Plant Physiol. (1988) 86, 857–862
0032-0889/88/86/0857/06/$01.00/0

Endogenous Gibberellins from Sporophytes of Two Tree Ferns, *Cibotium glaucum* and *Dicksonia antarctica*[1]

Received for publication June 16, 1987 and in revised form November 2, 1987

HISAKAZU YAMANE, SHOZO FUJIOKA, CLIVE R. SPRAY, BERNARD O. PHINNEY*, JAKE MACMILLAN, PAUL GASKIN, AND NOBUTAKA TAKAHASHI
Department of Biology, University of California, Los Angeles, California 90024 (H.Y., S.F., C.R.S., B.O.P.); *School of Chemistry, University of Bristol, Bristol BS8 ITS, United Kingdom* (J.MacM., P.G.); *and Department of Agricultural Chemistry, The University of Tokyo, Bunkyo-ku, Tokyo 113, Japan* (N.T.)

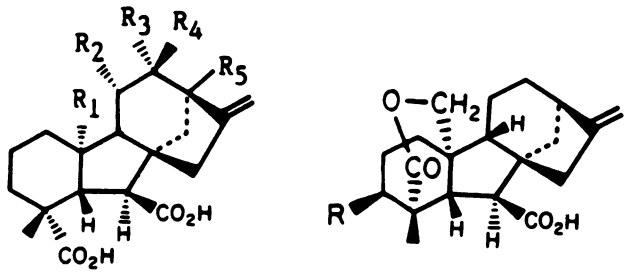
ABSTRACT

Gibberellin A_1 (GA_1), 3-epi-GA_1, GA_4, GA_9, 11α-hydroxyGA_{12}, 12α-hydroxyGA_{12}, GA_{15}, GA_{17}, GA_{19}, GA_{20}, GA_{25}, GA_{37}, GA_{40}, GA_{58}, GA_{69}, GA_{70}, and GA_{71} have been identified from Kovats retention indices and full scan mass spectra by capillary GC-MS analyses of purified extracts from sporophytes of the tree fern, *Cibotium glaucum*. Abscisic acid, dihydrophaseic acid, an epimer of 4′-dihydrophaseic acid, and the epimeric *ent*-6α,7α,16α,17-$(OH)_4$ and *ent*-6α,7α,16β,17-$(OH)_4$ derivatives of *ent*-16,17-dihydrokaurenoic acid, in addition to the epimeric 16α,17- and 16β,17-dihydroxy-16,17-dihydro derivatives of GA_{12}, were also identified in extracts of *C. glaucum*. An oxodihydrophaseic acid and a hydroxydihydrophaseic acid were also detected. In extracts of sporophytes of *Dicksonia antarctica*, GA_4,GA_9, 12α- and 12β-hydroxyGA_{12}, GA_{15}, GA_{25}, and GA_{37} were identified by the same criteria, as well as abscisic acid, phaseic acid, 8′-hydroxymethylabscisic acid and dihydrophaseic acid. This is the first time that GA_{40} has been identified in a higher plant; it is also the first report of the natural occurrence of the two gibberellins, 11α- and 12β-hydroxyGA_{12}. The total gibberellin (GA) content in *C. glaucum* (tall) was at least one order of magnitude greater than that of *D. antarctica* (dwarf) based on total ion current response in GC-MS and bioassay data. Abscisic acid was a major component of *D.antarctica* and the oxodihydrophaseic acid was a major component of *C. glaucum*.

In a previous paper (12) we reported the identification of 10 GAs,[2] including three new GAs (GA_{69}, GA_{70}, and GA_{71}) from young leaves (croziers) of the tree fern, *Cyathea australis*, family Cyatheaceae. We report here on the identification of endogenous GAs (Fig. 1) from croziers of two species of tree ferns, *Cibotium glaucum* (tall) and *Dicksonia antarctica* (dwarf), family Dicksoniaceae.

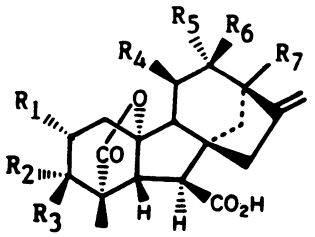
MATERIALS AND METHODS

Plant Material. Croziers of *C. glaucum* and *D. antarctica* were



	R_1	R_2	R_3	R_4	R_5
11α-OHGA_{12}	CH_3	OH	H	H	H
12α-OHGA_{12}	CH_3	H	OH	H	H
12β-OHGA_{12}	CH_3	H	H	OH	H
GA_{17}	CO_2H	H	H	H	OH
GA_{19}	CHO	H	H	H	OH
GA_{24}	CHO	H	H	H	H
GA_{25}	CO_2H	H	H	H	H
12α-OHGA_{25}	CO_2H	H	OH	H	H

	R
GA_{15}	H
GA_{37}	OH



	R_1	R_2	R_3	R_4	R_5	R_6	R_7
GA_1	H	H	OH	H	H	H	OH
3-epi-GA_1	H	OH	H	H	H	H	OH
GA_4	H	H	OH	H	H	H	H
GA_9	H	H	H	H	H	H	H
GA_{20}	H	H	H	H	H	H	OH
GA_{35}	H	H	OH	OH	H	H	H
GA_{40}	OH	H	H	H	H	H	H
GA_{58}	H	H	OH	H	OH	H	H
GA_{69}	H	H	H	H	H	OH	H
GA_{70}	H	H	H	H	OH	H	H
GA_{71}	H	H	OH	H	H	OH	H

FIG. 1. The structures of the gibberellins identified in the three species of tree fern discussed in this paper.

[1] Financial support from the following institutions is gratefully acknowledged: the National Science Foundation, Grant DMB85-06998 (B.O.P.) and the Agricultural and Food Research Council (J. MacM.).

[2] Abbreviations: GA, gibberellin; MeOH, methanol; EtOAc, ethyl acetate; AEA, acidic ethyl acetate; HOAc, acetic acid; RP-HPLC, reverse phase-HPLC; f.w., fresh weight; Rt, retention time; MeTMSi, methyl ester trimethylsilyl ether; Me, methyl ester; KRI, Kovats Retention Index.

collected from cultivated plants on the campus of the University of California, Los Angeles. They were weighed and immediately frozen and stored at −20°C until extraction. The material used in fractionation procedure A was collected in March 1985; the material used in fractionation procedure B was collected in February 1987.

Fractionation Procedure A. *Extraction and Solvent Partitioning.* The frozen croziers (1.0 kg f.w.) of *C. glaucum* were homogenized and extracted three times with 100% MeOH (1.5 L). The MeOH was evaporated *in vacuo* at 30°C and the aqueous residue was partitioned twice against *n*-hexane (0.5 L) at pH 5. The aqueous layer was then adjusted to pH 3 with 6 N HCl and extracted three times with EtOAc (0.7 L). The combined EtOAc extracts were partitioned three times against 5% (w/v) aqueous $NaHCO_3$ (0.7 L). The combined $NaHCO_3$ extracts were adjusted to pH 3 and extracted three times with EtOAc (0.7 L). The combined EtOAc extracts were dried over anhydrous Na_2SO_4, filtered and evaporated to give the acidic EtOAc-soluble (AEA) fraction (1.18 g).

The frozen croziers of *D. antarctica* (2.4 kg f.w.) were extracted and fractionated as for *C. glaucum* to give the AEA fraction (24.2 g). The AEA fraction, dissolved in EtOAc (50 ml), was left at 4°C for 24 h and then filtered from a white precipitate, which was washed twice with EtOAc (20 ml). The filtrate and washings were combined and evaporated to dryness (20.2 g).

Silicic Acid Partition Chromatography (10). The AEA fraction (1.18 g) from *C. glaucum* was adsorbed on Celite (3 g) and placed on a column of silica gel (20 g; Fisher Scientific Co., 100–200 mesh) impregnated with 0.5 M aqueous formic acid (12.5 ml). The column was eluted stepwise, with 60 ml portions of *n*-hexane-EtOAc mixtures (v/v), saturated with 0.5 M aqueous formic acid, as follows: Fractions 1 to 3 (0–20% EtOAc in 10% steps); fractions 4 to 13 (25–70% EtOAc in 5% steps); and fractions 14 to 16 (80–100% EtOAc in 10% steps). Following bioassay, fractions 3, 4, 5, 6, 10, 11, and 12 were recombined (219 mg) for DEAE-Sephadex chromatography.

Half (10.1 g) of the AEA fraction, from which the white precipitate had been removed, from *D. antarctica*, was chromatographed by a similar procedure to that used for *C. glaucum*, except that the scale was four times greater and the column was eluted in 200 ml fractions of increasing EtOAc in *n*-hexane. Following bioassay, the fractions eluting with 0 to 65% EtOAc in *n*-hexane were recombined (1.57 g) for DEAE-Sephadex chromatography.

DEAE-Sephadex Chromatography (5). The recombined fractions from *C. glaucum* (219 mg) were chromatographed on a column of DEAE-Sephadex A-25 (10 ml), eluted with 30 ml portions of the following solvents: MeOH, 0.25 N HOAc in MeOH; 0.5 N HOAc in MeOH; 0.75 N HOAc in MeOH; 1 N HOAc in MeOH; 3 N HOAc in MeOH; and 10 N HOAc in MeOH. Based on bioassay results, all the fractions, except that eluting with 10 N HOAc in MeOH, were recombined for RP-HPLC.

The recombined fractions from silicic acid partition chromatography of the *D. antarctica* extract (1.57 g) were subjected to DEAE-Sephadex chromatography as described above except that the scale was three times greater and that the column was eluted with 90 ml fractions. The fractions eluting with MeOH to 1 N HOAc in MeOH (1.22 g) were recombined and subjected to a second silicic acid partition column, as described above, using silica gel (24 g) impregnated with 0.5 M aqueous formic acid (15 ml). The column was eluted with 100 ml portions of the solvents, as described above. The fractions eluting from 20 to 45% EtOAc in *n*-hexane were recombined as one fraction for RP-HPLC.

Sep-Pak Cartridge Treatment. Each sample for Sep-Pak treatment was dissolved in MeOH:H_2O (v/v; 4:1; 3 ml) and loaded onto a Sep-Pak C_{18} cartridge (Waters Assoc.). The cartridge was eluted twice with MeOH:H_2O (v/v; 4:1; 3 ml). The eluates were combined and evaporated to dryness.

Reverse Phase-HPLC (RP-HPLC). Each fraction for RP-HPLC was dissolved in 1% (v/v) aqueous HOAc:MeOH (v/v; 7:3; 0.5 ml) and injected onto a 10 μm C_{18} column (25 cm × 10 mm i.d.; Alltech Assoc.). A linear gradient from 30 to 100% MeOH in 1% aqueous HOAc was applied over 28 min, starting 2 min after injection. The solvent flow rate was 3 ml/min. Following injection, fractions were collected every minute.

Bioassay. Each fraction was assayed using the dwarf rice (*Oryza sativa* L. cv Tan-ginbozu) micro-drop method (9). The fractions from silicic acid partition chromatography were assayed at dosages equivalent to 10 g f.w. of *C. glaucum* and 12 g f.w. of *D. antarctica*. After RP-HPLC the fractions were assayed at dosages equivalent to 18 g f.w. of *C. glaucum* and 20 g f.w. of *D. antarctica*.

Fractionation Procedure B. *Extraction and Solvent Partitioning.* The frozen croziers of *C. glaucum* (1.0 kg f.w.) and of *D. antarctica* (0.75 kg f.w.) were extracted twice with MeOH:H_2O (v/v; 4:1, about 10 L/kg f.w.). The MeOH was evaporated *in vacuo* at 35°C and the aqueous residue was adjusted to pH 7 and stirred with PVP (about 50 g/L) overnight. The PVP was then removed by filtration and the residue was washed with water at pH 8. The filtrate and washings were combined and subjected to the usual solvent partitioning (as described in Procedure A) to give the AEA fraction (*C. glaucum*, 1.026 g: *D. antarctica*, 8.095 g).

PVP Column Chromatography. The AEA fraction was dissolved in a small amount of phosphate buffer (0.1 M; pH 8) and placed on a column of PVP (column volumes, 70 ml for *C. glaucum*; 210 ml for *D. antarctica*). The column was eluted with phosphate buffer (0.1 M; pH 8) and the effluent in the range of Ve(elution volume)/Vt(total volume), 0.6 to 3.0 was collected. The effluent was adjusted to pH 3 and extracted three times with EtOAc. The combined EtOAc fractions were evaporated to dryness (*C. glaucum*, 0.621 g: *D. antarctica*, 6.008 g).

DEAE-Sephadex Chromatography (5). The material from the PVP column was dissolved in a small amount of MeOH and loaded onto a column of DEAE-Sephadex A-25. The column (volume; 100 ml) was eluted with 100 ml portions of the following solvents: MeOH, 0.25 N HOAc in MeOH, 0.5 N HOAc in MeOH, 0.75 N HOAc in MeOH, 1 N HOAc in MeOH, 3 N HOAc in MeOH. Based on preliminary experiments, the fractions eluting from 0.5 to 3 N HOAc in MeOH were combined and concentrated as the free GA fraction (*C. glaucum*, 53.3 mg: *D. antarctica*, 2.913 g).

Charcoal Adsorption Chromatography. The DEAE-Sephadex fraction from the *D. antarctica* extract was dissolved in a small amount of acetone:H_2O (v/v; 1:4) and loaded onto a column of charcoal (volume; 60 ml). The column was eluted with acetone:H_2O (v/v; 1:4, 300 ml) followed by acetone:H_2O (v/v; 9:1, 450 ml). The acetone:H_2O (v/v; 9:1) fraction was collected and evaporated to dryness (537.1 mg) and then repurified by DEAE-Sephadex A-25 column chromatography (column volume; 30 ml) as described above.

Sep-Pak Cartridge Treatment. The DEAE-Sephadex fraction from *C. glaucum* was passed through a Sep-Pak cartridge as described in Fractionation Procedure A.

HPLC. The fraction from Sep-Pak cartridge treatment for *C. glaucum* and the fraction from the second DEAE-Sephadex A-25 column for *D. antarctica* were each, separately, dissolved in 0.1% (v/v) HOAc in MeOH and injected onto a Nucleosil 5 $N(CH_3)_2$ column (10 cm × 10 mm i.d.). In each case the column was eluted isocratically with 0.1% (v/v) HOAc in MeOH, flow rate 3 ml/min. This purification was repeated. In the first HPLC the effluent was collected in the Rt range 5 to 60 min. The total eluate in this Rt range was concentrated and then subjected to

a second HPLC. Fractions were collected every 3 min from Rt: 5 to 35 min (10 fractions) with a final fraction (Rt: 35–50 min).

GC-MS. GC-MS analyses were performed using a computerized VG 7050 mass spectrometer (VG Analytical, Manchester, U.K.) and a DANI 3800 GC (Kontron Instruments, St. Albans, U.K.) fitted with a WCOT vitreous silica column (25 m × 0.2 mm i.d.) coated with a 0.25 μm layer of bonded OV-1. Each sample for GC-MS was methylated with ethereal diazomethane and trimethylsilylated with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide. The conditions of analysis were the same as those previously described (3).

RESULTS

Extraction A. The AEA fraction from a MeOH extract of croziers of *C. glaucum* was subjected to silicic acid partition chromatography (10). The fractions were bioassayed using the dwarf rice cv Tan-ginbozu microdrop method (9). Fractions 3, 4, 10, 11, and 12, which had GA-like activity, and fractions 5 and 6, which showed inhibition, were combined and chromatographed on a DEAE-Sephadex A-25 column. Fractions eluting with 0 to 3 N HOAc in MeOH were combined and the material which eluted from a Sep-Pak cartridge with MeOH:H_2O (v/v; 4:1) was fractionated by RP-HPLC. The resulting fractions were bioassayed using the dwarf rice cv Tan-ginbozu microdrop method (Fig. 2). Fractions which exhibited GA-like activity, and also fractions which were inhibitory, were derivatized and analysed by capillary GC-MS (Table I). (The fractions from *D. antarctica* with Rt less than 18 min were examined by GC and found to be too dirty for further analysis.)

Gibberellin A_4, 11α-hydroxyGA_{12}, 12α-hydroxyGA_{12}, GA_{20}, GA_{37}, GA_{58}, and GA_{71} were identified by comparison of the Kovats retention indices (7) and mass spectra of their MeTMSi derivatives with those of authentic samples. Gibberellin A_9, GA_{15}, GA_{25}, and possibly GA_{25}-7-aldehyde, were similarly identified as their Me esters. Other compounds identified by GC-MS are shown in Table II. They include ABA and several derivatives of ABA, and the epimeric 16,17-dihydrodiols of *ent*-kaurenoic acid and GA_{12}.

The AEA fraction from a MeOH extract of croziers of *D. antarctica* was almost 9 times greater in weight than the corresponding fraction from *C. glaucum*, on a f.w. basis. Removal of a white EtOAc-insoluble solid, characterization of which will be reported elsewhere, reduced the mass of the acidic fraction which was subjected to silicic acid partition chromatography. No GA-like bioactive fractions were obtained even after re-chromatography of fractions 1 to 12, which should contain bioactive GAs. The inhibitory fractions 3, 4, 5, 7, and 9, in which GA-like activity may have been masked, were combined, passed through a Sep-Pak column and the material which was eluted with MeOH:H_2O (v/v; 4:1) was fractionated by RP-HPLC. The bioactive and bioinhibitory fractions were derivatized and analysed by capillary GC-MS (Table I). Gibberellin A_4 was identified by comparison of the Kovats retention index and full scan mass spectrum of its MeTMSi derivative with those of an authentic sample. Gibberellin A_9, GA_{15}, and GA_{25} were similarly identified as their Me esters. ABA and DPA (dihydrophaseic acid) were also identified (Table II).

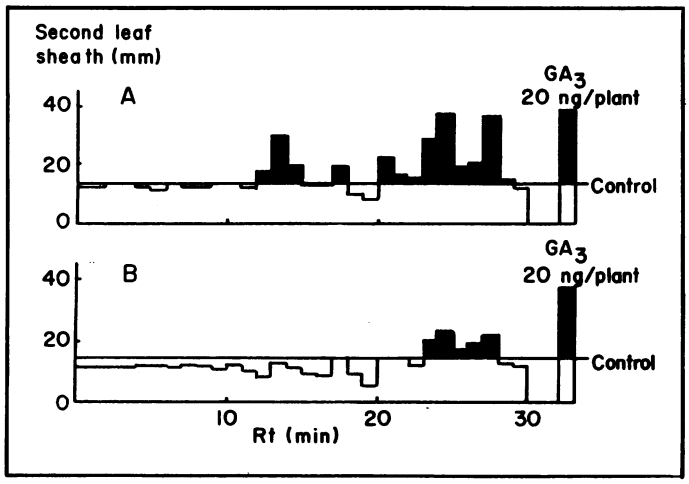


FIG. 2. Biological activity of the fractions from RP-HPLC (fractionation procedure A) of extracts from *C. glaucum* (A) and *D. antarctica* (B). Values for lengths of the second leaf sheath represent the average of six plants (dwarf rice cv Tan-ginbozu).

Extraction B. In view of the large mass of the AEA fraction from the sporophytes of *D. antarctica*, containing low levels of GAs and high levels of inhibitors, an alternative purification procedure was devised. This procedure was applied to repeat extracts of sporophytes of both *D. antarctica* and *C. glaucum*. It differed from procedure A in three respects. First, the crude AEA fraction was treated with PVP to reduce the levels of phenolic components. Second, to reduce the total mass of the *D. antarctica* extract, an additional step of charcoal adsorption chromatography was inserted between repeat DEAE-Sephadex chromatography. Third, HPLC on a Nucleosil $N(CH_3)_2$ column replaced RP-HPLC on a C_{18} column, in order to change the elution pattern of the GAs and phenols. None of these steps were monitored by bioassay. The fractions from HPLC on the Nucleosil $N(CH_3)_2$ column were derivatized and analysed by capillary GC-MS.

The GAs, identified by Kovats retention indices and full scan mass spectra, are shown in Table III. Gibberellin A_1 was present in very low amount in the fraction from *C. glaucum* eluting from 11 to 14 min. The identifications of 12α-hydroxyGA_{25} and GA_{25}-7-aldehyde from *C. glaucum* are only tentative since no authentic samples were available for comparison. The compounds listed in Table II were also identified from this extraction.

DISCUSSION

Nine GAs (GA_4, GA_9, 11α-hydroxyGA_{12}, 12α-hydroxyGA_{12}, GA_{15}, GA_{20}, GA_{25}, GA_{37}, and GA_{71}) of the 16 found to occur in sporophytes of *C. glaucum*, were identified using both the two adopted fractionation procedures A and B. However, one GA, GA_{58}, was identified in procedure A only, and six (GA_1, GA_{17}, GA_{19}, GA_{40}, GA_{69}, and GA_{70}) in procedure B only. In the case of *D. antarctica*, 7 gibberellins were identified. Only GA_4 was identified by both fractionation procedures; three (GA_9, GA_{15}, and GA_{25}) by procedure A, only; and three (12α-hydroxyGA_{12}, 12β-hydroxyGA_{12}, and GA_{37}) by procedure B, only.

There may be three reasons for the difference in the range of GAs detected in the two fractionation procedures. First, there could have been variation in the stage of development of the collected material; second, the purification steps were different; third, in extraction procedure A, fractions for GC-MS analysis were selected on the basis of their biological activity, whereas in extraction procedure B, all the fractions from HPLC were analysed by GC-MS. Thus, the results are a salutary warning that only a selection of the total GAs in a plant may be identified by any one fractionation method.

11α-HydroxyGA_{12} and 12β-hydroxyGA_{12} are new GAs, their natural occurrence being reported here for the first time. Both of these GAs were identified by comparison of their Kovats retention indices and mass spectra of their MeTMSi derivatives with those of the corresponding metabolites, obtained by incubation of the 11α- and 12β-hydroxy derivatives of *ent*-kaurenoic acid (4) in cultures of *Gibberella fujikuroi*, mutant B1-41a. Al-

Table I. *GAs Identified by GC-MS as Me or MeTMSi Derivatives in Fractions from RP-HPLC (Purification Procedure A)*

Plant Material	Retention Time	GA Activity/ kg f.w.	GA	KRI	Principal Ions and Relative Abundance
	min	*ng GA_3 equivalent, from bioassay*			*m/z (% base peak)*
C. glaucum	13–14	135	GA_{71}	2683	506 (M^+,44), 416 (98), 384 (95), 356 (100), 282 (77), 223 (63)
			GA_{58}	2727	506 (M^+,37), 416 (97), 384 (95), 356 (100), 282 (88), 223 (82)
	17–18	19	12α-$OHGA_{25}$[a]	2644	492 (M^+,absent), 400 (57), 372 (53), 310 (100), 282 (94), 223 (52)
	18–20	Inhibition	11α-$OHGA_{12}$	2496	448 (M^+,31), 388 (16), 345 (40), 298 (14), 239 (23), 167 (100)
	20–21	28	GA_{20}	2477	418 (M^+,100), 403 (19), 375 (53), 359 (14), 301 (14), 207 (38)
	23–24	107	12α-$OHGA_{12}$	2539	448 (M^+,0.4), 416 (33), 388 (34), 298 (100), 239 (50), 209 (40)
			GA_{37}	2747	432 (M^+,19), 342 (32), 310 (78), 284 (44), 237 (25), 129 (100)
	24–25	931	GA_4	2493	418 (M^+,20), 328 (25), 289 (44), 284 (100), 224 (70), 129 (26)
	25–27	36	GA_{25}	2429	404 (M^+,0.4), 372 (13), 312 (82), 284 (100), 253 (8), 225 (41)
			GA_{25}-7-aldehyde[a]	2447	374 (M^+,4), 342 (21), 314 (100), 284 (21), 254 (12), 225 (34)
	27–28	677	GA_9	2295	330 (M^+,6), 298 (100), 270 (99), 243 (52), 226 (61), 159 (37)
			GA_{15}	2594	344 (M^+,10), 312 (17), 298 (9), 284 (44), 239 (100), 195 (16)
D. antarctica	24–25	43	GA_4	2493	See above
	25–27	25	GA_{25}	2429	See above
	27–28	25	GA_9	2295	See above
			GA_{15}	2594	See above

[a] Tentative identification.

Table II. *Other Compounds, Identified or Tentatively Identified, in Croziers of Cibotium glaucum and Dicksonia antarctica (Purification Procedures A and B)*

Compound	Source	Retention Time Procedure		KRI	Principal Ions and Relative Abundance
		A	B		
		min			*m/z (% base peak)*
ABA	*C. glaucum*	18–20	8–11	2030	278 (M^+,2), 190 (100), 162 (71), 134 (72), 125 (72), 112 (34)
	D. antarctica	20–21	8–11		
			11–14		
PA	*D. antarctica*	—[a]	11–14	2080	294 (M^+,4), 125 (60), 122 (51), 121 (46), 55 (41), 43 (100)
DPA	*C. glaucum*	—	8–11	2151	368 (M^+,1), 159 (60), 125 (37), 121 (41), 117 (31), 43 (100)
	D. antarctica	—	11–14		
ξ-OHDPA	*C. glaucum*	—	8–11	2254	456 (M^+,absent), 336 (4), 276 (14), 159 (53), 125 (48), 43 (100)
8′-$HOCH_2$-ABA[b]	*D. antarctica*	—	11–14	2270	366 (M^+,2), 221 (18), 215 (18), 190 (100), 161 (44), 125 (46)
ξ-oxoDPA	*C. glaucum*	7–18	11–14	2318	382 (M^+,3), 268 (51), 159 (89), 125 (47), 117 (59), 43 (100)
		18–20	14–17		
			17–20		
ent-6α,7α,16α,17-$(OH)_4$-16,17-dihydrokaurenoic acid	*C. glaucum*	18–20	5–8	2953	670 (M^+,0.1), 567 (27), 477 (100), 387 (10), 269 (23), 209 (9)
ent-6α,7α,16β,17-$(OH)_4$-16,17-dihydrokaurenoic acid	*C. glaucum*	18–20	5–8	2978	670 (M^+,0.1), 567 (32), 477 (100), 387 (9), 269 (5), 191 (9)
16β,17-$(OH)_2$-16,17-dihydroGA_{12}	*C. glaucum*	20–21	5–8	2741	538 (M^+,absent), 479 (3), 435 (100), 388 (9), 375 (50), 285 (14)
	D. antarctica	—	8–11		
16α,17-$(OH)_2$-16,17-dihydroGA_{12}	*C. glaucum*	—	5–8	2780	538 (M^+,absent), 479 (3), 435 (100), 388 (9), 375 (50), 285 (14)
	D. antarctica	—	8–11		
ent-7α,16β,17-$(OH)_3$-16,17-dihydrokaurenoic acid	*C. glaucum*	20–21	—	2861	582 (M^+,absent), 479 (71), 402 (19), 389 (100), 329 (28), 239 (32)
ent-6α,7α,17-$(OH)_3$-16β,17-dihydrokaurenoic acid	*C. glaucum*	23–24	—	2841	582 (M^+,absent), 567 (43), 492 (6), 361 (20), 269 (22), 209 (24)
Fujenal triacid	*C. glaucum*	27–28	—	2533	406 (M^+,1), 255 (4), 227 (45), 195 (100), 167 (51), 107 (84)
ent-7α,16β,17-$(OH)_3$-16,17-dihydrokaurenolide	*C. glaucum*	20–21	—	2896	566 (M^+,2), 463 (100), 373 (61), 345 (12), 301 (7), 137 (21)

[a] Not detected. [b] The recently proposed new nomenclature (2) for ABA and its metabolites has been used here.

Table III. *GAs Identified by GC-MS as Me or MeTMSi Derivatives in Fractions from Nucleosil N(CH$_3$)$_2$ HPLC (Purification Procedure B)*

Plant Material	Retention Time	GA	KRI	Principal Ions and Relative Abundance
	min			*m/z (% base peak)*
C. glaucum	5–8	12α-OHGA$_{12}$	2537	See Table I
	8–11	11α-OHGA$_{12}$	2496	See Table I
		GA$_{37}$	2747	See Table I
	11–14	GA$_{25}$	2428	See Table I
		GA$_4$	2493	See Table I
		GA$_{17}$	2566	492 (M$^+$,29), 460 (25), 432 (25), 372 (18), 251 (15), 208 (100)
		GA$_1$ (trace)	2661	506 (M$^+$,100), 491 (9), 448 (18), 377 (12), 313 (9), 207 (22)
		GA$_{15}$	2594	See Table I
	14–17	GA$_9$	2295	See Table I
		12α-OHGA$_{25}$[a]	2644	See Table I
		GA$_{71}$	2683	See Table I
	17–20	GA$_9$	2295	See Table I
		GA$_{20}$	2477	See Table I
		GA$_{25}$-7-aldehyde[a]	2447	See Table I
		GA$_{40}$	2520	418 (M$^+$,absent), 371 (100), 343 (83), 299 (81), 284 (80), 225 (58)
		GA$_{69}$	2489	418 (M$^+$,17), 328 (37), 296 (93), 282 (44), 268 (100), 223 (64)
		GA$_{70}$	2542	418 (M$^+$,16), 328 (36), 296 (91), 282 (45), 268 (100), 223 (67)
	20–23	GA$_{69}$	2489	See above
		3-epi-GA$_1$	2781	506 (M$^+$,100), 491 (11), 459 (10), 448 (26), 376 (24), 207 (44)
	26–29	GA$_{25}$	2430	See above
		GA$_{19}$	2590	462 (M$^+$,7), 434 (100), 402 (37), 375 (47), 239 (32), 208 (31)
	35–50	GA$_{25}$	2430	See above
D. antarctica	8–11	12α-OHGA$_{12}$	2539	See Table I
		12β-OHGA$_{12}$	2553	448 (M$^+$,0.9), 416 (39), 388 (40), 298 (100), 239 (42), 209 (48)
		GA$_{37}$	2747	See Table I
		GA$_4$	2493	See Table I

[a] Tentative identification.

though the structures of 11α, 12α-, and 12β-hydroxyGA$_{12}$ identified in the present study are in no doubt, the allocation of GA numbers (8) to them must await the final confirmation of these structures either by rational synthesis or by the isolation of the pure GA from a natural source in amounts which permit the determination of a range of physical and chemical properties. The tentative identifications of 12α-hydroxyGA$_{25}$, also tentatively identified in seeds of *Cucurbita maxima* (1), and of GA$_{25}$-7-aldehyde are based solely on the interpretation of the mass spectral fragmentation of the MeTMSi derivatives.

The identification of GA$_{40}$ is the first report of the natural occurrence of a 2α-hydroxylated gibberellin in a higher plant. Previously, the three known 2α-hydroxylated gibberellins (GA$_{40}$, GA$_{47}$, and GA$_{56}$) have been identified only in cultures of the fungus, *G. fujikuroi*.

The dwarf tree fern, *D. antarctica*, contains about one-twentieth of the total GA-like bioactivity of the tall tree fern, *C. glaucum* (Fig. 2). The total ion currents, observed in the GC-MS analyses of the detected GAs, were also less for *D. antarctica* than for *C. glaucum*. Also, fewer GAs were detected in extracts of *D. antarctica*, but this may be a consequence of the high levels of inhibitory compounds present in the final HPLC fractions, even after pretreatment with PVP. These inhibitory substances included ABA, a major component, and several unidentified phenols. The level of phenolics and of ABA were much less in extracts of *C. glaucum*, in which an oxo-derivative of DPA was a major component.

The diversity of hydroxylation patterns of both the C$_{20}$- and C$_{19}$-GAs identified in this study and the previous one (12) on *Cyathea australis* is analysed in Table IV. For the C$_{20}$-GAs there are examples of nonhydroxylation and of 3β-, 11α-, 12α-, 12β-, and 13-monohydroxylation. In the C$_{19}$-GAs there are examples of nonhydroxylation, of 2α-, 3β-, 12α-, 12β-, and 13-monohydroxylation and of 3β,11β-, 3β,12α-, 3β,12β-, and 3β,13-dihydroxylation. The hydroxylation patterns suggest the presence of several pathways from GA$_{12}$-aldehyde to the C$_{19}$-GAs in sporophytes of tree ferns. However, on the basis of the identified GAs and their appropriate relative levels, the pathway shown in our previous paper (12) is probably the major one. Nevertheless

Table IV. *Hydroxylation Patterns in Native GAs of Cibotium glaucum, Dicksonia antarctica, and Cyathea australis*

Position of Hydroxylation	*C. glaucum*	*D. antarctica*	*C. australis* (12)
C$_{20}$-GAs			
None	GA$_{15}$, GA$_{25}$	GA$_{15}$, GA$_{25}$,	GA$_{15}$, GA$_{24}$
3β	GA$_{37}$	GA$_{37}$	—[a]
11α	11α-OHGA$_{12}$	—	—
12α	12α-OHGA$_{12}$ 12α-OHGA$_{25}$[b]	12α-OHGA$_{12}$	—
12β	—	12β-OHGA$_{12}$	—
13	GA$_{17}$, GA$_{19}$	—	—
G$_{19}$-GAs			
None	GA$_9$	GA$_9$	GA$_9$
2α	GA$_{40}$	—	—
3β	GA$_4$	GA$_4$	GA$_4$
12α	GA$_{70}$	—	GA$_{70}$
12β	GA$_{69}$	—	GA$_{69}$
13	GA$_{20}$	—	—
3β,11β	—	—	GA$_{35}$
3β,12α	GA$_{58}$	—	GA$_{58}$
3β,12β	GA$_{71}$	—	GA$_{71}$
3α,13	3-epi-GA$_1$	—	—
3β,13	GA$_1$	—	GA$_1$

[a] Not detected. [b] Tentative identification.

it is possible that GA_4 is formed from GA_9. Gibberellin A_9 and GA_4 co-occur in all three tree ferns examined by us and the conversion of GA_9 to GA_4 has been observed in cell-free enzyme preparations from seeds of *C. maxima* (6) and *Phaseolus vulgaris* (11). In the case of *C. maxima*, however, GA_9 has not been shown to occur naturally.

LITERATURE CITED

1. BLECHSCHMIDT S, U CASTEL, P GASKIN, P HEDDEN, JE GRAEBE, J MACMILLAN 1984 GC/MS analysis of the plant hormones in seeds of *Cucurbita maxima*. Phytochemistry 23: 553–558
2. BOYER GL, BV MILBORROW, PF WAREING, JAD ZEEVAART 1985 The nomenclature of abscisic acid and its metabolites. *In* M Bopp, ed, Plant Growth Substances 1985. Springer-Verlag, Berlin, pp 99–100
3. GASKIN P, SJ GILMOUR, JR LENTON, J MACMILLAN, VM SPONSEL 1984 Endogenous gibberellins and kauranoids identified from developing and germinating barley grain. J Plant Growth Regul 2: 229–242
4. GASKIN P, M HUTCHISON, N LEWIS, J MACMILLAN, BO PHINNEY 1984 Microbiological conversion of 12-oxygenated and other derivatives of *ent*-kaur-16-en-19-oic acid by *Gibberella fujikuroi*, mutant B1-41a. Phytochemistry 23: 559–564
5. GRÄBNER R, G SCHNEIDER, G SEMBDNER 1976 Gibberelline XLIII. Mitt. Fraktionierung von Gibberellinen, Gibberellinkonjugaten und anderen Phytohormonen durch DEAE-Sephadex-Chromatographie. J Chromatogr 121: 110–115
6. GRAEBE JE, P HEDDEN, W RADEMACHER 1980 Gibberellin biosynthesis. *In* JR Lenton, ed. Gibberellins—Chemistry, Physiology and Use, Monograph 5. British Plant Growth Regulator Group, Wantage, pp 31–37
7. KOVATS E 1958 Gas-chromatographische Charakterisierung organischer Verbindungen Teil 1: Retentions indices aliphatischer halogenide, alkohole, aldehyde und ketone. Helv Chim Acta 41: 1915–1932
8. MACMILLAN J, N TAKAHASHI 1968 Proposed procedure for the allocation of trivial names to the gibberellins. Nature 217: 170–171
9. MURAKAMI Y 1968 A new rice seedling test for gibberellins, 'microdrop method,' and its use for testing extracts of rice and morning glory. Bot Mag 81: 33–43
10. POWELL LE, KJ TAUTVYDAS 1967 Chromatography of gibberellins on silica gel partition columns. Nature 213: 292–293
11. TAKAHASHI M, Y KAMIYA, N TAKAHASHI, JE GRAEBE 1986 Metabolism of gibberellins in a cell-free system from immature seeds of *Phaseolus vulgaris* L. Planta 168: 190–199
12. YAMANE H, I YAMAGUCHI, M KOBAYASHI, M TAKAHASHI, Y SATO, N TAKAHASHI, K IWATSUKI, BO PHINNEY, CR SPRAY, P GASKIN, J MACMILLAN 1985 Identification of ten gibberellins from sporophytes of the tree fern, *Cyathea australis*. Plant Physiol 78: 899–903