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Communication

Identification of Endogenous Gibberellins from *Sorghum*[1]

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ABSTRACT

Gibberellins (GA) A_1, A_{19}, and A_{20} were identified in shoot cylinders containing the apical meristems from sorghum (*Sorghum bicolor* L.). Extracts were purified by sequential SiO_2 partition chromatography and reversed-phase C_{18} high performance liquid chromatography and biologically active (dwarf rice cv Tan-ginbozu microdrop assay) fractions were subjected to gas chromatography-selected ion monitoring. Based on the use of [^{3}H]GA and [^{2}H](d_2)GA internal standards, amounts of GA_1, GA_{19}, and GA_{20} were estimated to be 0.7, 8.8, and 1.5 namograms per gram dry weight of tissue, respectively.

Gibberellins characteristic of the early 13-OH biosynthetic pathway have been previously identified from a number of C_4 and tropical grasses. GA_{19}[2] and GA_{20} have been identified from bamboo (*Phyllostachys edulis*) (13); GA_1 and GA_{19} from rice (*Oryza sativa*) (9); GA_1, GA_{19}, and GA_{29} from sugarcane (*Saccharum* spp.) (8); and eight GA from the early 13-OH pathway have been identified from maize (*Zea mays*) (3, 4). Further, GA_1 has also been identified from a number of other cereal grasses (6). The physiological similarities between maize and sorghum (*Sorghum bicolor*) and the evolutionary relationship between sorghum and other C_4 and tropical grasses suggests that sorghum might also contain GA characteristic of the early 13-OH metabolic pathway. The present study was initiated to identify the endogenous GA of sorghum, a commercially important C_4 cereal for which GA-like substances have been previously reported (1) but not characterized.

MATERIALS AND METHODS

Plant Material. Forty-five d after seedling emergence, shoot cylinders containing the apical meristems were excised from sorghum (*Sorghum bicolor* L., hybrid Pride PF70) plants grown in field plots at Lethbridge, Alberta. The shoot cylinders were immediately frozen in liquid N_2 and subsequently lyophilized.

Extraction and Purification. A 300 g (dry weight) sample was ground at −20°C in H_2O:methanol (20:80). To allow for the determination of recovery efficiencies and to accurately establish chromatographic Rt, 1.8 kBq [1,2-^{3}H]GA_1 (1.21 TBq per mmol, Amersham) and 2 kBq [2,3-^{3}H]GA_{20} (49.9 GBq per mmol) (12) were added to the extract (*i.e.* 0.5 ng GA_1 and 13.3 ng GA_{20}). The methanol was removed *in vacuo* at 35°C after the addition of 0.5 M phosphate buffer (pH 8.0). The buffered aqueous extract was slurried with poly-*N*-PVP and filtered. The pH was raised to 9.0 with NaOH and Chl was removed by two extractions with diethyl ether. The pH was then reduced to 3.0 with HCl and the sample extracted 3 times with equal volumes of H_2O-saturated ethyl acetate. The ethyl acetate was frozen at −40°C the ice removed by filtering, and the ethyl acetate was subsequently removed *in vacuo* at 35°C. The acidic, ethyl acetate-soluble extract was purified on columns of charcoal:celite (1:1) eluted with acetone:water (80:20). This was followed by stepwise-elution SiO_2 partition chromatography (2, 16), and detection of GA-like activity using the dwarf rice cv Tan-ginbozu microdrop assay (11) modified by using 0.5 μl application droplets and 48 h of incubation. Biologically active SiO_2 fractions were then chromatographed on reversed-phase C_{18} HPLC (7, 15). Flow and solvent parameters were as previously described (7, 15), although the gradient from 0 to 70% MeOH was run over 60 rather than 30 min. Eighty 1-min fractions were collected and subsequently bioassayed at 3 dilutions (1/200, 1/400, 1/800 aliquots). Bioassay results after SiO_2 partition chromatography (see Fig. 1) are expressed as moving three-point averages to reduce experimental "noise," although the chromatographic peaks are broadened in so doing.

GC-MS. HPLC fractions showing GA-like activity were derivatized to the MeTMSi derivative using ethereal CH_2N_2 followed by silylation using BSTFA with 1% TMCS (Pierce Chemical Co.). For GC-SIM, a Hewlett-Packard 5790A series Gas Chromatograph and a 5970A series Mass Selective Detector (MSD) fitted with a direct capillary interface for on-column injection were used. The 15 m capillary column was a cross-linked 95% dimethyl-5% diphenyl polysiloxane with a film thickness 0.25 μm and i.d. 0.25 mm (DB-5-15N, J & W Scientific, Inc.). Capillary head pressure was 3 psi with a He carrier gas flow rate of 1.1 ml min^{-1}. The GC was programmed to maintain 60°C for 1 min and then rise at 25°C/min up to 250°C. The interface was maintained at 280°C and the MSD was operated with the electron multiplier at 2000 V.

To accurately determine Rt on capillary GC, and to quantitate SIM ion intensities, a known amount (10, 20, or 100 ng) of MeTMSi [^{2}H](d_2) GA_1, GA_{19}, or GA_{20} was added as an internal standard and co-injected with the appropriate HPLC fractions from the purified sorghum extracts. The [17,17-^{2}H]GA_1 and [17,17-^{2}H]GA_{20} (99.2% enrichment) were prepared from the 17-nor-16-ketones by a modification of the Nozaki procedure (10).

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[2] Abbreviations: GA, gibberellin; MeTMSi, methyl ester trimethylsilyl ether; MSD, mass selective detector; SIM, selected ion monitoring; Rt, retention time.

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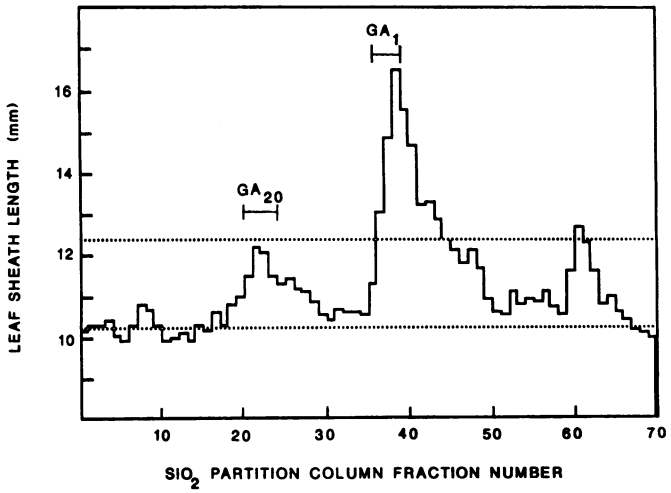

FIG. 1. Elution of GA-like substances from extracts of sorghum apical meristems with the cv Tan-ginbozu dwarf rice assay on fractions from stepwise-eluted SiO_2 partition columns. Elution regions of authentic [^{3}H] GA are shown above the profile of GA-like activity. The lower dashed line represents the leaf sheath length of control seedlings while the upper dashed line represents the response to 10^{-4} μg GA_3 per rice plant.

[17,17-^{2}H]GA_{19} was obtained following the incubation of [17,17-^{2}H] steviol (similarly obtained from the nor-ketone) in *Gibberella fujikuroi* (14). For GC-SIM, six ions were monitored representing three ions characteristic of the endogenous GA (17) and three of the deuterated GA. Then, additionally, six characteristic ions were monitored for the endogenous GA_{19}. Ion abundances were corrected for the contribution from the deuterated GA. Kovats retention indices for GA_1, GA_{19}, and GA_{20} were 2722, 2671, and 2573, respectively.

RESULTS AND DISCUSSION

Three regions of GA-like activity were observed from the stepwise-eluted SiO_2 partition columns (Fig. 1). The first co-chromatographed with authentic [^{3}H]GA_{20} while the second peak eluted slightly after authentic [^{3}H]GA_1. The third region of GA-like activity eluted in the methanol wash where glucosyl conjugates of GA are expected (7).

Peak 1 (Fig. 1) was subjected to reversed-phase C_{18} HPLC and eluted as a single peak of GA-like activity which co-chromatographed with authentic [^{3}H]GA_{20} Peak 2 (Fig. 1) was resolved through reversed-phase C_{18} HPLC into two regions of GA-like activity (data not presented); the first co-chromatographed with authentic [^{3}H]GA_1 while the second, larger peak of GA-like activity eluted at about the Rt of GA_{19} (5, 7). The GA-like peak in the methanol wash from the SiO_2 column eluted from HPLC as a single peak of GA-like activity slightly earlier than the GA_{19} (data not presented), a Rt that would be consistent with a glucoside or glucosyl ester of GA_{19}.

GS-SIM analysis of the GA_{20}-like peak from sequential SiO_2 partition and reversed-phase C_{18} HPLC confirmed the presence of GA_{20} (Table I). GC-SIM analyses of the other two GA-like peaks from HPLC confirmed the presence of GA_1 and GA_{19}, respectively (Table I, Fig. 2). Thus, GA_1, GA_{19}, and GA_{20} were identified from vegetative shoot cylinders of sorghum.

Quantities of GA_1 and GA_{20} in the initial sample can be estimated using the internal standard [^{3}H]GA to determine recovery efficiencies up to the point of derivatization. The internal standards of [^{2}H](d_2)GA added just prior to derivatization can be used to determine subsequent recovery as well as the MSD sensitivity. While no internal standard [^{3}H]GA_{19} was available, losses of both GA_1 and GA_{20} during sample workup were quite similar, and we have assumed similar losses of GA_{19}. (Final recoveries of GA_1 and GA_{20} were 4.78% and 4.96%, respectively, and most of the losses were accountable in aliquots removed throughout the purification and chromatographic procedures). Thus, levels of GA_1, GA_{19}, and GA_{20} were estimated to be 0.7, 8.8, and 1.5 ng/g dry weight, respectively. The bioassay data are consistent with these relative proportions as the GA_{19}-like peak was the largest biologically active region. Smaller amounts of GA_{20}-like activity, and still lesser amounts of the GA_1-like activity were observed.

The most direct comparison between quantitative estimates based on bioassay and GC-SIM, are determinations of GA-like levels in HPLC fractions just prior to sample derivatization. Encouragingly, the 177 ng of GA_{19} in the sample (determined from GC-SIM) was similar to that estimated by the Tan-ginbozu assay (160 ng).

GA_1, GA_{19}, and GA_{20} are members of the early 13-OH pathway which is observed in maize and probably exists in other tropical grasses. Given the presence of GA_1, GA_{19}, and GA_{20} in sorghum,

Table I. *Capillary GC-SIM of Authentic MeTMSi of [^{2}H](d_2)GA_1, -GA_{19}, or -GA_{20} Coinjected with Peaks Containing GA-Like Activity from Extracts of Sorghum Eluted from Sequential SiO_2 → C_{18}HPLC Columns (Figs. 1 and 2)*

GA	Retention Time	Relative Abundance of Peak (Percentage Abundance in Parenthesis) at *m/z*:					
		508	493	450	506	491	448
	min						
Sorghum putative							
GA_1 + [^{2}H](d_2)GA_1	11.54	387	41	69	21	3	10
[^{2}H](d_2)GA_1	11.54	387 (100%)	31 (8%)	48 (12%)	2	0	6
Corrected intensities for sorghum GA_1					19 (100%)	3 (16%)	4 (21%)
		464	436	376	462	434	374
Sorghum putative							
GA_{19} + [^{2}H](d_2)GA_{19}	12.67	5	31	29	8	54	42
[^{2}H](d_2)GA_{19}	12.67	2 (6%)	31 (100%)	19 (61%)	0	0	3
Corrected intensities for sorghum GA_{19}					8 (15%)	54 (100%)	39 (72%)
		420	405	377	418	403	375
Sorghum putative							
GA_{20} + [^{2}H](d_2)GA_{20}	12.77	209	35	148	24	5	17
[^{2}H](d_2)GA_{20}	12.77	209 (100%)	26 (12%)	121 (58%)	1	1	2
Corrected intensities for sorghum GA_{20}					23 (100%)	4 (17%)	15 (65%)

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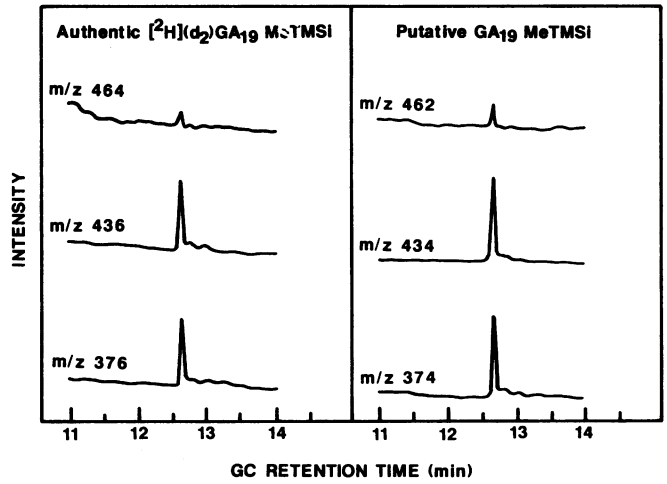

FIG. 2. Capillary GC-SIM of authentic $[^2H](d_2)GA_{19}$ MeTMSi coinjected with putative GA_{19} MeTMSi from sorghum.

it is probable that other characteristic GA of this early 13-OH pathway are also native, although less abundant.

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LITERATURE CITED

1. DUNLAP JR, AH HANKS, PW MORGAN 1981 Preflowering levels of phytohormones in sorghum. I. Analytical procedure. Crop Sci 21: 82–88
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. HEDDEN P, BO PHINNEY, R HEUPEL, S COHEN, D FUJII, P GASKIN, J MACMILLAN 1982 Hormones of young tassels of *Zea mays*. Phytochemistry 21: 390–393
4. HEUPEL RC, BO PHINNEY, CR SPRAY, P GASKIN, J MACMILLAN, P HEDDEN, JE GRAEBE 1985 Native gibberellins and the metabolism of [^{14}C]gibberellin A_{53} and of [17-^{13}C, 17-$^{3}H_2$]gibberellin A_{20} in tassels of *Zea mays*. Phytochemistry 24: 47–53
5. JONES MG, JD METZGER, JAD ZEEVAART 1980 Fractionation of gibberellins in plant extracts by reverse phase high performance liquid chromatography. Plant Physiol 65: 218–221
6. KAUFMAN PB 1980 Physiology and biochemistry of gibberellins in developing shoots of grasses. *In* SS Purohit, ed, Aspects of Physiology and Biochemistry of Plant Hormones. Kalyani Publishers, Ludhiana, India, pp 125–137
7. 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
8. KUHNLE JA, PH MOORE, WF HADDON, MM FITCH 1983 Identification of gibberellins from sugarcane plants. J Plant Growth Regul 2: 59–71
9. KUROGOCHI S, N MUROFUSHI, Y OTA, N TAKAHASHI 1979 Identification of gibberellins in the rice plant and qualitative changes of gibberellin A_{19} throughout its life cycle. Planta 146: 185–191
10. LOMBARDO L 1982 Methylenation of carbonyl compounds with Zn-CH_2Br_2-$TiCl_4$. Tetrahedron Lett 23: 4293–4296
11. 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
12. MUROFUSHI N, RC DURLEY, RP PHARIS 1977 Preparation of radioactive gibberellins A_{20}, A_5, and A_8. Agric Biol Chem 41: 1075–1079
13. MUROFUSHI N, S IRIUCHIJIMA, N TAKAHASHI, T TAMURA, J KATO, Y WADA, E WATANABE, T AOYAMA 1966 Isolation and structure of a novel C_{20} gibberellin in bamboo shoots. Agric Biol Chem 30: 917–924
14. MUROFUSHI N, Y SHIGEMATSU, S NAGURA, N TAKAHASHI 1982 Metabolism of steviol and its derivatives by *Gibberella fujikuroi*. Agric Biol Chem 46: 2305–2311
15. ROOD SB, M KOSHIOKA, TJ DOUGLAS, RP PHARIS 1982 Metabolism of tritiated gibberellin A_{20} in maize. Plant Physiol 70: 1614–1618
16. ROOD SB, RP PHARIS, M KOSHIOKA 1983 Reversible conjugation of gibberellins *in situ* in maize. Plant Physiol 73: 340–346
17. TAKAHASHI N, I YAMAGUHI, H YAMANE 1986 Gibberellins. *In* N Takahashi, ed, Chemistry of Plant Hormones. CRC Press, Boca Raton, FL, pp 57–151

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