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GIBBERELLIN A_{58} AND *ENT*-6α,7α,12α-TRIHYDROXYKAUR-16-EN-19-OIC ACID FROM SEEDS OF *CUCURBITA MAXIMA*

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Key Word Index—*Cucurbita maxima*; Cucurbitaceae; endosperm; gibberellin A_{58}; *ent*-6α,7α,12α-trihydroxykaur-16-en-19-oic acid; DCCC; GC/MS; MS; ^1H and ^{13}C NMR.

Abstract—The isolation of gibberellin A_{58} and *ent*-6α,7α,12α-trihydroxykaurenoic acid from a cellulase-hydrolysed extract of endosperm of *Cucurbita maxima* is described. The two compounds are characterized by their MS, ^1H NMR and ^{13}C NMR.

INTRODUCTION

In the preceding two papers [1, 2] the GC/MS identification of several new 12α-hydroxy-gibberellins and *ent*-12α-hydroxykaurenoids in the seeds of *Cucurbita maxima* L. was described. This paper reports the isolation from the endosperm of these seeds of two of these compounds: 12α-hydroxyGA_4 (**1**) allocated [3, 4] the GA-number, GA_{58}; and *ent*-6α,7α,12α-trihydroxykaur-16-en-19-oic acid (**10**).

RESULTS AND DISCUSSION

As reported in the preceding papers [1, 2], GA_{58} (**1**) occurs in the endosperm of seeds of *C. maxima*, both in the free and conjugated state. To maximize the yield of free GA_{58} (**1**), the homogenized endosperm, pooled from many fruits of different maturity, was treated with a cellulase preparation, then extracted with ethyl acetate. The acidic fraction of this extract was chromatographed on a silica gel column. The fraction found by GC/MS to contain GA_{58} (**1**) was separated by droplet counter-current chromatography (DCCC).

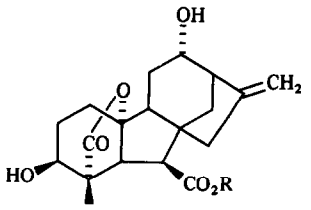
Gibberellin A_{58} (**1**) was obtained as an intractable gum which was homogeneous by TLC and by GC of the MeTMSi derivative. The acid was characterized by MS and by ^1H and ^{13}C NMR; the methyl ester (**2**) was characterized by MS and ^1H NMR. A comparison (Table 1) of the chemical shift differences of the carbon atoms in rings C and D of GA_{58} (**1**), GA_4 (**3**) and GA_1 (**4**) shows the presence of a 12-hydroxyl in GA_{58} (**1**); as noted by Yamaguchi *et al.* [5], the large up-field shift of C-16 and down-field shift of C-17, compared to GA_4 (**3**) and GA_1 (**4**) are characteristic of a 12-hydroxyGA. The α-stereochemistry of the 12-hydroxyl in GA_{58} (**1**) is indicated by the chemical shift of the 17-protons (Table 2). As pointed out by Fukui *et al.* [6], for 12β-hydroxyGAs but not for 12α-hydroxyGAs, one of the 17-proton signals is shifted to lower-field in C_5D_5N solutions compared to $CDCl_3$ solutions. The data (Table 2) for the methyl esters of GA_{58} (**1**), GA_{39} (**9**), GA_{48} (**5**) and GA_{49} (**6**), and for GA_{47} and GA_{48} diacetates (**7** and **8**) confirm the 12α-hydroxyl stereochemistry in GA_{58} (**1**).

A second constituent was isolated from the DCCC separation and characterized as *ent*-6α,7α,12α-trihydroxykaurenoic acid (**10**) based on the following data. The MS of the acid (**10**) and its methyl ester (**11**) did not contain an $[M]^+$ ion. However accurate mass measurements of the $[M-18]^+$ ion for both compounds were in accord with the molecular composition, $C_{20}H_{26}O_3$, for the acid (**10**). The TMSi derivative of the methyl ester (**11**) showed a strong (base) peak at *m/z* 269, also present in the MS of the MeTMSi derivative of *ent*-6α,7α-dihydroxykaurenoic acid (**12**) and characteristic of the 6,7- and 9,10-cleavage in MeTMSi derivatives of 6,7-diols of *ent*-kaurenoic acids unsubstituted in ring A. The ^1H NMR spectrum of the acid (**10**) and *ent*-6α,7α-dihydroxykaurenoic acid (**12**), were very similar except for an additional hydroxymethine proton in the acid (**10**) and for the 17-proton chemical shifts. As for 12-hydroxyGAs, and as initially indicated by Jefferies and Retallack [7] for *ent*-hydroxykaurenoic acids, *ent*-12α- and *ent*-12β-hydroxykaurenoic acids can be distinguished by the chemical shift differences of the 17-protons in C_5D_5N and $CDCl_3$ solutions. A comparison (Table 2) of the chemical shifts of these protons for the new acid (**10**) with those for the known [8, 9] reference compounds **12**–**14** confirms the *ent*-12α-hydroxyl stereochemistry in the acid (**10**) from *C. maxima*. Due to the small amount of material isolated, full interpretation of the ^{13}C NMR spectrum of **10** was not possible. However the signals for carbons 6, 7, 12, 16 and 17 were discernible and are shown in Table 3 with the corresponding data for the reference compounds **12**–**14**. The chemical shift of C-17 (δ106.6) confirms the *ent*-12α (12β) stereochemistry in **10** (cf. δ106.4 in **13** and δ104.6 in **14**).

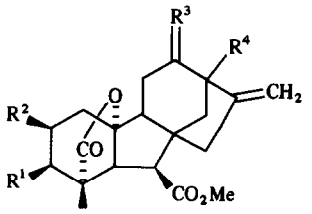
EXPERIMENTAL

For general experimental details see ref. [11]. ^1H and ^{13}C NMR were obtained in the specified solvents using Jeol FX90Q and FX200 instruments. For DCCC a Tokyo Rikakikai instrument was used containing 350 tubes (40 × 0.2 cm).

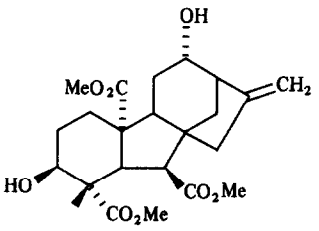
Isolation of gibberellin A_{58} (**1**) *and* ent-6α,7α,12α-*trihydroxykaur*-16-*en*-19-*oic acid* (**10**). Homogenized endosperm (650 ml)



R
1 H
2 Me



	R^1	R^2	R^3	R^4
3	OH	H	H_2	H
4	OH	H	H_2	OH
5	OH	OH	H,βOH	H
6	OH	OH	H,αOH	H
7	OAc	OAc	H,βOH	H
8	OAc	OAc	H,αOH	H



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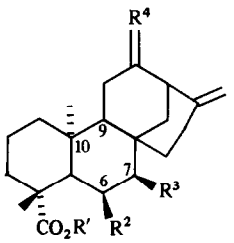

	R^1	R^2	R^3	R^4
10	H	OH	OH	H,βOH
11	Me	OH	OH	H,βOH
12	H	OH	OH	H_2
13	H	H	H	H,βOH
14	H	H	H	H,αOH

Table 1. ^{13}C chemical shifts [δ (ppm)] for rings C and D of GA_{58}(1), GA_4(3) and GA_1(4)

C	GA_{58} (1)	GA_4 (3)	GA_1 (4)
8	51.6	51.5	49.8
9	50.7*	54.0	53.5
1	27.4	16.5	18.0
12	75.1	31.8	39.9
13	50.2*	39.4	77.9
14	34.6	37.4	46.3
15	45.3	45.0	44.0
16	153.0	157.7	159.2
17	109.5	107.2	106.5

*Assignments may be reversed.

Table 2. ^1H chemical shifts (δ TMS) of 17-protons in some 12-hydroxyGAs and *ent*-12-hydroxykaurenoic acids

Compound	$CDCl_3$	C_5D_5N	Ref.
GA_{58} (1)	*	4.98, 5.08	—
GA_{58} Me ester (2)	5.00, 5.10	5.02, 5.10	—
5	5.10	5.10, 5.30	[6]
6	4.90, 5.00	5.00	[6]
7	5.16	5.20, 5.41	[10]
8	5.05, 5.15	†	[10]
9	4.96, 5.02	4.96, 5.01	[6]
10	*	5.11, 5.29	—
12	*	4.90	—
13	4.90	4.97, 5.13	—
14	4.81, 4.88	4.94	—

*Insoluble.
†Data not recorded in ref. [10].

from 28 fruits of *C. maxima* was adjusted to pH 4.5 with 5 M HCl and treated with cellulase (6.5 g) at 35° for 22 hr. The pH was then adjusted to 8.0 with 5 M KOH and the mixture was extracted with EtOAc (4 × 250 ml). The aq. residue was adjusted to pH 2.5 with 5 M HCl and extracted with EtOAc (4 × 250 ml), which was washed with H_2O (500 ml) and then evaporated to dryness under vacuum. This acidic extract (747 mg) was fractionated on a column of silica gel (30 g), eluted with petrol (40–60°, 100 ml) containing EtOAc increasing in 10% steps. The fraction (35.0 mg) eluted with 60% EtOAc in petrol was found by

Table 3. ^{13}C chemical shifts [δ (ppm, C_5D_5N)] of carbons 6, 7, 12, 16 and 17 for some *ent*-hydroxykaurenoic acids

Compound	C-6	C-7	C-12	C-16	C-17
10	71.8*	81.2	71.6*	151.9	106.6
12	71.8	81.9	33.9	155.7	103.6
13	22.6	40.6	71.2	153.3	106.4
14	22.7	41.7	71.5	153.8	104.6

*Assignments may be interchanged.

GC/MS to contain GA_{58}. It was further purified by DCCC using CH_2Cl_2–MeOH–H_2O (5:6:4). Fractions (*ca* 7 ml) of the mobile phase were collected and were monitored by TLC.

No material was eluted when the descending mobile phase was organic. The mixture (26 mg) recovered from the tubes was rechromatographed with the same solvent system but with the aq. phase as the ascending mobile phase. Gibberellin A_{58} (**1**) was obtained from fractions 20–26 (140–180 ml) as a gum (5.5 mg) (Found: $[M]^+$ 348.158. $C_{19}H_{24}O_6$ requires $[M]^+$ 348.157); MS *m/z*: 348 $[M]^+$ (8%), 330 (66), 312 (61), 302 (57), 294 (17), 284 (100), 266 (38), 240 (41), 195 (43), 105 (35); *m/z* (MeTMSi derivative): see preceding paper; ^1H NMR C_5D_5N: δ1.62 (*s*, 18-H_3), 3.23 (*d*, J = 11 Hz, 6-H), 3.91 (*d*, J = 11 Hz, 5-H), 4.10 (*m*, 3- and 12-H), 4.98 and 5.08 (each *br*, 17-H_2); ^{13}C NMR C_5D_5N: δ15.4 (*q*, 18-C), 27.4 (*t*, 11-C), 27.9 (*t*, 1-C), 28.8 (*t*, 2-C), 34.6 (*t*, 14-C), 45.3 (*t*, 15-C), 51.6 (*s*, 8-C), 50.2, 50.7, 52.1 and 52.6 (each *d*, 5, 6, 9, 13-C), 55.6 (*s*, 4-C), 69.6 (*d*, 3-C), 75.1 (*d*, 12-C), 94.3 (*s*, 10-C), 109.5 (*t*, 17-C), 153 (*s*, 16-C), 175.3 (*s*, 7-C) and 179.7 (*s*, 19-C). The Me ester (prepared with CH_2N_2) was a gum; ^1H NMR $CDCl_3$: δ1.15 (*s*, 18-H_3), 2.73 (*d*, J = 11 Hz, 6-H), 3.22 (*d*, J = 11 Hz, 5-H), 3.72 (*s*, OMe), 3.73 (*t*, J = 4.5 Hz, 12-H), 3.85 (*br*, 3-H), 5.00 and 5.10 (*br*, 17-H_2); ^1H NMR C_5D_5N: δ1.50 (*s*, 18-H_3), 3.08 (*d*, J = 11 Hz, 6-H), 3.81 (*d*, J = 11 Hz, 5-H), 3.67 (*s*, OMe), *ca* 4.10 (*m*, 3- and 12-H), and 5.00 (17-H_2); MS: *m/z* 362 $[M]^+$ (18%), 344 (47), 330 (36), 312 (37), 302 (52), 284 (37), 164 (100), 162 (29), 103 (30), 91 (27).

From the DCCC separation, fractions 37–43 (260–300 ml), *ent*-6α,7α,12α-hydroxykaur-16-en-19-oic acid (**10**) (3.5 mg) was obtained as a gum (Found: $[M-18]^+$ = 332.1989; $C_{20}H_{28}O_4$ $[M-H_2O]^+$ requires 332.1987); ^1H NMR C_5D_5N: δ1.24 (*s*, 20-H_3), 1.87 (*s*, 18-H_3), 4.03 (*d*, J = 2.5 Hz, 7-H), 4.10 (*m*, 12-H), 4.85 (*dd*, J = 2.5, 10.5 Hz, 6-H), 5.11 and 5.29 (both *br*, 17-H_2); ^{13}C NMR: see Table 3.

The methyl ester (**11**) had $[M-18]^+$ = 346.2128, $C_{21}H_{30}O_4$ $[M-H_2O]^+$ requires 346.2144; *m/z* (Tris-TMSi ether): see previous paper [2].

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