	431 (8)	414 (2)
Authentic iso-GA_3	16[c]	—	11.9–12.0[c]	504 (100)[c]	489 (10)	473 (3)
				445 (16)	414 (3)	370 (12)
Putative iso-GA_3	16–17	17–18	11.93	504 (100)	489 (23)	473 (trace)
				445 (18)	414 (trace)	370 (11)
Authentic GA_{19}	16	48–50	11.31	462 (8)	434 (100)	402 (38)
				374 (77)	345 (38)	315 (31)
Putative GA_{19}	16–17	49–50	11.31	462 (11)	434 (100)	402 (40)
				374 (81)	345 (15)	315 (28)
Authentic GA_{20}	9–10	46	10.51	418 (100)	403 (17)	387 (3)
				375 (75)	359 (21)	301 (24)
Putative GA_{20}	9–10	46–47	10.48	418 (100)	403 (13)	387 (3)
				375 (78)	359 (22)	301 (31)

[a] Data from Crozier and Durley (1). [b] Data from Takahashi *et al.* (15). [c] Estimates and data from Moore *et al.* (5).

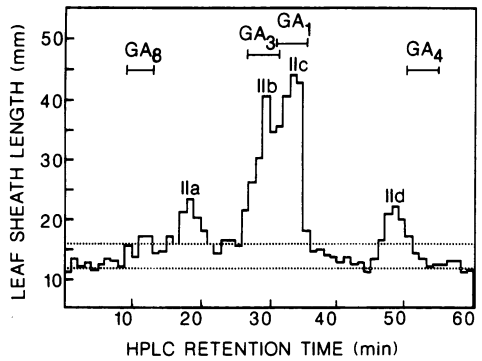

FIG. 2. Elution of GA-like substances as determined with the cv Tanginbozu dwarf rice microdrop assay, from a gradient-eluted reverse-phase C_{18} HPLC column loaded with SiO_2 region II (Fig. 1) which originated from semipurified extracts from oilseed rape stems. The lower dashed line represents the leaf sheath length of control seedlings while the upper dashed line represents the response to 10^{-4} μg GA_3 per rice plant.

bioassays and for exogenous applications in physiological studies and hence, it is always possible that its appearance in an extract may result from contamination rather than from its native occurrence. While this is a possibility for oilseed rape since we routinely use substantial amounts of GA_3 in our laboratory, no GA_3 had been previously observed in extracts from other plants which had been analyzed during the same period in the same laboratory using similar experimental procedures and equipment (10, 12). Thus, the occurrence of GA_3 in oilseed rape does not appear to be the result of contamination, although the occurrence of iso-GA_3 may be an artifact resulting from degradation of GA_3 during work-up (5, 9).

The identification of GA_1, GA_{19}, and GA_{20} from oilseed rape suggests synthesis through the early 13-OH biosynthetic pathway which probably involves $GAs_{1,8,12,17,19,20,29,44,53}$. Of these, $GA_{1,19,20}$ are very active in most GA bioassays (1) and common in higher plants (15), including another member of the Cruciferae, *Thlapsi arvense* (4).

LITERATURE CITED

1. CROZIER A, RC DURLEY 1983 Modern methods of analysis of gibberellins. *In* A Crozier, ed, The Biochemistry and Physiology of Gibberellins, Vol 1. Praeger Publishers, New York, pp 485–560
2. DURLEY RC, A CROZIER, RP PHARIS, GE MCLAUGHLIN 1972 Chromatography of 33 gibberellins on a gradient eluted silica gel partition column. Phytochemistry 11: 3029–3033
3. KOSHIOKA M, J HARADA, K TAKENO, M NOMA, T SASSA, K OGIYAMA, JS TAYLOR, SB ROOD, RL LEGGE, RP PHARIS 1983 Reversed-phase C_{18} high-performance liquid chromatography of acidic and conjugated gibberellins. J Chromatogr 256: 101–115
4. METZGER JD, MC MARDAUS 1986 Identification of endogenous gibberellins in the winter annual weed *Thlapsi arvense* L. Plant Physiol 80: 396–402
5. MOORE PH, RP PHARIS, M KOSHIOKA 1986 Gibberellins in apical shoot meristems of flowering and vegetative sugarcane. J Plant Growth Regul 5: 101–109
6. MURAKAMI Y 1968 A new rice seedling bioassay for gibberellins, "Microdrop Method," and its use for testing extracts of rice and morning glory. Bot Mag Tokyo 81: 33–43
7. MUROFUSHI N, RC DURLEY, RP PHARIS 1977 Preparation of radioactive gibberellins A_{20}, A_5, and A_8. Agric Biol Chem 41: 1075–1079
8. PHARIS RP, RW KING 1985 Gibberellins and reproductive development in seed plants. Annu Rev Plant Physiol 36: 517–568
9. PRYCE RJ 1973 Decomposition of aqueous solutions of gibberellic acid upon autoclaving. Phytochemistry 12: 507–514
10. ROOD SB, NJ BATE, LN MANDER, RP PHARIS 1987 Identification of gibberellins A_1 and A_{19} from hybrid poplar, *Populus balsamifera x P. deltoides*. Phytochemistry. In press
11. ROOD SB, M KOSHIOKA, TJ DOUGLAS, RP PHARIS 1982 Metabolism of tritiated gibberellin A_{20} in maize. Plant Physiol 70: 1614–1618
12. ROOD SB, KM LARSEN, LN MANDER, H ABE, RP PHARIS 1986 Identification of endogenous gibberellins from *Sorghum*. Plant Physiol 82: 330–332
13. ROOD SB, RP PHARIS, M KOSHIOKA 1983 Reversible conjugation of gibberellins *in situ* in maize. Plant Physiol 73: 340–346
14. SUGE H, H TAKAHASHI 1982 The role of gibberellins in the stem elongation and flowering of Chinese cabbage, *Brassica campetris* var. *pekinensis* in their relation to vernalization and photoperiod. Rep Inst Agr Res, Tohoku Univ 33: 15–34
15. TAKAHASHI N, I YAMAGUCHI, H YAMANE 1986 Gibberellins. *In* N Takahashi, ed, Chemistry of Plant Hormones. CRC Press, Boca Raton, FL, pp 57–151
16. WITTWER SH, MJ BUKOVAC 1957 Gibberellin effects on temperature and photoperiodic requirements for flowering of some plants. Science 126: 30–31
17. ZEEVAART JAD 1983 Gibberellins and flowering. *In* A Crozier, ed, The Biochemistry and Physiology of Gibberellins, Vol 2. Praeger Publishers, New York, pp 333–374