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GC-MS Identification of GA_{20}-13-*O*-Glucoside Formed from GA_{20} in Normal Plants and Dwarf-1 Mutants of *Zea mays* L.

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Abstract. After feeding GA_{20} to excised seedlings of *Zea mays* L. normals (N) and dwarf-1 mutants (d1), GA_{20}-13-*O*-glucoside (*9*) was identified by HPLC and by GC-MS of its permethylated derivative. The glucosylation rate of GA_{20} was found to be higher in the dwarf-1 mutant (26%) than in the normal plant (3.6%). This article includes a GC-MS study in which diagnostic fragments from the spectra of permethylated synthetic GA glucosides have been selected that proved to be useful for the identification of permethylated GA glucosides.

In *Zea mays*, GA_{20} is the immediate precursor of the active gibberellin, GA_1 (Phinney 1984). Thus the metabolism of GA_{20} is expected to be precisely controlled in the plant. Apart from conversion into GA_1, two possibilities exist for the metabolism of GA_{20} (see Fig. 1): (1) 2β-hydroxylation, which results in GA_{29}, and (2) conjugation, leading to, e.g., glucosyl derivatives.

In the dwarf-1 mutant (d1) of *Zea mays*, where the conversion of GA_{20} into GA_1 is blocked (Phinney 1984), GA_{29} and the GA_{20} conjugates are expected to be primary metabolites (Fig. 1). The 2β-hydroxylation step in the pathway has been proved to occur (Heupel et al. 1985). However, GA_{20} conjugates, e.g., GA_{20} glucosyl conjugates, have not yet been identified unequivocally in *Zea mays*, although some investigations on presumptive GA_{20} conjugates have been reported (Rood et al. 1983a,b).

The aim of our experiments is to gain evidence for the metabolic transformation of GA_{20} into GA_{20}-13-*O*-glucoside (*9*) in both the normal plant and the dwarf-1 mutant of *Zea mays* L.

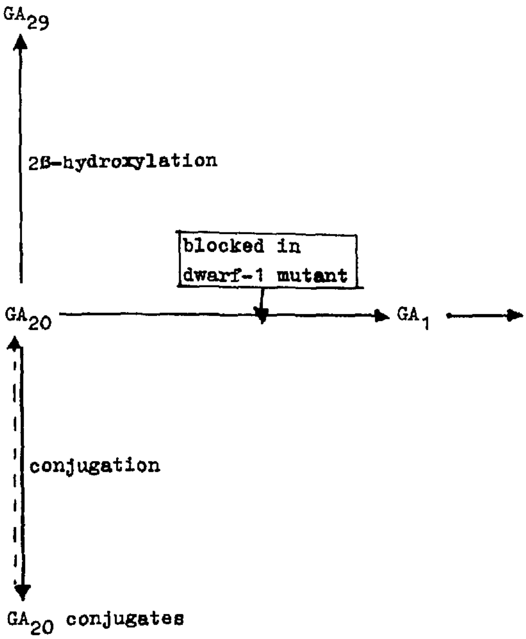


Fig. 1. Presumptive metabolic pathway of GA_{20} in *Zea mays* L.

Materials and Methods

Plant Material and Incubation Conditions

Caryopses of *Zea mays* L. normal (N) and *Zea mays* L. dwarf-1 mutant (d1) were germinated and grown for 7 days at 25°C. Excised shoots of each were incubated with 5.6 mg GA_{20} (Beale et al. 1980) dissolved in 2 ml of an ethanol-water (15:85) mixture for 48 h at room temperature.

Extraction and Purification

The incubated shoots of *Zea mays* N (fresh weight; 16 g) and d1 (fresh weight; 10.5 g) were macerated in 150 and 100 ml, respectively, 80% MeOH. After 4 h at 4°C the extracts were filtered and the residue reextracted with 100 ml MeOH for 12 h. The pooled first and second extracts were treated with 1 g PVP overnight and each spiked with 4.17×10^4 Bq (0.5 μg) of $[^3H]GA_{20}$-13-*O*-glucoside (*9*). After the evaporation of the MeOH, the aqueous residue was purified by extraction with *n*-hexane and subsequently taken to dryness (N, 640 mg; d1, 360 mg). The extracts were adsorbed onto Celite, subjected to a 25 ml DEAE-Sephadex A-25 column (Gräbner et al. 1976) and eluted with 25 ml portions of MeOH, 0.25 N HOAc/MeOH, 0.5 N HOAc/MeOH, 0.75 N HOAc/MeOH, 1 N HOAc/MeOH, 2 N HOAc/MeOH, 4 N HOAc/MeOH, and 8 N HOAc/MeOH. Fractions 6–8 (2 N HOAc to 8 N HOAc), which contained the marker activity, were pooled (N, 52 mg; d1, 28 mg) and rechromatographed on 18g silica

Table 1. Retention times [t_R (min)] and number of theoretical plates/m (N) of permethylated GA glucosides on SP-2100 and QF-1.

Permethylated samples	Formula no.	SP-2100		QF-1	
		t_R	N	t_R	N
GA_1-3-*O*-glucoside	(*1*)	48.6	2690	14.2	1650
GA_1-13-*O*-glucoside	(*2*)	41.1	2710	11.9	1870
GA_4-3-*O*-glucoside	(*5*)	35.1	3420	9.7	1440
GA_5-13-*O*-glucoside	(*6*)	31.5	2760	10.3	2100
GA_8-2-*O*-glucoside	(*7*)	61.8	2350	16.9	1540
GA_8-13-*O*-glucoside	(*8*)	53.7	2620	13.5	1560
GA_{20}-13-*O*-glucoside	(*9*)	31.5	3140	9.9	1960

For experimental details see Materials and Methods.

(Woelm, 100–200 mesh) with chloroform:MeOH:HOAc in the following ratios 50:0:0, 45:5:0, 40:10:0, 40:10:0.5, 40:10:1, and 40:10:2 (v:v:v, 10 ml fractions). The radioactivity was recovered from fractions 17–21 (N, 8 mg; d1, 8 mg). The samples were then purified by preparative HPLC (for experimental details see Schneider et al. 1984) on an RP 18 column (4.6 × 250 mm) eluted with MeOH:0.2% HOAc (40:60) (1 ml/min). The GA_{20}-glucoside fraction (t_R = 12–14 min) was collected and taken to dryness.

HPLC Quantitation

Analyses were carried out with a Milichrom OB 4 (Academy of Sciences of the USSR, Novosibirsk) (Baram et al. 1983) fitted with a Silica RP 18 (5 μm) column (2 × 62 mm), detector set on 206 nm, eluent: MeOH:0.1%H_3PO_4 (50:50, 100 μl/min). Synthetic GA_{20}-13-*O*-glucoside (*9*) (Schneider et al. 1984) was used as a standard for calibration (t_R = 5.1 min).

The content of the HPLC purified GA_{20}-glucoside fractions from the feeding was calculated on the basis of the peak area and the recovered radioactivity (Table 3). For conditions for radiocounting see Lattke and Schneider (1985).

Gas Chromatography and GC-MS

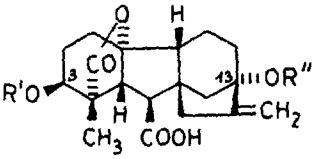
Gibberellin glucoside samples (Schneider 1981) were permethylated according to Rivier et al. (1981). The ethyl acetate extracts were subjected to preparative TLC (Silica 60 Merck) with toluene:ethyl acetate:HOAc [60:40:5 (v:v:v)]. Zones between R_F 0.60 and 0.70 were scraped off and eluted with ethyl acetate. The dried extracts were dissolved in MeOH and subjected to GC or GC-MS.

GC analyses were performed on an HP 5700 equipped with a glass column (180 cm × 2 mm i.d.) containing 3% SP-2100 on Supelcoport (100–120 mesh) or 3% QF-1 on GasChrom (80–100 mesh), carrier gas nitrogen, flow rate 50 ml/min, 250° isothermal, FID detection. The GC-MS measurements were car-

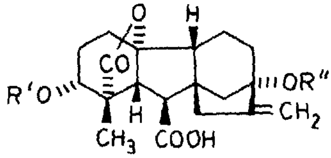
Table 2. Diagnostic ions [m/z (%)] of permethylated gibberellin glucosides.

Permethylated sample		M	M^+	M-175^+	M-235^+	M-267^+	M-279^+
GA_1-3-*O*-glucoside[a]	*(1)*	594	594 (1.3)	—	359 (4.6)	—	315 (11)
GA_4-3-*O*-glucoside[a]	*(5)*	564	—	—	329 (5.8)	—	285 (9.2)
GA_8-2-*O*-glucoside[b]	*(7)*	624	624 (0.5)	449 (4.1)	389 (4.5)	—	345 (4.2)
GA_1-13-*O*-glucoside[b]	*(2)*	594	—	419 (2.4)	359 (15)	327 (3.1)	—
GA_5-13-*O*-glucoside[b]	*(6)*	562	—	387 (6.2)	327 (22)	295 (5.4)	—
GA_8-13-*O*-glucoside[b]	*(8)*	624	—	449 (2.3)	389 (16)	357 (4.2)	—
GA_{20}-13-*O*-glucoside[b]	*(9)*	564	—	389 (3.0)	329 (15)	297 (3.5)	—
[$^2H_2$]GA_{20}-13-*O*-glucoside[b]	*(10)*	566	—	391 (3.6)	331 (13)	299 (3.6)	—
3-epi-GA_1-3-*O*-glucoside[b]	*(3)*	594	594 (1.3)	419 (3.6)	359 (9.4)	—	315 (15)
3-epi-GA_1-13-*O*-glucoside[b]	*(4)*	594	—	419 (5.6)	359 (25)	327 (11)	315 (2.7)
GA_{20}-13-*O*-glucoside from the feeds[b]		564	—	389 (4.5)	329 (17)	297 (4.9)	—

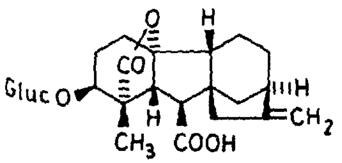
[a] Base peak: m/z *101 (100%)*.
[b] Base peak: m/z 88 (100%).



1: R'=Gluc; R''=H
2: R'=H; R''=Gluc



3: R'=Gluc; R''=H
4: R'=H; R''=Gluc



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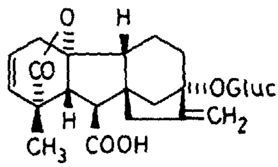

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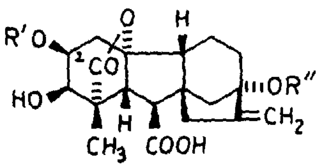
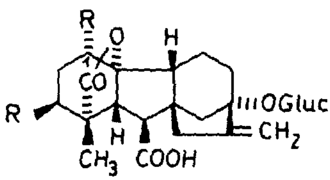


7: R'=Gluc; R''=H
8: R'=H; R''=Gluc



9: R = H
10: R = ^{2}H

Fig. 2. *Structures of the compounds referred to in the text.*

ried out on a Varian Mat 111 fitted with a glass column (180 cm × 2 mm) containing 3% QF-1 on GasChrom Q (125–160 mesh), carrier gas helium 15 ml/min, 250° isothermal, MS electron energy 80 eV.

Results and Discussions

GC-MS of Permethylated GA-O-Glucosides

As the basis for the identification of GA-*O*-glucosides by GC-MS we reinvestigated the permethylation of gibberellins and their conjugates originally described by Rivier et al. (1981). Permethylated derivatives are especially promising for compounds with a relatively high molecular weight, like gibberellin

Table 3. Quantification of GA_{20}-13-*O*-glucoside (*9*) formed from GA_{20} in normal plants (N) and dwarf-1 mutants (d1) of *Zea mays* L.

	Recovered		Specific activity (Bq/μmol)	Total amount (μg)	Metabolic rate (%)
	Amount (μg) HPLC	Activity (Bq)			
Normal plants (N)	190	2.67×10^4	6.79×10^4	297	3.6
Dwarf-1 mutants (d1)	1300	2.56×10^4	9.35×10^3	1227	25.9

glucosyl conjugates. A series of synthetic gibberellin glucosides including GA_{20}-13-*O*-glucoside (*9*) was permethylated. Preparative TLC proved to be useful for purifying the crude product. The GC on SP-2100 and QF-1 resulted in distinct peaks for all permethylated glucosides, the retention times of which are summarized in Table 1.

The permethylated standard compounds were then subjected to GC-MS. The resulting MS spectra were compared in order to select diagnostic fragments for identification. The most prominent ions originate from the permethylated sugar moiety (m/z 75, 101, 155, 187, 219) (Kochetkov et al. 1963, 1965). One of the most characteristic ions for all permethylated gibberellin glucosides indicates the radical loss of a permethylglucosyloxy group ($M-235^+$; Table 2). In the case of the ring A-glucosylated gibberellins *1, 3, 5, 7*, an additional loss of CO_2 leads to the ion at $M-219^+$ which is not present in the spectra of 13-*O*-glucosylated gibberellins (*2, 4, 6, 8, 9*). For the latter glucosides the ion at $M-267^+$ is characteristic, which is formed by the loss of MeOH from the $M-235^+$ ion. These differences may be of value for the differentiation of the investigated ring A 3-*O*-glucosides and 13-*O*-glucosides.

Metabolic Formation of GA_{20}-13-O-Glucoside (9)

Excised shoots of 7-day-old N and d1 *Zea mays* seedlings were incubated with GA_{20}. After 48 h the shoots were extracted with methanol. The pooled extracts were spiked with [^{3}H]GA_{20}-13-*O*-glucoside and subsequently purified by DEAE-Sephadex chromatography, silica chromatography, and preparative reverse-phase HPLC. The identity of the isolated GA_{20}-13-*O*-glucoside (*9*) was established by the HPLC retention time (t_R = 5.1 min), which was shown to be the same as that of authentic GA_{20}-13-*O*-glucoside (*9*).

The GA_{20}-13-*O*-glucoside fractions from both feeds were permethylated and examined by GC-MS. The mass spectra obtained at the retention time of authentic permethylated GA_{20}-13-*O*-glucoside (*9*) were found to be identical with that of the authentic compound. Both contained diagnostic ions (m/z 389, 329, 297), and their abundances coincided with those of the standard substance (Table 2).

In the literature there are reports dealing with GA_{20} metabolism in *Zea mays* where polar fractions formed in cobs and apical meristem of hybrids were assumed to be GA glucoside-like (Rood et al. 1983a,b). Our data demonstrate that GA_{20}-13-*O*-glucoside (*9*) is metabolically formed in *Zea mays*. This is true for both the N and the d1 mutant. This also indicates that *Zea mays*, like *Vicia faba* (Lattke and Schneider 1985), possesses enzymes for GA-13-*O*-glucosylation.

As far as the amount of glucosides is concerned, we found striking differences between the N and d1 feed. In d1 seedlings up to six times more GA_{20}-13-*O*-glucoside was formed than in normal plants (Table 3). The total glucosylation rate of 26% in d1 seedlings has to be considered exceptionally high.

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