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Endogenous Gibberellins and Kauranoids Identified from Developing and Germinating Barley Grain*

Paul Gaskin,[1] Sarah Jane Gilmour,[1] John R. Lenton,[2] Jake MacMillan,[1] and Valerie M. Sponsel[1]

[1]Agricultural Research Council Research Group, School of Chemistry, The University of Bristol, BS8 1TS, UK
[2]Rothamsted Experimental Station, Harpenden, Hertfordshire. AL5 2JQ, UK

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Abstract. Several gibberellins (GAs) and kauranoids were identified in extracts of barley (*Hordeum vulgare*) by combined capillary gas chromatography-mass spectrometry (GC-MS). A partially purified acidic ethyl acetate extract from 21-day postanthesis developing barley grain (cv. Proctor) contained GA_1 (trace), GA_4 (trace), GA_8 (trace), GA_{12}, GA_{17}, GA_{20} (tentative) (trace), GA_{25}, GA_{34}, GA_{48}, 18-hydroxy-GA_4, 12β-hydroxy-GA_9, and 18-hydroxy-GA_{34} (tentative). A hydrolyzed butanol extract contained GA_{17}, GA_{20}, GA_{48}, and 18-hydroxy-GA_{34} (tentative). An acidic ethyl acetate extract from 3-day-old germinating barley grain (cv. Maris Otter) contained GA_1, GA_3 (possibly a contaminant), GA_{17}, GA_{19}, GA_{20}, GA_{34}, GA_{48}, and 18-hydroxy-GA_{34} (tentative). A hydrolyzed butanol extract contained GA_{34}, GA_{48}, and 18-hydroxy-GA_{34} (tentative). In germinating grain, levels of all GAs were very low. Two hydroxylated kauranoic acids and a number of other kauranoids were also detected in the above extracts. 1β-Hydroxylated GAs previously found in wheat were not found in barley in this study.

Abbreviations: GA, gibberellin; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; GC-MS, combined gas chromatography-mass spectrometry; EtOAc, ethyl acetate; KRI, Kovats Retention Index; TMSi, trimethylsilyl.

* This work has been reported in a poster demonstration (Gaskin et al. 1982).

Introduction

It has been known for some time that gibberellin (GA)-like substances are present in immature (Jones et al. 1963, Radley 1966) and germinating (Radley 1959, Yomo 1960) barley grain. Attempts have been made to identify these substances using a variety of techniques. Jones et al. (1963) found two GA-like substances in immature barley grain and considered one to be GA_3 based on TLC, bioassay, and fluorimetry. The second GA-like substance was later shown by Radley (1966) to co-chromatograph with GA_4/GA_7. Subsequently, Radley (1967, 1968) used TLC and bioassay as evidence for the presence of GA_1 and GA_3 in immature and germinating barley grain. Murphy and Briggs (1973) reported two fractions from germinating barley that co-chromatographed with GA_3 and GA_7, and after methylation the relevant fractions co-crystallized with GA_3 and GA_7 methyl esters. Faull et al. (1974) found GA_1-like and GA_3-like substances in the roots and shoots of 8-, 11-, and 15-day-old barley seedlings. Their data were based on chromatographic, electrophoretic, and bioassay techniques. Recently Atzorn and Weiler (1982) have presented immunoassay evidence that GA_4 is a major GA in germinating barley.

In view of these conflicting results, an investigation of the GAs in developing and germinating barley grain was undertaken using C_{18} reverse phase HPLC as a separating technique and capillary GC-MS as the analytical method.

Materials and Methods

Plant Materials

Barley (*Hordeum vulgare* cv. Proctor) grown in the field at Rothamsted during 1981 was harvested 3 weeks after anthesis (milk ripeness) and freeze-dried immediately.

Approximately 4,000 mature grains of barley (cv. Maris Otter) were surface sterilized by soaking for 20 min in an 8% aqueous solution of household bleach ("Domestos"). The grains were washed well with water and germinated for 3 days on damp paper towels at 25°C in the dark. The whole seedlings were taken for extraction as described below.

Extraction Procedure

To minimize contamination by synthetic GAs, all plant extractions were carried out in a laboratory dedicated to plant extraction only. All glassware was cleaned with chromic acid before use, and all solvents were redistilled.

The immature grains from 50 ears (approximately 1,350 grains, 40 g dry weight) and approximately 4,000 3-day-old germinating grain (300 g fresh weight) were extracted three times with 80% aqueous MeOH at 4°C. The methanolic extract was reduced to an aqueous phase *in vacuo* below 40°C. The aqueous phase was adjusted to pH 8.0 with solid $NaHCO_3$ and slurried three times with insoluble polyvinylpyrrolidone (PVP, GAF Co. Ltd., 1 g/10 ml extract) (Glenn et al. 1972). The extract was partitioned three times against a one-third volume of petroleum ether (b.p. 60–80°C), then three times against an

equal volume of EtOAc. The petroleum and EtOAc fractions were discarded. The aqueous fraction was then adjusted to pH 2.5 with 5.5 *N* HCl and partitioned three times against EtOAc. The aqueous fraction was further partitioned three times against an equal volume of water-saturated n-butanol. The combined acidic EtOAc and combined butanol fractions were evaporated to dryness *in vacuo*. A small volume of water (approximately 1 ml/100 ml of solvent) was added prior to evaporation to ensure that residual acid was removed as an azeotrope.

Enzymatic Hydrolysis of Butanol Fraction

The butanol fractions were hydrolyzed by the following modified method of Frydman and MacMillan (1975). Pectolytic enzyme (Boots Co. Ltd., Nottingham, England) was removed from its kieselguhr support by stirring with 50 m*M* K-Pi buffer, pH 4.0 (1 g enzyme/5 ml buffer), and filtering off the kieselguhr. The dried butanolic extract was dissolved in the enzyme solution (100 ml enzyme solution/g extract) and allowed to stand at 30°C for 2 days. The solution was then adjusted to pH 2.5 with 5.5 *N* HCl and partitioned three times with EtOAc. The combined EtOAc fraction was evaporated to dryness *in vacuo*.

Reverse Phase HPLC

The method employed was based on that of Jones et al. (1980). The dried acidic EtOAc extracts were dissolved in 30% aqueous MeOH and filtered through a 0.45 μM-type HA Millipore filter. The filtered extract (1–2 ml) was injected via a Waters model U6K injector fitted with a 2-ml loop onto a Shandon column (250 × 8 mm i.d.) packed with ODS-Hypersil (5 μm). A linear gradient of 30–100% MeOH in 1% aqueous acetic acid was applied via two Waters type 6000A pumps controlled by a Waters 660 solvent programmer. The gradient was run at 2.5 ml/min over 25 min. Fractions were collected at 1 minute intervals for 30 min and dried in a fume hood.

Bioassays

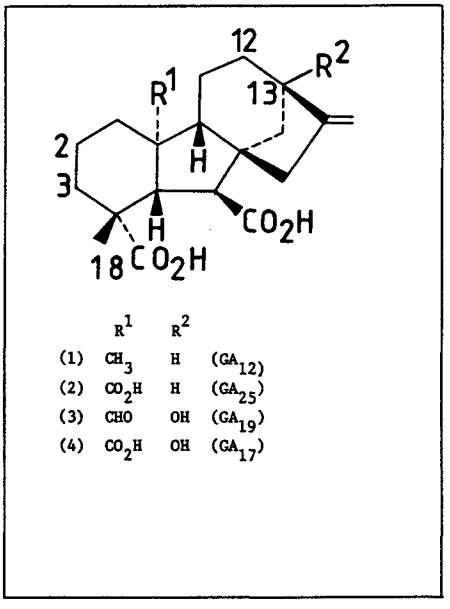
A small portion of each HPLC fraction was removed and bioassayed by a modified barley endosperm bioassay (Jones and Varner 1967).

Grains of barley cv. Himalaya (grown at Rothamsted during 1977) were surface sterilized for 4 min in 0.1% mercuric chloride, rinsed in distilled water, then cut transversely in half. Three embryoless half seeds were placed in vials each containing the sample in 1 ml of distilled water. The capped vials were incubated at 28°C in the dark for 36–48 h. After incubation, each bioassay solution was diluted to 10 ml and a 0.2 ml aliquot was taken for colorimetric analysis of reducing sugars (Nelson 1944, Somogyi 1952). A standard curve of

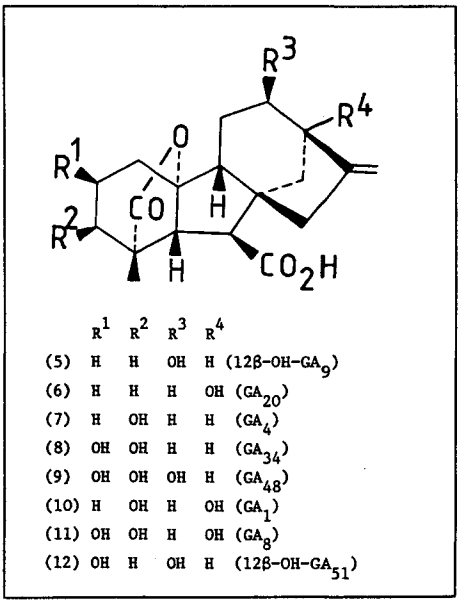
GA_3 (0.1–10.0 ng) versus absorbance at 560 nm was constructed for each set of bioassays.

Derivatization and GC-MS

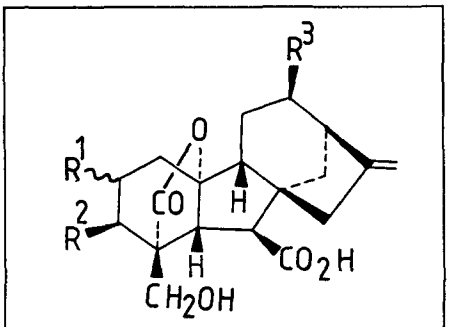
Pooled HPLC fractions were dissolved in MeOH and methylated with excess ethereal diazomethane. The fractions were then dried under vacuum and trimethylsilyl (TMSi) ethers of the methyl esters were prepared using N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) (Pierce). Derivatized samples were chromatographed on a DANI-3800 GC using a vitreous silica wall coated open tubular (WCOT) capillary column (25 m × 0.2 mm i.d.) coated with OV-1. Samples were injected onto the column at 30°C in the Grob splitless mode, and the injector purge was activated after 40 sec. The column temperature was taken rapidly to 60°C and, after a 2 min isothermal hold, was programmed at 13°C per min to 150°C and subsequently at 3°C per min to 280°C with a 10-min isothermal hold at the end of the program. The pressure of the He carrier gas was 2 bar. The column effluent was led directly into the ion source of a VG 7050 computerized GC-MS with a source temperature of 220°C and an interface temperature of 250°C. The ionizing potential was 24 eV and mass spectra were recorded at 0.7 sec per decade (1.3 sec per cycle). A mixture of n-alkanes (C_{16}–C_{34}, approximately 1–15 ng of each) (BDH and "Parafilm," Gallenkamp) (Gaskin et al. 1971) was coinjected with each sample in order to provide relative retention times for each GC peak. Kovats Retention Indices (KRIs) (Kovats 1958) were obtained from a calibration curve of alkane carbon number versus retention time.



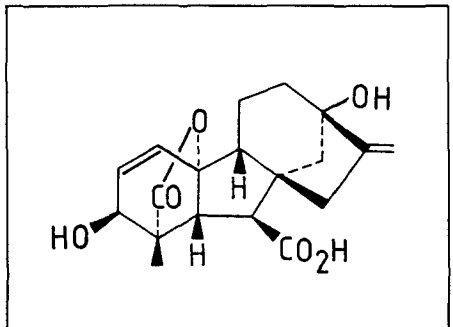
	R^1	R^2	
(1)	CH_3	H	(GA_{12})
(2)	CO_2H	H	(GA_{25})
(3)	CHO	OH	(GA_{19})
(4)	CO_2H	OH	(GA_{17})



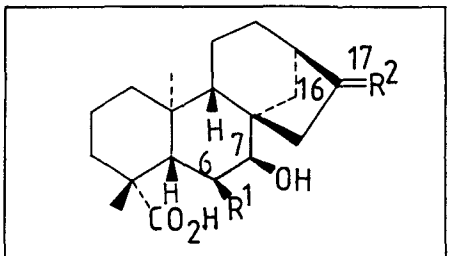
	R^1	R^2	R^3	R^4	
(5)	H	H	OH	H	(12β-OH-GA_9)
(6)	H	H	H	OH	(GA_{20})
(7)	H	OH	H	H	(GA_4)
(8)	OH	OH	H	H	(GA_{34})
(9)	OH	OH	OH	H	(GA_{48})
(10)	H	OH	H	OH	(GA_1)
(11)	OH	OH	H	OH	(GA_8)
(12)	OH	H	OH	H	(12β-OH-GA_{51})



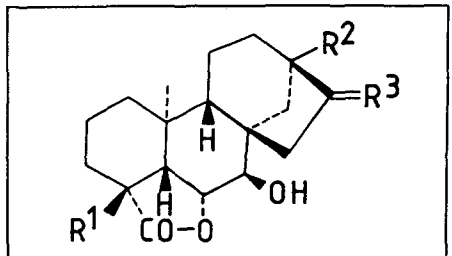
	R^1	R^2	R^3	
(13)	H	OH	H	(18-OH-GA_4)
(14)	H,β-OH	OH	H	(18-OH-GA_{34})
(15)	H,α-OH	OH	H	(18-OH-GA_{47})
(16)	H,β-OH	OH	OH	(18-OH-GA_{48})



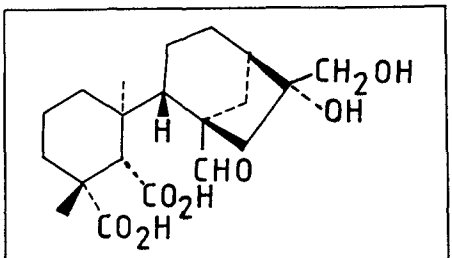
(17) (GA_3)



	R^1	R^2	
(18)	H	CH_2OH, α-OH(7β,16α,17-triOH-kauranoic acid)	
(19)	OH	CH_2OH, α-OH(6β,7β,16α,17-tetraOH-kauranoic acid)	



	R^1	R^2	R^3	
(20)	CH_2OH	H	CH_2	(7β,18-diOH-kaurenolide)
(21)	CH_3	OH	CH_2	(7β,13-diOH-kaurenolide)
(22)	CH_3	H	CH_2OH,	β-OH(7β,16β,17-triOH-kauranolide)
(23)	CH_3	H	CH_2OH,	α-OH(7β,16α,17-triOH-kauranolide)



(24) (16α,17-diOH-fujanoic acid)

Scheme 1

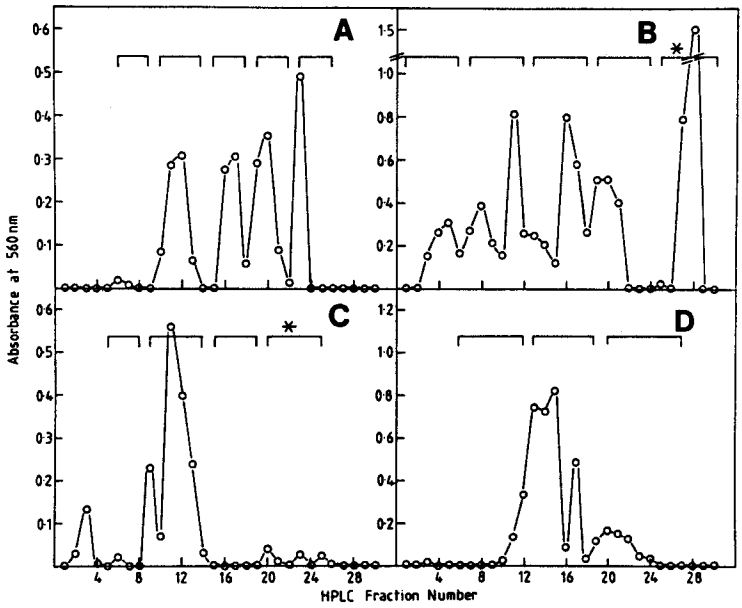


Fig. 1. Barley aleurone bioassays of HPLC fractions of acidic EtOAc and enzyme-hydrolyzed butanol extracts of 3-week postanthesis developing barley grain (cv. Proctor) and 3-day-old germinating barley grain (cv. Maris Otter). A. Developing barley, acidic EtOAc extract—27 grain equivalents were bioassayed; 1 ng and 10 ng GA_3 equivalents gave absorbances of 0.61 and 0.83, respectively. B. Developing barley, hydrolyzed butanol extract—272 grain equivalents were bioassayed; 1 ng and 10 ng GA_3 equivalents gave absorbances of 1.09 and 1.49, respectively. C. Germinating barley, acidic EtOAc extract—80 grain equivalents were bioassayed; 1 ng and 10 ng GA_3 equivalents gave absorbances of 0.40 and 0.69, respectively. D. Germinating barley, hydrolyzed butanol extract—312 grain equivalents were bioassayed; 1 ng and 10 ng GA_3 equivalents gave absorbances of 1.05 and 1.55, respectively. Fractions were pooled as shown and examined by GC-MS. No GAs were identified in those zones indicated by * because they were too heavily contaminated with polyhydroxylated fatty acids.

Results

Reverse phase C_{18} HPLC was used to fractionate acidic EtOAc and enzyme-hydrolyzed acidic butanol extracts obtained from both developing barley grain (cv. Proctor) 3 weeks after anthesis and germinating barley grain (cv. Maris Otter). The HPLC fractions were bioassayed by the barley aleurone bioassay (see Fig. 1) and groups of fractions were pooled as shown in Fig. 1, derivatized, and examined by capillary GC-MS. Table 1 lists the GAs and kauranoids identified by capillary GC-MS from both types of plant material. The following compounds were identified as their Me esters TMSi ethers by comparison with reference spectra (Binks et al. 1969, library of reference spectra at Bristol) and from their relative retention times on capillary GC: GA_1 (Scheme 1, structure

Table 1. Gibberellins and kauranoids identified in barley.

21-day postanthesis developing barley grain (cv. Proctor)				
	Acidic EtOAc extract HPLC fractions where found		Hydrolyzed butanol extract HPLC fractions where found	
C_{19} GAs	10–14	GA_1(10) (trace)	13–18	GA_{20}(6)
	[a]18–26	GA_4(7) (trace	7–12	GA_{48}(9)
	6–9	GA_8(11) (trace)	13–18	[c]18-OH-GA_{34}(14)
	[a]14–20	[b]GA_{20}(6) (trace)	1–6	[c]18-OH-GA_{48}(16)
	15–18, 19–22	GA_{34}(8)	7–12	
	6–9, 10–14	GA_{48}(9)		
	19–22	18-OH-GA_4(13)		
	10–14	12β-OH-GA_9(5)		
	15–18	[c]18-OH-GA_{34}(14)		
	6–9	[c]18-OH-GA_{48}(16)		
	10–14	[c]12β-OH-GA_{51}(12)		
C_{20}GAs	23–26	GA_{12}(1)	13–18	GA_{17}(4)
	15–18, 19–22	GA_{17}(4)		
	19–22	GA_{25}(2)		
Kauranoids	6–9	16α,17-diOH-fujanoic acid(24)	13–18	16α,17-diOH-fujanoic acid(24)
	15–18	7β,16α,17-triOH-kauranoic acid(18)	1–6, 7–12, 13–18	7β,16α,17-triOH-kauranoic acid(18)
	[a]8–17, 18–26	6β,7β,16α,17-tetraOH-kauranoic acid(19)	13–18	[d]7β,13-diOH-kaurenolide(21)
	19–22	[d]7β,18-diOH-kaurenolide(20)		
	15–18, 19–22	7β,16β,17-triOH-kauranolide(22)		

[a] These values were taken from subsequent extractions.
[b] Very weak spectrum.
[c] Tentative identification.
[d] Possibly due to contamination.
[e] All GAs were present in very low levels in germinating grain.

10), GA_3(17), GA_4(7),* GA_8(11), GA_{12}(1), GA_{17}(4), GA_{19}(3), GA_{20}(6), GA_{25}(2), GA_{34}(8), GA_{48}(9). Relative retention times and KRIs for these compounds are shown in Table 2. The identification of GA_{20} in the acidic EtOAc fraction from developing barley grain was tentative because of its weak spectrum.

* Although not detected initially, a trace amount of GA_4 was subsequently detected in an extract of developing barley grain.

Table 1. (cont'd)

3-day-old germinating barley grain[e] (cv. Maris Otter)				
	Acidic EtOAc extract HPLC fractions where found		Hydrolyzed butanol extract HPLC fractions where found	
C_{19} GAs	9–14	GA_1(10)	20–27	GA_{34}(8)
	9–14	[d]GA_3(17)	13–19	GA_{48}(9)
	15–19	GA_{20}(6)	13–19 / 20–27	[c]18-OH-GA_{34}(14)
	15–19	GA_{34}(8)		
	9–14	GA_{48}(9)		
	15–19	[c]18-OH-GA_{34}(14)		
C_{20} GAs	15–19	GA_{17}(4)		
	15–19	GA_{19}(3)		
Kauran-oids	9–14	16α,17-diOH-fujanoic acid(24)	6–12	16α,17-diOH-fujanoic acid (24)
	9–14	16α,17-diOH-fujanoic acid(24)	13–19 / 20–27	6β,7β,16α,17-tetraOH-kauranoic acid(19)
	15–19	7β,16α,17-triOH-kauranoic acid(18)	13–19	7β,16α,17-triOH-kauranolide(23)
	15–19	7β,16β,17-triOH-kauranolide(22)	20–27	[d]7β,13-diOH-kauranolide(21)

[a] These values were taken from subsequent extractions.
[b] Very weak spectrum.
[c] Tentative identification.
[d] Possibly due to contamination.
[e] All GAs were present in very low levels in germinating grain.

12β-Hydroxy-GA_9(5) was identified in the acidic EtOAc fraction from developing grain by comparison with the mass spectrum and relative retention time of 12β-hydroxy-GA_9 obtained from a feed of 12β-hydroxy-kaurenoic acid to the mutant, B1-41a of the fungus *Gibberella fujikuroi* (Gaskin et al. 1984).

18-Hydroxy-GA_4(13) was also identified in the acidic EtOAc fraction from developing barley grain by comparison with the mass spectrum and relative retention time of 18-hydroxy-GA_4 obtained from a feed of 18-hydroxy-GA_{12}-7-aldehyde to mutant B1-41a of *G. fujikuroi* (Beeley 1975). It is interesting to note that an intense M^+-103 peak was not observed in the mass spectrum of this compound (or other 18-hydroxylated compounds identified) even though the presence of a hydroxyl attached to a methyl group has been observed to give this ion due to loss of $CH_2 = {}^+O\text{-TMSi}$ (Binks et al. 1969). Gibberellin "A" numbers (MacMillan and Takahashi 1968) will be assigned to these two compounds when they have been isolated and fully characterized.

A compound that appeared in extracts from both types of plant material was tentatively assigned the structure 18-hydroxy-GA_{34} (14) on the following basis. The mass spectrum of the MeTMSi derivative was identical with that of a minor product from a feed of 18-hydroxy-GA_{12}-7-aldehyde to mutant B1-41a

Table 2. Relative retention times on capillary GC and Kovats Retention Indices (KRI) of a number of GAs and kauranoids, including those identified in barley

Compound	Relative Retention Time (alkane carbon number + time (min:sec))	KRI
GA_{12}	C_{23} + 0:36	2323
GA_{25}	C_{24} + 0:43	2430
GA_{20}	C_{24} + 1:47	2474
12β-OH-GA_9	C_{24} + 2:07	2489
GA_4	C_{24} + 2:18	2497
GA_{17}	C_{25} + 1:33	2567
GA_{19}	C_{25} + 2:09	2594
18-OH-GA_4	C_{26} + 0:44	2634
7β,18-diOH-kaurenolide	C_{26} + 0:59	2645
[a]12β-OH-GA_{51}	C_{26} + 1:06	2649
GA_{34}	C_{26} + 1:16	2657
GA_1	C_{26} + 1:22	2662
7β,13-diOH-kaurenolide	C_{26} + 1:34	2672
12β-OH-GA_4	C_{26} + 1:51	2683
GA_3	C_{26} + 1:54	2686
[a]18-OH-GA_{47}	C_{27} + 0:12	2710
[a]18-OH-GA_{34}	C_{27} + 1:35	2773
GA_{48}	C_{27} + 1:53	2788
GA_8	C_{28} + 0:20	2816
7β,16α,17-triOH-kauranoic acid	C_{28} + 1:16	2862
7β,16α,17-triOH-kauranolide	C_{28} + 1:55	2895
[a]18-OH-GA_{48}	C_{29} + 0:00	2900
6β,7β,16α,17-tetraOH-kauranoic acid	C_{29} + 1:35	2978
16α,17-diOH-fujanoic acid	C_{30} + 0:31	3026
7β,16β,17-triOH-kauranolide	C_{30} + 0:50	3041

[a] Tentative identification.

of *G. fujikuroi* (Beeley 1975). This latter compound has been tentatively assigned the structure 18-hydroxy-GA_{47} (15) on the basis of the fragment ions in its mass spectrum. From the identical mass spectra and different KRIs of the putative 18-hydroxy-GA_{34} (KRI 2773) and the putative 18-hydroxy-GA_{47} (KRI 2710) it is tentatively concluded that the two compounds are 2-epimers.

A compound with an M^+ m/z 682, which was eluted early on reverse phase HPLC, was tentatively assigned the structure 18-hydroxy-GA_{48} (16). Its molecular ion at m/z 682 indicated that it was the MeTMSi derivative of a tetrahydroxy-GA_9. The presence of an intense ion at m/z 191 ($(CH_3)_3SiO\overset{+}{C}H = {}^+OSi(CH_3)_3$) supported this conclusion, as it is indicative of a polyhydroxyl compound. Some sugars also show this ion (DeJongh et al. 1969). A strong fragment ion at M^+-18 (loss of H_2O from the lactone) gave some indication that the compound could possibly be 18-hydroxylated (Beeley and Gaskin unpublished work). Based on the other GAs found in barley, the most logical assignment of structure was 18-hydroxy-GA_{48}.

The MeTMSi derivative of a compound with M^+ m/z 506 and KRI 2649, which had a different relative retention time from 12β-hydroxy-GA_4 (KRI

Table 3. Mass spectra of methyl esters/TMSi ethers of some GAs and kauranoids identified from barley

Compound	Diagnostic ions m/z (% relative intensity)
12β-OH-GA_9(5) (Gaskin et al. 1984)	418(M^+, 7), 403(12), 386(9), 372(16), 358(19), 343(10), 328(21), 296(72), 282(47), 268(100), 251(25), 223(95), 75(42) and 73(31).
[a]12β-OH-GA_{51}(12)	506(M^+, absent), 491(3), 416(16), 401(10), 384(18), 356(23), 326(35), 294(51), 282(40), 266(51), 223(54), 221(63), 75(33) and 73(100).
18-OH-GA_4(13) (Beeley 1975)	506(M^+, 9), 491(38), 488(33), 474(71), 459(27), 431(13), 416(10), 384(52), 377(44), 369(57), 341(74), 326(78), 317(90), 294(57), 282(68), 280(57), 266(63), 223(100), 221(68), 195(47), 131(61) and 73(46).
[a]18-OH-GA_{34}(14)	594(M^+, 43), 579(10), 576(4), 562(8), 547(5), 504(13), 489(13), 370(18), 317(31), 75(100) and 73(33).
[a]18-OH-GA_{48}(16)	682(M^+, 8), 664(3), 650(8), 635(2), 592(9), 560(8), 502(17), 412(20), 386(12), 191(56), 75(100) and 73(39).
16α,17-di-OH-fujanoic acid(24)	554(M^+, absent), 539(1), 507(2), 464(1), 451(91), 419(30), 373(14), 327(40), 237(38), 227(38), 195(95), 167(45), 107(50), 75(100) and 73(28).
7β,16α,17-tri-OH-kauranolide(23) and 7β,16β,17-tri-OH-kauranolide(22)	566(M^+, absent), 551(1), 463(55), 419(9), 373(22), 345(16), 137(18), 75(100) and 73(14).
7β,16α,17-tri-OH-kauranoic acid(18)	582(M^+, absent), 567(2), 479(71), 402(19), 389(100), 329(28), 239(32), 75(14) and 73(16).
6β,7β,16α,17-tetra-OH-kauranoic acid (19) (Beeley et al. 1975)	670(M^+, absent), 655(7), 567(24), 477(100), 417(5), 387(11), 269(19), 209(9), 191(13), 75(19) and 73(24).

[a] Tentative identification.

2683), was also observed. It could conceivably be 12β-hydroxy-GA_{51} (12), as it was also found to be produced from a feed of GA_{51} to developing barley grain. Elucidation of the structure of this compound, as well as the putative 18-hydroxy-GA_{34} and 18-hydroxy-GA_{48}, must await the availability of authentic compounds.

The mass spectra of 12β-hydroxy-GA_9, 18-hydroxy-GA_4, and the tentatively identified 18-hydroxy-GA_{34}, 18-hydroxy-GA_{48}, and 12β-hydroxy-GA_{51} are listed in Table 3.

Great care was taken to minimize contamination that would occur from the

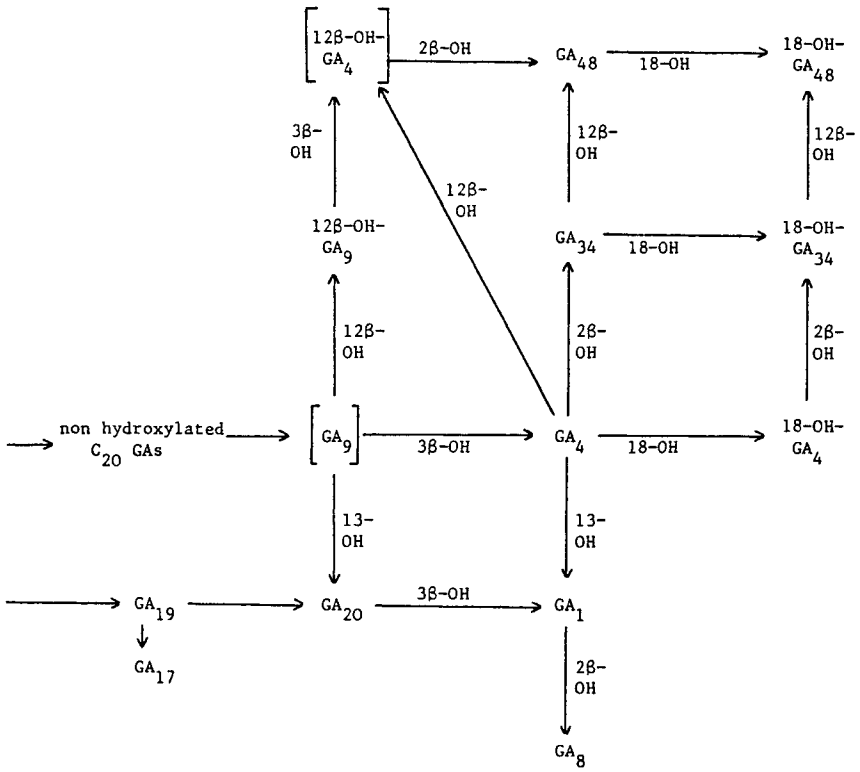


Fig. 2. Hypothetical metabolic grid of GA interconversions in developing barley grain. GAs shown in parentheses were not detected in developing barley grain in the present investigation.

presence of synthetic and fungal GAs under study in an adjacent laboratory. However, GA_3 was occasionally found in all HPLC fractions and occasionally in one or two that were not consistent with its retention time. Therefore, the evidence presented herein for the presence of GA_3 in germinating barley extracts is questionable.

On the basis of the intensity of the ions in the mass spectra, the major GAs in developing barley grain 3 weeks after anthesis were probably 18-hydroxy-GA_4 (13) and the putative 18-hydroxy-GA_{34} (14). GA_1, GA_4, GA_8, and GA_{20} were present only in trace amounts. The levels of all GAs in germinating grain were much lower than in developing grain.

A number of kauranoids were also found in the barley extracts (see Table 1). Their mass spectra are listed in Table 3. Some of these had an intense ion at M^+-103, which is characteristic of 16,17-dihydroxylated compounds. The M^+-103 ion is formed by loss of $CH_2 = {}^+OTMSi$ from a hydroxymethyl group, as mentioned for 18-hydroxylated compounds. In the case of 7β,16β,17-trihydroxykauranolide (22) the base peak (m/z 463) was M^+-103. In the cases of 16α,17-dihydroxy-fujanoic acid (24) and 7β,16α,17-trihydroxykauranoic acid (18), M^+-103 was the most intense peak after the base peak.

The stereochemistry of the 16-hydroxyl group of 7β,16α,17-trihydroxykauranoic acid (18) and 6β,7β,16α,17-tetrahydroxykauranoic acid (19) is assumed to be α. This is based on the identical spectra but different relative retention times from the authentic 16β-epimers. No 16α-epimers were available for direct comparison. However, the 16-hydroxyl group is assumed to be β in 7β,16β,17-trihydroxykauranolide (22). This is based on the fact that this compound gives the same mass spectrum with a different relative retention time from 7α,16α,17-trihydroxykauranolide (23) (see Table 2). It is interesting to note that developing grain at 3 weeks after anthesis contains 7β,16β,17-trihydroxykauranolide only, whereas the 16α-epimer is also present in 3-day-old germinating grain.

7β,18-Dihydroxykaurenolide (20) (Hedden et al. 1973) and 7β,13-dihydroxykaurenolide (21) (Bearder et al. 1975) detected in extracts of developing barley grain (Table 1) have not been observed in two subsequent extractions. Unfortunately these two compounds may be contaminants originating from large-scale synthetic work conducted concurrently in an adjacent laboratory.

The GAs identified in the hydrolyzed butanol fractions may have been present in the original extracts as conjugates (e.g. with glucose). However, it is also possible that these GAs appeared in the butanol phase as "spillover" due to incomplete extraction into EtOAc. This would happen particularly with the more polar GAs.

The two cultivars of barley (Proctor and Maris Otter) used in this study were obtained from two different laboratories. However they are closely related in their genetic background in that Maris Otter originated from a cross between Proctor and Pioneer (Bell et al. 1964). Thus it is unlikely that they differ qualitatively in their GA and kauranoid composition.

Discussion

A diverse number of GAs and kauranoids have been found to be present in developing and germinating barley grain (Table 1). Consideration of specific GA metabolic pathways from GA_{12}-7-aldehyde must be speculative until metabolic work has been carried out. However, based on the GAs identified there is some evidence for the early 13-hydroxylation pathway from GA_{12}-7-aldehyde that has been established in seeds of pea (*Pisum sativum*) (Kamiya and Graebe 1983) and that has been adduced for maize (*Zea mays*) (Hedden et al. 1982), broad bean (*Vicia faba*) (Sponsel et al. 1979), spinach (*Spinacia oleracea*) (Metzger and Zeevaart 1980), and corn cockle (*Agrostemma githago*) (Jones and Zeevaart 1980). There is no evidence for any other early hydroxylation pathway suggesting that multiple hydroxylations occur at the C_{19} level. C_{19}-gibberellins hydroxylated at the 2β-, 3β-, 12β, 13-, and 18-positions were detected in developing barley grain, for which a hypothetical metabolic grid shown in Fig. 2 has been constructed. It is noteworthy that GA_9 and 12β-hydroxy-GA_4 were not detected in this study. Nevertheless, preliminary metabolic studies in this laboratory have shown that GA_9 is converted to 12β-hydroxy-GA_9, and GA_4 is converted to 12β-hydroxy-GA_4, in developing grain.

1β-Hydroxylated C_{19}-GAs, characteristic of developing wheat grain (Gaskin et al. 1980), were not detected in developing barley grain.

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