

Communication

Identification of Endogenous Gibberellins from *Sorghum*¹

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ABSTRACT

Gibberellins (GA) A₁, A₁₉, and A₂₀ were identified in shoot cylinders containing the apical meristems from sorghum (*Sorghum bicolor* L.). Extracts were purified by sequential SiO₂ partition chromatography and reversed-phase C₁₈ 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 [³H]GA and [³H](d₂)GA internal standards, amounts of GA₁, GA₁₉, and GA₂₀ were estimated to be 0.7, 8.8, and 1.5 nanograms per gram dry weight of tissue, respectively.

Gibberellins characteristic of the early 13-OH biosynthetic pathway have been previously identified from a number of C₄ and tropical grasses. GA₁₉² and GA₂₀ have been identified from bamboo (*Phyllostachys edulis*) (13); GA₁ and GA₁₉ from rice (*Oryza sativa*) (9); GA₁, GA₁₉, and GA₂₉ from sugarcane (*Saccharum* spp.) (8); and eight GA from the early 13-OH pathway have been identified from maize (*Zea mays*) (3, 4). Further, GA₁ 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 C₄ 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 C₄ 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 N₂ and subsequently lyophilized.

Extraction and Purification. A 300 g (dry weight) sample was ground at -20°C in H₂O:methanol (20:80). To allow for the

determination of recovery efficiencies and to accurately establish chromatographic Rt, 1.8 kBq [1,2-³H]GA₁ (1.21 TBq per mmol, Amersham) and 2 kBq [2,3-³H]GA₂₀ (49.9 GBq per mmol) (12) were added to the extract (*i.e.* 0.5 ng GA₁ and 13.3 ng GA₂₀). 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 H₂O-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 SiO₂ 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 SiO₂ fractions were then chromatographed on reversed-phase C₁₈ 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 SiO₂ 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 CH₂N₂ 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 [³H](d₂) GA₁, GA₁₉, or GA₂₀ was added as an internal standard and co-injected with the appropriate HPLC fractions from the purified sorghum extracts. The [17,17-²H]GA₁ and [17,17-²H]GA₂₀ (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.

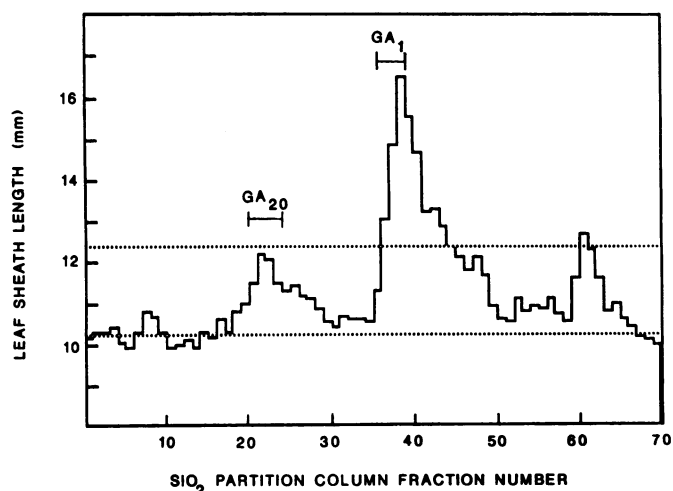


FIG. 1. Elution of GA-like substances from extracts of sorghum apical meristems with the cv Tan-ginbozu dwarf rice assay on fractions from stepwise-eluted SiO₂ partition columns. Elution regions of authentic [³H] GA are shown above the profile of GA-like activity. The lower dashed line represents the leaf sheath length of control seedlings while the upper dashed line represents the response to 10⁻⁴ μg GA₃ per rice plant.

[17,17-²H]GA₁₉ was obtained following the incubation of [17,17-²H] 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 GA₁₉. Ion abundances were corrected for the contribution from the deuterated GA. Kovats retention indices for GA₁, GA₁₉, and GA₂₀ were 2722, 2671, and 2573, respectively.

RESULTS AND DISCUSSION

Three regions of GA-like activity were observed from the stepwise-eluted SiO₂ partition columns (Fig. 1). The first co-chromatographed with authentic [³H]GA₂₀ while the second peak eluted slightly after authentic [³H]GA₁. 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 C₁₈ HPLC and eluted as a single peak of GA-like activity which co-chromatographed with authentic [³H]GA₂₀. Peak 2 (Fig. 1) was resolved through reversed-phase C₁₈ HPLC into two regions of GA-like activity (data not presented); the first co-chromatographed with authentic [³H]GA₁, while the second, larger peak of GA-like activity eluted at about the Rt of GA₁₉ (5, 7). The GA-like peak in the methanol wash from the SiO₂ column eluted from HPLC as a single peak of GA-like activity slightly earlier than the GA₁₉ (data not presented), a Rt that would be consistent with a glucoside or glucosyl ester of GA₁₉.

GS-SIM analysis of the GA₂₀-like peak from sequential SiO₂ partition and reversed-phase C₁₈ HPLC confirmed the presence of GA₂₀ (Table I). GC-SIM analyses of the other two GA-like peaks from HPLC confirmed the presence of GA₁ and GA₁₉, respectively (Table I, Fig. 2). Thus, GA₁, GA₁₉, and GA₂₀ were identified from vegetative shoot cylinders of sorghum.

Quantities of GA₁ and GA₂₀ in the initial sample can be estimated using the internal standard [³H]GA to determine recovery efficiencies up to the point of derivatization. The internal standards of [²H](d₂)GA added just prior to derivatization can be used to determine subsequent recovery as well as the MSD sensitivity. While no internal standard [³H]GA₁₉ was available, losses of both GA₁ and GA₂₀ during sample workup were quite similar, and we have assumed similar losses of GA₁₉. (Final recoveries of GA₁ and GA₂₀ 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 GA₁, GA₁₉, and GA₂₀ 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 GA₁₉-like peak was the largest biologically active region. Smaller amounts of GA₂₀-like activity, and still lesser amounts of the GA₁-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 GA₁₉ in the sample (determined from GC-SIM) was similar to that estimated by the Tan-ginbozu assay (160 ng).

GA₁, GA₁₉, and GA₂₀ are members of the early 13-OH pathway which is observed in maize and probably exists in other tropical grasses. Given the presence of GA₁, GA₁₉, and GA₂₀ in sorghum,

Table I. Capillary GC-SIM of Authentic MeTMSi of [²H](d₂)GA₁, -GA₁₉, or -GA₂₀ Coinjected with Peaks Containing GA-Like Activity from Extracts of Sorghum Eluted from Sequential SiO₂ → C₁₈HPLC Columns (Figs. 1 and 2)

GA	Retention Time min	Relative Abundance of Peak (Percentage Abundance in Parenthesis) at m/z:					
		508	493	450	506	491	448
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%)

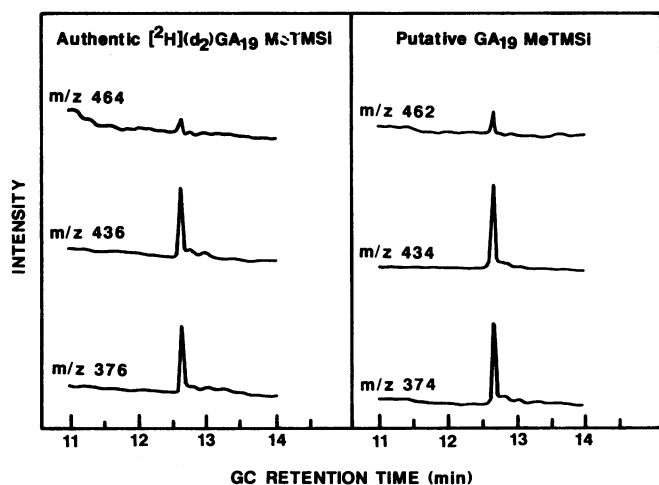


FIG. 2. Capillary GC-SIM of authentic $[^2\text{H}](\text{d}_2)\text{GA}_{19}$ MeTMSi coinjected with putative GA_{19} MeTMSi from sorghum.

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

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