J Plant Growth Regul (1983) 2:59–71

Journal of
Plant
Growth
Regulation
© 1983 Springer-Verlag

Identification of Gibberellins from Sugarcane Plants*,**

J. A. Kuhnle,[1]*** P. H. Moore,[2] W. F. Haddon,[1] and M. M. Fitch[2]

[1]Western Regional Research Center, USDA/ARS, Berkeley, California 94710 USA
[2] Experiment Station Hawaiian Sugar Planters' Association, USDA/ARS, Aiea, Hawaii 96701 USA

Received February 9, 1982; accepted April 5, 1983

Abstract. Five GAs, GA_1, GA_3, GA_{19}, GA_{20}, and GA_{29}, were identified in extracts from mature leaf and shoot apical meristem of flowering and non-flowering sugarcane (*Saccharum* spp. hybrids) by combined GC/MS. The presence of ABA was also confirmed.

Sugarcane stalk length, weight, and sucrose content increase with GA_3 applications (Bull 1964). This increase in growth varies with environment and genotype (Moore and Buren 1978), possibly because of differences in GA metabolism. Most and Vlitos (1966) reported GA-like activity in extracts from a single sugarcane clone at three different ages and grown under different environmental conditions. They concluded from bioassay of fractions after celite

*Contribution from the USDA ARS and the Hawaiian Sugar Planters' Association Experiment Station, Aiea, Hawaii 96701 USA. Published with the approval of the Director as Paper No. 550 in the Journal Series of the Experiment Station, Hawaiian Sugar Planters' Association.

**Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

*** Present address: Experiment Station HSPA, P.O. Box 1057, Aiea, Hawaii 96701 USA

Abbreviations. eV, electron volt; GA, gibberellin; MS, mass spectrometry; dw, dry weight; G, relative centrifugal force (gravity); ABA, abscisic acid; PVP, insoluble polyvinylpyrrolidone; GLC, gas liquid chromatography; GC/MS, combined gas chromatography-mass spectrometry; MeTMSi, methyl ester-trimethylsilyl ether; SIM, single ion monitoring; RT, retention time; TIC, total ion current; TLC, thin-layer chromatography; IR, infrared.

column chromatography and TLC that GA_1, GA_3, GA_5, and an unidentifiable GA were present in the extracts at specific developmental stages. These conclusions were without basis of chemical structural data necessary for GA identification. Correa et al. (1972) also reported GA-like activity in extracts from sugarcane shoot apical meristems. Correa concluded that nonflowering meristems contained more GA-like substances than did flowering meristems at time of induction and at floral differentiation.

These earlier findings suggest genotypic and environmental control of GA metabolism; yet, conclusive evidence for the structures of endogenous GAs of sugarcane is lacking. In this paper, we report the GC/MS identification of the more abundant endogenous GAs of leaf and shoot apical meristem tissues from flowering and nonflowering plants of three sugarcane cultivars.

Materials and Methods

Plant Material

Six-month-old sugarcane plants (*Saccharum* spp. hybrids) of three cultivars, H54-775, H57-5174 and H59-3775, were field grown and harvested during flowering and nonflowering developmental stages. Mature leaf tissue and apical meristem tissues were collected, extracted, and analyzed at the two developmental stages. The leaf tissue sample for both flowering and nonflowering plants consisted of the four uppermost fully expanded leaf blades (mid-rib removed) of 20 plants. The apical meristem sample differed between flowering and nonflowering plants but in each case consisted of shoot apices from 50 plants. For nonflowering plants, the meristem sample consisted of a 10-cm segment composed of the nine youngest internodes with attached immature leaf bases up to 2 cm in length. For flowering plants, the meristem sample was a 15-cm segment containing a 2-cm panicle in addition to the nine youngest internodes with attached leaf bases.

All harvested tissue was immediately frozen in liquid nitrogen, lyophilized, ground with a Wiley Mill, and stored at −4°C until used.

Gibberellin Extraction

The general procedure used for extraction of native GAs has been described previously (Kamienska and Pharis 1975). All solvents were either nanograde quality or distilled. Ten-gram dw batches of each sample were extracted with 100 ml cold (0°C) methanol:water (80:20) with a Polytron tissue homogenizer at medium speed for 1 min. After filtration, the residue was reextracted with 50 ml of cold (0°C) 100% methanol. The filtered extracts were combined and the methanol evaporated on a rotary evaporator at 30°C. An equal volume of 0.5 M, pH 8.0, phosphate buffer was added to the aqueous residue. The pH was adjusted to 9.0 and the mixture centrifuged at 8,000 *g* for 5 min. The supernatant was partitioned five times with a 3/5 volume of diethyl ether. The pH of the remaining aqueous phase was adjusted to between 2.8 and 3.0. The aqueous phase was partitioned five times with a 3/5 volume of ethyl acetate to give the acidic ethyl acetate fraction.

Preliminary purification of the acidic ethyl acetate fraction was by PVP (Polyclar-AT Powder, GAF, NY) chromatography on a 2.54 cm × 30 cm column eluted with 0.1 M, pH 8.0, phosphate buffer (Glenn et al. 1972).

Final purification was by silica gel (Woelm Pharma, ICN Nutritional Biochemicals, St. Louis, Mo, USA) partition chromatography on a 1.25 cm × 29.5 cm column (Durley et al. 1972). The column was gradient-eluted using a Varigrad gradient maker. Chambers 1 to 4 contained the following solvent mixtures: 136 ml 50/50, 130 ml 65/35, 122 ml 85/15, and 115 ml 100/0 (v/v) ethyl acetate/hexane, saturated with formic acid. Thirty 20-ml fractions were collected.

Bioassay

The 30 fractions from silica gel partition chromatography were assayed for GA-like activity by the tan-ginbozu dwarf rice microdrop bioassay (Murakami 1968). Solvent was evaporated from each 20-ml fraction under a stream of nitrogen. The residue was lyophilized and then dissolved in 100 μl or 300 μl of ethanol; 0.5 μl was applied to each plant. Ten rice plants were used for each fraction.

Gas Chromatography-Mass Spectrometry (GC/MS)

Biologically active fractions were analyzed as MeTMSi derivatives (Binks et al. 1969). Methyl esters were prepared with ethereal diazomethane. Trimethylchlorosilane and hexamethyldisilazane in dry pyridine (1:1:5 v/v) (Pierce Chemical Co., Rockford, Ill, USA) were used to prepare trimethylsilyl ethers of the methyl esters.

The following glass columns (on-column injection) and conditions were used for GLC: (a) 1.32 m × 2 mm i.d. packed with 1% XE-60 on Gas Chrom Q (100–120 mesh, Alltech Assoc.), 170°C isothermal for 4 min, then programmed 4°C per min to 205°C, or 175°C isothermal for 4 min, then programmed 4°C per min to 210°C; (b) 1.8 m × 2 mm i.d., packed with 2% SE-30 on Gas Chrom Q (AW-DMCS, 100–120 mesh, Varian Aerograph) 195°C isothermal for 6 min, then programmed 2°C per min to 220°C; and (c) 1.32m × 2 mm i.d. packed with 1% QF-1 on Gas Chrom Q (100–120 mesh, Alltech Assoc.), 200°C isothermal.

GC/MS data were obtained with a Hewlett-Packard 5830A gas chromatograph interfaced by an all-glass jet separator to a Micromass MM 70/70F (VG-Organic Ltd., Altrincham, Cheshire, England) double-focusing, magnetic-sector, mass spectrometer using electron ionization at 70 eV. Spectra were recorded continuously at a rate of one spectrum every 5 sec (5 sec/scan) into a computer system based on an IBM 1800 (Haddon et al. 1970). For full scans, extracts from the equivalent of 1 to 2 g dw of plant tissue were used for each GC/MS run. SIM was used for selected samples (Feneslau 1977).

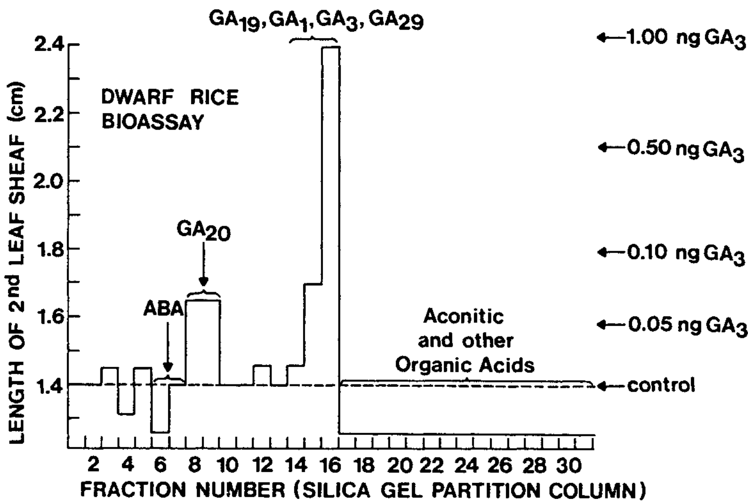

Fig. 1. Histogram of tan-ginbozu dwarf rice bioassay for gibberellin-like activity from 0.05 g dry weight mature leaf tissue, cv. H59-3775, which was separated by silica gel partition chromatography. Values for lengths of the second leaf sheath are the average of 10 plants.

Results and Discussion

Bioassay

The tan-ginbozu dwarf rice seedling test was used to bioassay fractions from the silica gel partition column. The purpose of the bioassay was to determine which fractions from the various cultivars, tissues, and stages of development contained sufficient GA-like activity for GC/MS identification. The silica gel column separated each of the sugarcane extracts into fractions which characteristically promoted, did not affect, or inhibited rice seedling growth. Figure 1 is a histogram showing the GA-like and inhibitory substances characteristic of our sugarcane extracts. The bioassay of each sugarcane extract always showed GA-like activity of compounds in fractions 14-16 and inhibitors in fractions 6-7 and in most fractions above 17. Other fractions which showed viable amounts of GA-like activity as a function of cultivar, tissue, and developmental stage were fractions 3-4, 8-9, and 10-13. A qualitative summary of GA-like activity found in all samples is given in Table 1.

A white residue remained in fractions 17-30 after solvent evaporation. The amount of residue was greater in the meristem samples than in the leaf samples. An IR spectrum of the residue indicated that the white solid consisted primarily of aconitic acid, the major organic acid of sugarcane. Samples with the least amount of residue and the most GA-like activity were selected for GA identification.

GC/MS Identification of GAs

Combined silica gel partition fractions selected for GA identification were methylated, followed by trimethylsilization, and examined by GC/MS using

Table 1. Summary of dwarf rice seedling bioassay[a] and residue[b] of sugarcane tissue extracts partitioned on a silica gel column.

Sugarcane cultivar	Developmental stage	Tissue	Column fraction numbers 3–4	6–7	8–9	10–13	14–16	17–30
H54-775	flowering	meristem	+	–	++	+++	+++++++++	*****
		leaf	+	–	++	+++	++++	***
	nonflowering	meristem	+	–	++	+++	+++	*****
		leaf	+	–	++	+++	++	*
H57-5174	flowering	meristem	+	–	++	+++	+++	*****
		leaf	+	–	0	0	+++++++++	*
	nonflowering	meristem	+	–	++	+++	++	*****
		leaf	0	–	0	++	+++++++++	*
H59-3775	flowering	meristem	+	–	++	++++	+++++	*****
	nonflowering	leaf	+	–	+++	++	+++++	***

[a] The bioassay data are presented as 0 = neither promotion nor inhibition, – = inhibition, and + = growth promotion. The number of + represents the amount of sheath elongation relative to increasing amounts of GA_3. + = least growth; ++, +++ = moderate growth, +++++++++ = most growth.

[b] The residue data are presented as * = just visible residue from 20-ml fraction after solvent evaporation. The number of * is equal to estimates of quantities of residue relative to that just visible.

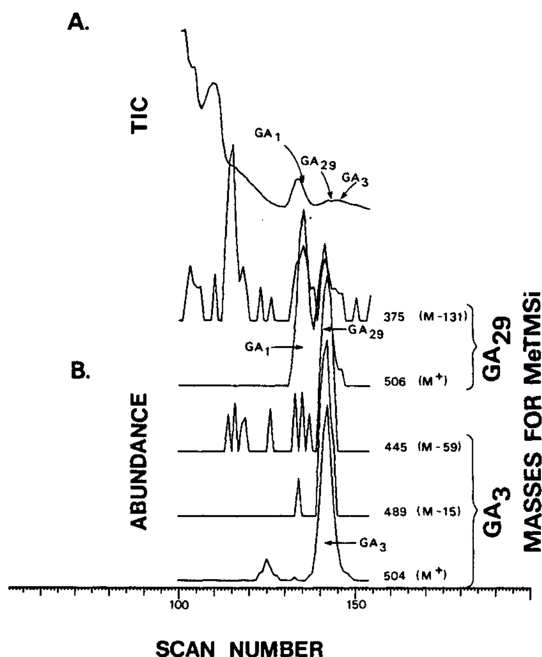

Fig. 2. GC/MS using a 2% SE-30 column of the MeTMSi derivatives, combined fractions 14-16, after silica gel partition chromatography of the extract from sugarcane, mature leaf tissue, flowering stage, cv. H57-5174. A. TIC chromatogram. B. Mass chromatograms for GA_3 MeTMSi and GA_{29} MeTMSi.

2% SE-30, 1% XE-60, or 2% QF-1 columns. TIC chromatograms, in Fig. 2a, 3a, and 4a did not show well-resolved peaks for the GA MeTMSi derivatives, since the limited purification of the extracts resulted in mixtures containing small quantities of GAs compared to the total amount of acidic compounds in the mixtures. The presence of the GA MeTMSi derivatives in the mixtures was determined by the use of mass chromatograms. Full mass spectra were obtained by background subtraction from the scans containing the GA MeTMSi derivatives. Although inaccuracies appeared in some of the MS spectra at lower masses, we were able to use the upper portions of mass spectra, mass chromatograms, and GLC retention on three columns compared to authentic GAs or previously published results to identify the sugarcane GAs. We eliminated other known GA isomers by comparison of our data with published results.

Identification of GA_1, GA_3, GA_{19}, and GA_{29} in Combined Fractions 14-16

The combined fractions 14-16 after silica gel partition chromatography from extracts from mature leaf, flowering stage H57-5174, were used for the identification of GA_1, GA_3, GA_{19}, and GA_{29}.

Mass chromatograms of the GA MeTMSi derivatives after chromatography on the 2% SE-30 column are shown in Fig. 2b. The molecular ions and characteristic mass fragments of GA_{29} MeTMSi, m/e (M+) 506 and 375 in scan 139 (RT, 11.6 min), and GA_3MeTMSi, m/e (M+) 504, 489, and 445 in scan 141 (RT, 11.8 min), were present. The molecular ion of GA_1 MeTMSi, m/e (M+)

506, appeared in scan 134 (RT, 11.2 min). GLC retention times for authentic GA_1 MeTMSi, GA_{29} MeTMSi, and GA_3 MeTMSi corresponded to scans 134, 139, and 141, respectively. GA_3 MeTMSi and GA_{29} MeTMSi were unresolved so that the spectrum obtained from scan 140 of the plant extract after background subtraction was composed of a mixture of ions from both compounds. A spectrum was obtained for a mixture of authentic GA_3 MeTMSi and GA_{29} MeTMSi derivatives under the same GC/MS conditions used for the plant extract. The relative intensities of the ions for GA_{29} MeTMSi from both the plant extract and the authentic mixture, as well as authentic GA_{29} MeTMSi alone, were in agreement (Table 2). A spectrum identical to that of authentic GA_3 MeTMSi was obtained in scan 142 (RT, 11.8 min) from the plant extract with the absence of contributing peaks from GA_{29} MeTMSi (Table 2). A spectrum of GA_1 MeTMSi was not obtained after chromatography on the 2% SE-30 column because of a background contribution at the lower masses.

Mass chromatograms obtained from the same tissue sample chromatographed on 1% XE-60 contained the molecular ion and characteristic mass fragments for GA_1 MeTMSi, m/e (M+) 506, 491, 447, and 377 in scan 116 (RT, 9.7 min), (Fig. 3b). These fragments were also characteristic for GA_{29} MeTMSi, except that GA_{29} did not have a 377 but did have both 303 and 375 ions. These data also suggest the presence of GA_{29} MeTMSi in scan 118 (RT, 9.8 min). GLC retention times under the same conditions for authentic GA_1 MeTMSi and GA_{29} MeTMSi corresponded to scans 116 and 118, respectively. The spectrum from scan 115 after appropriate background subtraction is given in Table 2 along with data from an authentic sample of GA_1 MeTMSi. The spectrum was that of GA_1 MeTMSi with the presence of the 448 and 377 ions and the absence of the 303 which would be present for GA_{29} MeTMSi. A spectrum of scan 119 after background subtractions was that of GA_{29} MeTMSi, m/e (M+) 506 (100), 491 (16), 375 (19), and 303 (18), with the absence of 448. Mass chromatograms in Fig. 3b also indicate the presence of GA_3 MeTMSi as its (M + 2) 506 in scan 129. A spectrum of scan 128 was identical to that of authentic GA_3 MeTMSi.

Thus, the two GA MeTMSi derivatives with a molecular ion, m/e 506, GA_1 MeTMSi and GA_{29} MeTMSi, were identified by a combination of data which included mass chromatograms, mass spectra, and GLC retention times on SE-30 and XE-60 columns as compared with authentic samples. The presence of other known GA MeTMSi derivatives with molecular ions m/e 506, *epi*-GA_1, GA_{34}, GA_{35}, and GA_{16} MeTMSi were eliminated by comparison with reported GLC retention times relative to reported data for GA_1 MeTMSi and GA_{29} MeTMSi (Durley et al. 1973, 1979, Reeve et al. 1975) and published mass spectra (Binks et al. 1969). The compound with the molecular ion m/e 504, GA_3 MeTMSi, was identified by identical mass spectra and GLC retention times on both XE-60 and SE-30 columns as compared with an authentic GA_3 MeTMSi sample. The other two known GA MeTMSi derivatives with molecular ion m/e 504, GA_{22} MeTMSi and GA_{30} MeTMSi, were eliminated by comparison of reported GLC retention times on the XE-60 and SE-30 columns and reported reference spectra. (Binks et al. 1969, Durley et al. 1973).

GA_{19} MeTMSi was detected in scan 104 (RT 8.7 min) after chromatography on the XE-60 column by nine mass chromatograms which included the molec-

Table 2. GC/MS data obtained from sugarcane extracts and authentic samples. Fraction numbers refer to fractions eluted from silica gel partition column.

GA	Fraction or gibberellin derivative	Column	Scan no.	Peaks in mass spectrum with relative abundance in parentheses (m/e values)							
GA_{29}MeTMSi	Fr. 14–16	2% SE-30	140	506 (M^+,100)	491 (19)	447 (4)	416 (0)	375 (8)	303 (17)		
	GA_3MeTMSi + GA_{29}MeTMSi	2% SE-30		506 (M^+,100)	491 (13)	447 (8)	416 (2)	375 (9)	303 (18)		
	GA_{29}MeTMSi	1% XE-60		506 (M^+,100)	491 (12)	447 (6)	416 (2)	375 (10)	[illegible]		
GA_3MeTMSi	Fr. 14–16	2% SE-30	142	504 (M^+,100)	489 (10)	445 (12)	414 (6)	208 (12)	207 (23)		
	Fr. 14–16	1% XE-60	129	504 (M^+,100)	489 (9)	445 (12)	414 (5)	208 (21)	207 (20)		
	GA_3MeTMSi	probe		504 (M^+,100)	489 (10)	445 (9)	414 (4)	208 (48)	207 (16)		
GA_1MeTMSi	Fr. 14–16	1% XE-60	115	506 (M^+,100)	491 (13)	447 (12)	448 (19)	416 (0)	377 (16)	207 (31)	
	GA_1MeTMSi	1% XE-60		506 (M^+,100)	491 (11)	447 (7)	448 (15)	416 (4)	377 (12)	207 (30)	
GA_{19}MeTMSi	Fr. 14–16	1% XE-60	104	462 (M^+,9)	402 (44)	434 (100)	375 (55)	374 (51)	208 (19)	207 (95)	
	GA_{19}MeTMSi*										
GA_{20}MeTMSi	Fr. 8–9	1% XE-60		418 (M^+,100)	403 (18)	375 (66)	359 (19)	208 (13)	207 (39)		
	GA_{20}MeTMSi	1% XE-60		418 (M^+,100)	403 (19)	375 (56)	359 (17)	208 (15)	207 (34)		

* Not available. Data in agreement with Binks et al. (1969).

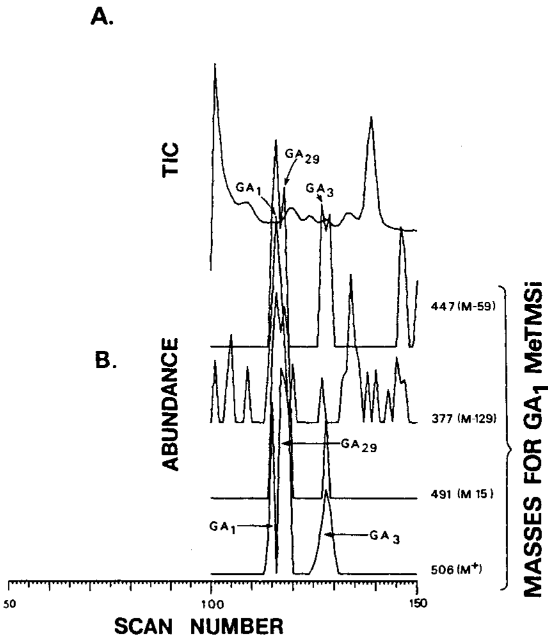

Fig. 3. GC/MS using a 1% XE-60 column of the MeTMSi derivatives, combined fractions 14-16, after silica gel partition chromatography of the extract from sugarcane, mature leaf tissue, flowering stage, cv. H57-5174. A. TIC chromatogram. B. Mass chromatograms for GA_1 MeTMSi.

ular ion (Fig. 4b). Data for the spectrum obtained from scan 104 after background subtraction are given in Table 2. An authentic sample of GA_{19} was not available, but the relative intensities of the characteristic ions for GA_{19} MeTMSi reported in Table 2 were in agreement with published spectra (Binks et al. 1969). GA_{36} MeTMSi, an isomer of GA_{19} MeTMSi, was eliminated on the basis of published reference spectra (Bearder and MacMillan 1973).

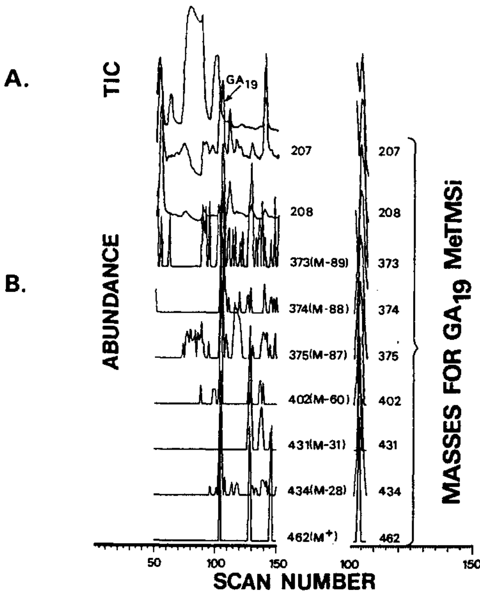

Fig. 4. GC/MS using a 1% XE-60 column of the MeTMSi derivative, combined fractions 14-16, after silica gel partition chromatography of the extract from sugarcane, mature leaf tissue, flowering stage, cv. H57-5174. A. TIC chromatogram. B. Mass chromatograms for GA_{19} MeTMSi.

Identification of GA_{20} in Combined Fractions 8 and 9

Data from GC/MS on 1% XE-60 of the MeTMSi derivative of combined fractions 8 and 9 from immature sugarcane stem tissue, cv. H59-3775, suggested the presence of GA_{20}. The spectrum obtained for GA_{20} MeTMSi was at a retention time identical to that of authentic GA_{20} MeTMSi. The low level of this GA made identification of full spectrum scans tenuous, even though the characteristic ions in Table 2 are in agreement with the authentic sample. Further evidence for GA_{20} MeTMSi was obtained by GLC on 1% QF-1 by continuously monitoring the 418 molecular ion by SIM to obtain increased sensitivity. The plant sample and authentic GA_{20} MeTMSi produced well-defined 418 peaks at identical retention times. The absence of a 284 peak at the retention time of our unknown in both full spectrum scans and SIM strongly suggested that the 418 was not that of GA_4 MeTMSi. The 284 has 100% relative abundance in the mass spectrum of authentic GA_4 MeTMSi determined under the same conditions on our instrument. Thus, if the 284 were present, it should have been easily detected in the plant sample. Even though our spectrum of GA_{20} MeTMSi was weak, it appeared that other known GA MeTMSi derivatives with molecular ions, m/e 418, GA_{40} MeTMSi, and GA_{45} MeTMSi could be eliminated by comparison with published reference spectra (Bearder et al. 1976, Martin et al. 1977). Additionally, the presence of GA_{29} and the documented metabolic conversion of GA_{20} to GA_{29} (Frydman and MacMillan 1975, Railton et al. 1974) further supported the evidence that GA_{20} is a sugarcane GA.

Fraction 8-9 was also examined by mass chromatograms and GC/SIM for GA_5 MeTMSi and GA_7 MeTMSi, molecular ion 416. We could not detect the presence of any 416; therefore, if present, these GAs were below our limit of detection.

Other Possible Sugarcane GAs

Even though the bioassay showed GA-like substances in some cultivars, growth stages, or plant parts in fractions 11-13, in a rapid screening of these fractions by mass chromatograms for masses of known GAs likely to be detected in these fractions, we could not detect any known GAs (Table 1). Lack of detection resulted from interfering substances in these fractions. Fractions above 17, in which one expects to find the more polar, trihydroxylated GAs, were not screened because the large amounts of organic acids other than GAs would have made the detection of GA MeTMSi derivatives difficult. Further purification of these fractions is necessary for GC/MS identification of GAs.

GC/MS Confirmation of ABA in Combined Fractions 6 and 7

Fractions 6 and 7, regardless of cultivar, growth stage, or plant tissue, always showed growth inhibition in the bioassay. Data from GC/MS of methyl esters of combined fractions 6 and 7 on 1% XE-60 (195°C isothermal) from immature stem segments, reproductive stage, H59-3775, confirmed the presence of ABA (Most 1971). Mass chromatograms for characteristic mass fragments of methyl

Table 3. Distribution of gibberellins GA_1, GA_3, GA_{19}, GA_{20}, and GA_{29} in sugarcane leaf or meristem tissues at vegetative and reproductive stages.

Cultivar	Developmental stage	Tissue(s)	Gibberellins				
			GA_{20}	GA_{19}	GA_1	GA_3	GA_{29}
H57-5174	Vegetative	Leaf		X	X	X	X
		Meristem		X	X	X	O
	Reproductive	Leaf		X	X	X	X
		Meristem		X	X	X	O
H54-775	Vegetative	Leaf		X	X	X	O
		Meristem		X	X	X	O
	Reproductive	Leaf		X	X	X	X
		Meristem		X	X	X	X
H59-3775	Vegetative	Leaf	X	X	X	X	X
		Meristem					
	Reproductive	Leaf					
		Meristem	X	X			

X = Gibberellin detected.
O = Gibberellin looked for but not detected.
Blank = Gibberellin not looked for.

abscisate appeared in scan 53 (RT, 4.4 min), the identical retention time for authentic methyl abscisate. A spectrum identical to that of the authentic compound was obtained from scan 53 of the plant extract.

Gibberellins Characteristic of Saccharum spp.

Samples initially bioassayed were examined by GC/SIM monitoring for GAs identified in the leaf, flowering stage of H57-5174. SIM monitoring of combined silica gel fractions 14-16 suggested the presence of GA_1, GA_3, GA_{19}, and GA_{29} in different quantities in all cultivars screened (Table 3). The first three GAs were found in all tissues that were monitored while GA_{29} was below the detectable limit in four of the nine samples. These data illustrate the importance of screening several tissue and developmental stages for the identification of endogenous GAs.

The fifth GA, GA_{20}, was found in mature leaf tissue and the apical meristem of cv. H59-3775. GC/MS data were not available for GA_{20} in the two other cultivars; however, bioassay of chromatographic fractions 8 and 9, which would contain GA_{20}, gave growth stimulation in eight of the ten samples. Thus, the five GAs for which we have evidence can be considered characteristic of *Saccharum* spp., even though all five GAs were not detected in all samples. Therefore, the identification of these sugarcane giberrellins provide the basis to quantitate the changes in GAs with respect to environment and genotype.

The sugarcane GAs identified to date are all C-13 hydroxylated GAs and all have been previously identified in the grass family, Poaceae. Recently, four of the five sugarcane GAs, GA_1, GA_{19}, GA_{20}, and GA_{29}, have been identified in *Zea mays* L. tassels (Hedden et al. 1982). Also, GA_{19} has been identified in *Oryza sativa* (Kurogochi et al. 1979) and *Phyllostachys edulis* (Murofushi et

al. 1966), while GA_1 and GA_3 have been identified in the vegetative tissues of *Secale cereale* (Eckert et al. 1978), *Oryza sativa* (Kurogochi et al. 1979), and *Triticum aestivum* (Eckert et al. 1978). Although GA_3 was identified in Avena, GA_1 was not (Kaufman et al. 1976); while GA_1 was identified in *Zea mays* L., GA_3 was not (Hedden et al. 1982). Metabolic pathways involving GA_{20}, GA_1, and GA_{29} have been reported (Frydman and MacMillan 1975, Lance et al. 1976, Railton et al. 1974), and additional data suggest the occurrence of GA_{19} in this metabolic sequence (Crozier et al. 1970, Hedden et al. 1982).

Acknowledgements. The authors acknowledge useful correspondence, discussions, and gifts of GA_1, GA_5, and GA_{20} from Dr. R. P. Pharis (University of Calgary, Canada) and GA_{29} from W. M. Proebsting (Oregon State University, Corvallis, Ore, USA).

References

Bearder JR, MacMillan J (1973) Fungal products. IX. Gibberellins A_{16}, A_{36}, A_{37}, A_{41} and A_{42} from *Gibberella fujikuroi*. J. C. S. Perkins 1:2824–2830

Bearder JR, Frydman VM, Gaskin P, Hatton IK, Harvey WE, MacMillan J, Phinney BO (1976) Fungal products. XVII. Microbiological hydroxylation of gibberellin A_9 and its methyl ester. J. C. S. Perkins 1:178–183

Binks R, MacMillan J, Pryce RJ (1969) Plant hormones. VIII. Combined gas chromatography-mass spectrometry of the methyl esters of gibberellins A_1 to A_{24} and their trimethylsilyl ethers. Phytochem 8:271–284

Bull TA (1964) The effects of temperature, variety, and age on the response of *Saccharum* spp. to applied gibberellic acid. Aust J Agric Res 15:77–84

Correa NS, Antich EP, Antoni H (1972) Estudios preliminares sobre sustancias tipo giberelinas en apices de caña de azucar y su relacion con la induccion *fo* toperiodica. Rev. ind. y Agricola de Tucuman 49:1–11

Crozier A, Reid DM (1970) Gibberellin metabolism in the roots of *Phaseolus coccineus* seedlings. In: Carr DJ (ed) Plant growth substances 1970. Springer-Verlag, Berlin Heidelberg New York, pp 414–419

Durley RC, Crozier A, Pharis RP, McLaughlin GE (1972) Chromatography of 33 gibberellins on a gradient eluted silica gel partition column. Phytochem 11:3029–3033

Durley RC, Pharis RP (1973) Interconversion of gibberellin A_4 to gibberellins A_1 and A_{34} by dwarf rice, cultivar tan-ginbozu. Planta (Berl.) 109:357–361

Durley RC, Sassa T, Pharis RP (1979) Metabolism of tritiated gibberellin A_{20} in immature seeds of dwarf pea, cv. Meteor. Plant Physiol 64:214–219

Eckert H, Schilling G, Podlesak W, Franke P (1978) Extraktion und Identifizierung von Gibberellinen (GA_1 and GA_3) aus *Triticum aestivum* L. und *Secale cereale* L. sowie die Veränderung der Gehalte im Laufe der Ontogenese. Biochem Physiol Pflanzen 172:475–486

Feneslau C (1977) The mass spectrometer as a gas chromatograph detector. Anal Chem 49:563A–570A

Frydman VM, MacMillan J (1975) The metabolism of gibberellins A_9, A_{20} and A_{29} in immature seeds of *Pisum sativum* cv. Progress No. 9. Planta (Berl.) 125:181–195

Glenn JL, Kuo CC, Durley RC, Pharis RP (1972) Use of insoluble polyvinylpyrrolidone for purification of plant extracts and chromatography of plant hormones. Phytochem 11:345–351

Haddon WF, Black DR, Elsken RH (1970) Acquisition of mass spectral data with a time-sharing computer system. Proc 18th Ann Conf on Mass Spectrometry, San Francisco: B450

Hedden P, Phinney BO, Heupel R, Fujii D, Cohen H, Gaskin P, MacMillan J, Graebe JE (1982) Hormones of young tassels of *Zea mays*. Phytochem 21:391–393

Kamienska A, Pharis RP (1975) Endogenous gibberellins of pine pollen. II. Changes during germination of *Pinus attenuata, P. coulteri,* and *P. ponderosa* pollen. Plant Physiol 56:655–659

Kaufman PB, Ghosheh NS, Nakosteen L, Pharis RP, Durley RC, Morf W (1976) Analysis of native gibberellins in the internode, nodes, leaves, and influorescence of developing *Avena* plants. Plant Physiol 58:131–134

Kurogochi S, Murofushi N, Ota Y, Takahashi N (1979). Identification of gibberellins in the rice plant and quantitative changes of gibberellin A_{19} throughout its life cycle. Planta (Berl.) 146: 185–191

Lance B, Durley RC, Reid DM, Thorpe TA, Pharis RP (1976) Metabolism of [^{3}H] gibberellin A_{20} in light- and dark-grown tobacco callus culture. Plant Physiol 58:387–392

Martin GC, Dennis FG Jr, Gaskin P, MacMillan J (1977) Identification of gibberellins A_{17}, A_{25}, A_{45}, abscisic acid, phaseic acid, and dihydrophaseic acid in seeds of *Pyrus communis*. Phytochem 16:605–607

Moore PH, Buren LL (1978) Gibberellin studies with sugarcane. I. Cultivar differences in growth responses to gibberellic acid. Crop Sci 17:443–446

Most BH, Vlitos AJ (1966) Gibberellins of sugarcane. Plant Physiol 41:1090–1094

Most BH (1971) Abscisic acid in immature apical tissue of sugar cane and in leaves of plants subjected to drought. Planta (Berl.) 101:67–75

Murakami Y (1968) A new rice seedling test for gibberellins, "micro-drop method," and its use for testing extracts of rice and morning glory. Bot Mag Tokyo 81:33–43

Murofushi N, Iriuchijima S, Takahashi N, Tamura S, Kato J, Wada Y, Watanabe E, Aoyama T (1966) Isolation and structure of a novel C_{20} gibberellin in bamboo shoots. Agric Biol Chem 30:917–924

Railton ID, Murofushi H, Durley RC, Pharis RP (1974) Interconversion of gibberellin A_{20} to gibberellin A_{29} by etiolated seedlings and germinating seeds of dwarf *Pisum sativum*. Phytochem 13:793–796

Reeve DR, Crozier A, Durley RC, Reid DM, Pharis RP (1975) Metabolism of ^{3}H-gibberellin A_1 and ^{3}H-gibberellin A_4 by *Phaseolus coccineus* seedlings. Plant Physiol 55:42–44