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IDENTIFICATION OF GA_{113}, GA_{114}, GA_{115} AND GA_{116} AND FURTHER NOVEL GIBBERELLINS IN *RAPHANUS SATIVUS*

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Key Word Index—*Raphanus sativus*; Brassicaceae; Capparidales; 12-hydroxy C_{20}-gibberellins, GA_{111}, GA_{112}, GA_{113}, GA_{114}, GA_{115}, GA_{116}; 3-*epi*-GA_{34}; 2α-hydroxy gibberellins, GA_{40}; GA_{20} ethyl ester.

Abstract—Endogenous gibberellins of seeds and shoots of *Raphanus sativus* have been reanalyzed by GC-MS. Six 12-hydroxy C_{20}-gibberellins (GA_{111} (12α-hydroxy GA_{12}), GA_{112} (12β-hydroxy GA_{12}), 12α-hydroxy GA_{15}, 12β-hydroxy GA_{15}, 12α-hydroxy GA_{24} and 12β-hydroxy GA_{24}), and GA_5, GA_{40}, GA_{95}-isolactone and 3-*epi*-GA_{34} were identified as their methyl ester and TMSi ether derivatives. Of these gibberellins, 12α-hydroxy GA_{15}, 12β-hydroxy GA_{15}, 12α-hydroxy GA_{24} and 12β-hydroxy GA_{24} were allocated the descriptors GA_{113}, GA_{114}, GA_{115} and GA_{116}, respectively. In addition, GA_{20} ethyl ester was identified as its TMSi ether derivative.

INTRODUCTION

We have been investigating the bolting of Japanese radish (*Raphanus sativus*) and have accumulated evidence that endogenous gibberellins (GAs) are significantly involved in this process [1, 2]. In addition to the GAs that are characteristic of early-13-hydroxylation and early-non-hydroxylation pathways, two novel GAs, 12-hydroxy GA_{15} [2] and 12-hydroxy GA_{24} [1, 3] were tentatively identified in seeds or shoots of *R. sativus* by GC-MS comparisons with the metabolites of 12β-hydroxy-*ent*-kaurenoic acid obtained with the *Gibberella fujikuroi* B1-41a mutant [4]. In addition, GA_{40} (**7**), GA_{47} (**8**) and GA_{81} (**9**), all of which possess a 2α-hydroxyl, also appeared to be present in *R. sativus* [2]. Until GA_{81} was identified in pea (*Pisum sativum*) [5], GAs with a 2α-hydroxy group, including GA_{40} and GA_{47}, had been found only in fungi. In this study of the analysis of endogenous GAs in *R. sativus*, we have sought to confirm the presence of the 2α-hydroxy GAs and, with the recent availability of several new C_{20}-GA methyl esters obtained by synthesis from gibberellic acid [6], to establish the structures of the 12-hydroxy C_{20}-GAs.

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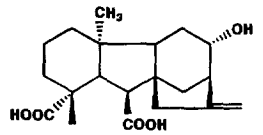
RESULTS

Seeds

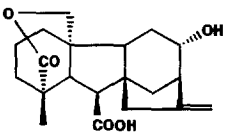
Our previous studies on *R. sativus* had shown that the putative 12-hydroxy GA_{24} occurred in the mature seeds and were eluted at almost the same R_t as that of ABA on ODS-HPLC [3], while the putative 12-hydroxy GA_{15} occurred in the shoots and were eluted later than ABA on ODS-HPLC [2]. We therefore analyzed the corresponding ODS-HPLC fractions of mature seeds extract using the R_t of authentic ABA as a marker to supplement the bioassay profile. GA_5 (**12**), GA_{40} (**7**) and GA_{95}-isolactone (**13**), as well as the novel GA, 12β-hydroxy GA_{24} (**6**), were clearly identified as their methyl ester TMSi ether derivatives; neither of the 12-hydroxy GA_{15} epimers (**2** or **5**) could be detected in the seeds extract (Table 1). A GA-like compound with a parent ion of *m/z* 432 was detected and definitively identified as GA_{20} ethyl ester (**16**) by direct comparison of its TMSi ether derivative (Table 1) with an authentic sample.

Shoots

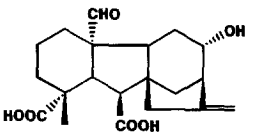
We separated the DEA column eluate in order to identify 12-hydroxy GAs based on the movement of these in their methylated or non-methylated form on



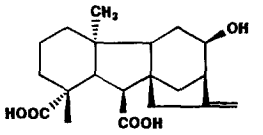
*12α-hydroxy GA_{12} (GA_{111}) (1)



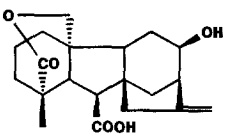
*12α-hydroxy GA_{15} (GA_{113}) (2)



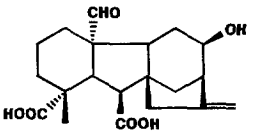
*12α-hydroxy GA_{24} (GA_{115}) (3)



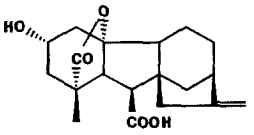
*12β-hydroxy GA_{12} (GA_{112}) (4)



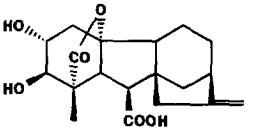
*12β-hydroxy GA_{15} (GA_{114}) (5)



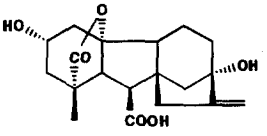
*12β-hydroxy GA_{24} (GA_{116}) (6)



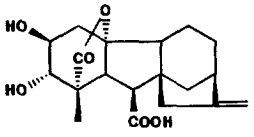
*GA_{46} (7)



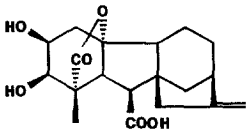
GA_{47} (8)



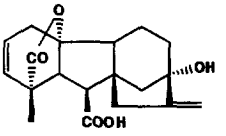
GA_{81} (9)



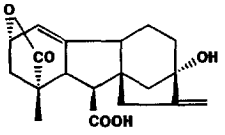
*3-*epi*-GA_{34} (10)



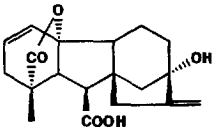
GA_{34} (11)



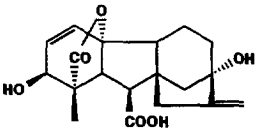
*GA_5 (12)



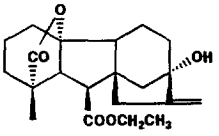
*GA_{95}-isolactone (13)



GA_{95} (14)



GA_3 (15)



*GA_{20} ethyl ester (16)

Structures of GAs. GAs identified in this study are indicated by asterisk.

chromatography. Our previous studies had indicated also that GA_{47} (**8**) and GA_{81} (**9**) occurred in the shoots and that they were eluted at almost the same R_f as that of the putative 12-hydroxy GA_{15} on ODS-HPLC [2]. One half of the DEA column eluate of the shoots extract was subjected to ODS-HPLC, while monitoring the fractions by the R_f of ABA and bioassay as before. The identified fractions were methylated and purified by TLC according to the movement of the methyl esters of authentic GA_{47}, GA_{81} and the 12-hydroxy GA_{15} epimers. Three novel GAs, 12α-hydroxy GA_{15} (**2**), 12α-hydroxy GA_{24} (**3**) and 3-*epi*-GA_{34} (**10**) (for preparation, see [7]) as well as GA_{112} (12β-hydroxy GA_{12}) (**4**) were identified as their methyl ester

Table 1. Identification of endogenous GAs in mature seeds of *Raphanus sativus*

HPLC R_t (min)	GAs	KRI	Characteristic ions m/z (relative intensity %)
28–34	[a] GA_{116} (12β-hydroxy GA_{24})	2711	462 ([M$^+$], 14), 430 (13), 402 (17), 374 (8), 344 (21), 312 (100), 284 (79), 258 (27), 225 (39), 199 (29)
	GA_{95}-isolactone	2600	416 ([M$^+$], 100), 401 (15), 387 (29), 371 (13), 357 (29), 343 (26), 299 (8), 269 (8), 238 (48)
36–38	GA_5	2564	416 ([M$^+$], 100), 401 (17), 372 (9), 357 (12), 343 (13), 321 (13), 299 (32), 275 (8), 207 (24)
38–40	[b] GA_{20} ethyl ester	2619	432 ([M$^+$], 100), 417 (12), 403 (6), 389 (41), 359 (23), 345 (5), 343 (5), 301 (12), 235 (8), 207 (27), 193 (12)
40–44	GA_{40}	2617	418 ([M$^+$], 0), 403 (16), 371 (100), 343 (72), 299 (68), 284 (51), 225 (47), 181 (16)
Synthetic GAs			
—	GA_5	2564	416 ([M$^+$], 100), 401 (15), 372 (5), 357 (14), 343 (13), 321 (5), 299 (32), 275 (9), 207 (27)
—	GA_{40}	2617	418 ([M$^+$], 0), 403 (16), 371 (100), 343 (77), 299 (85), 284 (64), 225 (54), 181 (20)
—	GA_{95}-isolactone	2601	416 ([M$^+$], 100), 401 (13), 387 (27), 371 (8), 357 (24), 343 (18), 299 (12), 269 (7), 238 (25)
—	[a] GA_{115} (12α-hydroxy GA_{24})	2708	462 ([M$^+$], 6), 430 (7), 402 (13), 374 (6), 344 (16), 312 (100), 284 (83), 258 (21), 225 (42), 199 (200)
—	[a] GA_{116}	2711	462 ([M$^+$], 6), 430 (10), 402 (20), 374 (10), 344 (17), 312 (100), 284 (81), 258 (28), 225 (40), 199 (28)
—	[b] GA_{20} ethyl ester	2619	432 ([M$^+$], 100), 417 (11), 403 (6), 389 (48), 359 (26), 345 (5), 343 (4), 301 (12), 235 (5), 207 (31), 193 (9)

Under these HPLC conditions, ABA was eluted at 34.5 min. KRIs and MS spectra were measured by GC-MS using temp program 1 as methyl ester TMSi ether derivatives.

[a] KRI values were each measured three times by GC-MS using temp program 2.

[b] KRIs and MS spectra were measured as TMSi ether derivatives.

TMSi ether derivatives by GC-MS comparisons (Table 2). GA_{20} ethyl ester was also identified as its TMSi ether derivative. The other half of the DEA column eluate of the shoots extract was methylated first and purified by TLC and HPLC according to the movement of authentic GA methyl esters, resulting in the identification of GA_{111} (12α-hydroxy GA_{12}) (**1**) and 12β-hydroxy GA_{15} (**5**) as their methyl ester TMSi ether derivatives by GC-SIM and GC-MS, respectively (Table 3). The four new C_{20}-GAs have been assigned [8] the following numbers: 12α-hydroxy GA_{15} as GA_{113}, its 12β-epimer as GA_{114}, 12α-hydroxy GA_{24} as GA_{115}, and its 12β-epimer as GA_{116}. Neither GA_{47} nor GA_{81} could be detected from the shoots.

Note on GC-MS *or* GC-SIM *analysis*

In this study, the identification of endogenous GAs was carried out by direct GC-MS comparisons on TMSi ether or methyl ester TMSi ether derivatives of endogenous GAs with those of authentic samples, including the newly available synthetic 12-hydroxy C_{20}-GAs [6]. The KRI values of 12-hydroxy C_{20}-GAs obtained by using DB-1 (100% dimethyl polysiloxane column), which is widely used for GAs analysis, are as follows: GA_{111}, 2537; GA_{112}, 2552; GA_{113}, 2789; GA_{114}, 2794; GA_{115}, 2634; GA_{116}, 2635. The HP-5 (5% diphenyl-95% dimethyl polysiloxane column) was used in this study because the 12-hydroxy GA_{24} epimers (GA_{115} and GA_{116}) methyl ester TMSi ethers could not be sufficiently separated to allow definitive identification by using DB-1. We note that KRI values are not absolute ones but depend on analytical conditions, including the column property and the temperature program.

In order to make certain that the endogenous 12-hydroxy GA_{24} epimers in mature seeds and shoots were the 12α- and 12β-epimers, respectively, KRI values of methyl ester TMSi ether derivatives of both naturally occurring and authentic 12α- and 12β-hydroxy GA_{24} were each measured three times. In the case of the identification of GA_{115} in shoots, KRI values of GA_{115} were as follows: naturally occurring GA_{115}: 2708, 2709 and 2709; synthetic GA_{115}: 2708, 2709 and 2709; synthetic GA_{116}: 2711, 2711 and 2712. In the case of the identification of GA_{116} in seeds, KRI values of GA_{116} were as follows: naturally occurring GA_{116}: 2710, 2711 and 2711; synthetic GA_{115}: 2708, 2708 and

Table 2. Identification of endogenous GAs in shoots of *Raphanus sativus* (I)

HPLC R_t (min)	TLC R_f value	GAs	KRI	Characteristic ions m/z (relative intensity %)
30–34	0.31–0.50	GA_{113} (12α-hydroxy GA_{15})	2899	432 ([M$^+$], 86), 400 (22), 372 (19), 342 (32), 310 (56), 296 (37), 282 (89), 237 (100), 225 (35), 197 (37)
	0.60–0.74	GA_{112} (12β-hydroxy GA_{12})	2625	448 ([M$^+$], 5), 416 (62), 388 (54), 373 (13), 356 (18), 326 (28), 298 (100), 283 (22), 239 (46)
		[a] GA_{115} (12α-hydroxy GA_{24})	2709	462 ([M$^+$], 11), 430 (12), 402 (15), 374 (7), 344 (17), 312 (100), 284 (94), 258 (27), 225 (52), 199 (22)
		[b] GA_{20} ethyl ester	2620	432 ([M$^+$], 100), 417 (12), 403 (6), 389 (51), 359 (23), 345 (3), 343 (2), 301 (14), 235 (5), 207 (22), 193 (8)
34–38	0.20–0.39	3-*epi*-GA_{34}	2869	506 ([M$^+$], 100), 431 (6), 416 (8), 341 (9), 313 (14), 283 (13), 239 (17), 223 (25), 147 (54)
Synthetic GAs				
—	0.68	GA_{112}	2625	448 ([M$^+$], 8), 416 (55), 388 (46), 373 (9), 356 (13), 326 (22), 298 (100), 283 (17), 239 (53)
—	0.43	GA_{113}	2899	432 ([M$^+$], 88), 400 (23), 372 (24), 342 (38), 310 (56), 296 (42), 282 (95), 237 (100), 225 (43), 197 (40)
—	0.61	[a] GA_{115}	2709	462 ([M$^+$], 6), 430 (7), 402 (13), 374 (6), 344 (16), 312 (100), 284 (83), 258 (21), 225 (42), 199 (20)
—	0.61	[a] GA_{116} (12β-hydroxy GA_{24})	2711	462 ([M$^+$], 6), 430 (10), 402 (20), 374 (10), 344 (17), 312 (100), 284 (81), 258 (28), 225 (40), 199 (28)
—	—	3-*epi*-GA_{34}	2870	506 ([M$^+$], 100), 431 (8), 416 (9), 341 (9), 313 (17), 283 (12), 239 (14), 223 (25), 147 (59)
—	0.65	[b] GA_{20} ethyl ester	2619	432 ([M$^+$], 100), 417 (11), 403 (6), 389 (48), 359 (26), 345 (5), 343 (4), 301 (12), 235 (5), 207 (31), 193 (9)

Under these HPLC conditions, ABA was eluted at 28.9 min. TLC R_f values were measured as methyl ester derivatives. KRIs and MS spectra were measured by GC-MS using temp program 1 as methyl ester TMSi ether derivatives.

[a] KRI values were each measured three times by GC-MS using temp program 2.

[b] TLC R_f values were measured as ethyl esters. KRIs and MS spectra were measured as TMSi either derivatives.

Table 3. Identification of endogenous GAs in shoots of *Raphanus sativus* (II)

HPLC R_t (min)	TLC R_f value	GAs	KRI	Characteristic ions m/z (relative intensity %)
25–26	0.33–0.52	GA_{114} (12β-hydroxy GA_{15})	2915	432 ([M$^+$], 80), 400 (26), 372 (18), 342 (28), 310 (40), 296 (32), 282 (89), 237 (100), 225 (40), 197 (43)
35–36	0.64–0.76	[a] GA_{111} (12α-hydroxy GA_{12})	2606	448 ([M$^+$], 12), 416 (78), 388 (93), 373 (13), 356 (8), 326 (17), 298 (100), 283 (36), 239 (60)
Synthetic GAs				
35.3	0.67	[a] GA_{111}	2607	448 ([M$^+$], 11), 416 (84), 388 (66), 373 (13), 356 (16), 326 (27), 298 (100), 283 (17), 239 (46)
25.4	0.43	GA_{114}	2915	432 ([M$^+$], 75), 400 (25), 372 (18), 342 (33), 310 (52), 296 (38), 282 (86), 237 (100), 225 (37), 197 (29)

HPLC R_t and TLC R_f value were measured as methyl ester derivatives. KRIs and MS spectra were measured by GC-MS using temp program 1 as methyl ester TMSi ether derivatives.

[a] Analyzed by GC-SIM.

2709; synthetic GA_{116}: 2710, 2711 and 2711. The representative values are described in Tables 1 and 2. Furthermore, the separation of each naturally occurring 12-hydroxy GA_{24} epimer was confirmed by co-injection with each of the synthetic 12-epimers (data not shown).

DISCUSSION

Six 12-hydroxy C_{20}-GAs (**1**)–(**6**) have been identified as their methyl ester TMSi ether derivatives by GC-MS in seeds or shoots of *R. sativus*. Of these six GAs, 12α-hydroxy GA_{15} (**2**), 12β-hydroxy GA_{15} (**5**), 12α-hydroxy GA_{24} (**3**) and 12β-hydroxy GA_{24} (**6**) are novel GAs, and according to convention [8], are now identified as GA_{113}, GA_{114}, GA_{115} and GA_{116}, respectively. Because of the coincidence of fractions eluted on ODS-HPLC, the 12-hydroxy GA_{15} and the 12-hydroxy GA_{24} isolated originally correspond to the 12α-epimer (GA_{113}) and 12β-epimer (GA_{116}), respectively [1–3]. We had already shown that the GA profile in *R. sativus* is consistent with both the early-13-hydroxylation and early-non-hydroxylation pathways [1–3]. It is conceivable that the respective groups of GA_{111}, GA_{113} and GA_{115}, and GA_{112}, GA_{114} and GA_{116} may be indicative of early 12-hydroxylation pathways in *R. sativus*, but such a view is unattractive in the absence of the simple 12-hydroxylated C_{19}-GAs, GA_{69} and GA_{70}. We note that GA_{111} has been detected previously in *Cucurbita maxima* [9] and the tree ferns *Cibotium glaucum* and *Dicksonia antarctica* [10], while GA_{112} has been found in *D. antarctica* [10] and *Matthiola incana* [11].

The occurrence of GA_{40} (2α-hydroxy GA_9) in *R. sativus* appears to be only the third observation of the occurrence of a 2α-hydroxy GA in higher plants. The discovery of 2α-hydroxy GAs in three kinds of plants, belonging to quite different orders (GA_{40}: *Raphanus sativus*, Brassicaceae, Capparidales; Ga_{47}: *Orobanche minor*, Orobanchaceae, Polemoniates [12]; GA_{81}: *Pisum sativum*, Leguminosae, Rosales [57]), may indicate that the distribution of 2α-hydroxy GAs in higher plants will prove to be more extensive than originally thought [13]. Given the ease with which GA_{95} is converted into its isolactone form during purification procedures [14], the occurrence of GA_{95}-isolactone (**13**) probably signals the presence of GA_{95} (**14**) itself in *R. sativus*. Having shown that the 3-*epi*-GA_1 and 3-*epi*-GA_4 previously identified in shoots of *R. sativus* were not artifacts from epimerization of the 3β-hydroxyl of GA_1 and GA_4, but naturally occurring [1], we are confident that 3-*epi*-GA_{34} (**10**) is similarly not an artifact from epimerization of the 3β-hydroxyl of GA_{34} (**11**), especially as 2β, 3β-dihydroxy GAs, such as GA_{34} and GA_8, are more stable to base than GA_1 and GA_4.

It seems strange that a neutral GA, GA_{20} ethyl ester, was detected after the purification procedure which concentrated free GAs. GA_{20} ethyl ester might therefore exist in the open-lactone form in the plant tissues. The occurrence of native GA alkyl esters is observed in *Lygodium* ferns (methyl esters of GA_9, GA_{20}, GA_{70}, GA_{73}, GA_{88}, 3-*epi*-GA_{88} and GA_{96}) [15–17] and *Cucumis sativus* (GA_3 *n*-propyl ester) [18]. In ferns, these GA methyl esters are significantly involved in sexual development as antheridiogens [15–17]. GA_{20} ethyl ester also could be expected to undertake some biological role in *R. sativus*.

EXPERIMENTAL

Plant materials

Mature seeds of Japanese radish (*Raphanus sativus* cv. Taibiyo-sobutori and cv. Wase-shijunichi) were purchased from Takii Seed Co. Ltd, Japan. The seeds of Wase-Shijunichi were sown on a mixture of soil and sand (1:1) and grown in a chamber kept at 20° under 8 h day length with an illumination from metal-halide lamp (470 μmol m^{-2} s^{-1}). Then the shoots were harvested after 30 days after sowing.

Purification of seeds extract

Mature seeds of Taibiyo-sobutori (140 g) were extracted with MeOH and the extract was red. to the aq. phase *in vacuo*. The aq. residue was adjusted to pH 3 and extracted with EtOAc. The EtOAc fraction was partitioned with 0.5 M Pi buffer (pH 8.3). The buffer fraction was adjusted to pH 3 and extracted with EtOAc to obtain the acidic ethyl acetate (AE) fraction. The AE fraction was loaded on a PVP column and eluted with 0.1 M phosphate buffer (pH 8.3). The eluate was adjusted to pH 3 and passed through an ODS column. The column was washed with 0.1% HOAc and eluted with 80% aq. MeOH (0.1% HOAc). The eluate was dissolved in MeOH and loaded onto a Bondesil DEA column. The column was eluted with MeOH and 0.5% HOAc in MeOH. The eluate of 0.5% HOAc in MeOH was subjected to TLC using a solvent system of chloroform–EtOAc–HOAc (7:12:1). The region from R_f 0.3–0.8 was scraped off and extracted with MeOH. The extract was dissolved in 20% aq. MeOH (0.1% HOAc) and charged onto a Sep-Pak (ODS) cartridge, washed with 20% aq. MeOH (0.1% HOAc) and eluted with MeOH. The eluate was dissolved in 30% MeOH (0.1% HOAc) and injected onto a Develosil ODS column (15 × 1 cm i.d.) attached to a Guard-pak in a Waters 600 HPLC system. The eluting conditions were as follows: solvent, 0–4 min, 30% MeOH (0.1% HOAc); 4–50 min, 30–80% MeOH (0.1% HOAc); 50–60 min, 80% MeOH (0.1% HOAc); 60–85 min 100% MeOH (0.1% HOAc); flow rate, 2 mil min^{-1}; column temperature, 40°. Following a 4 min delay after the injection, frs were collected every 2 min to give 30 frs. The frs were dried *in vacuo* and bioassayed by a dwarf rice (cv. Tan-ginbozu) microdrop procedure [19]. Frs of R_f 28–34 min, R_f 36–38 min, R_f 38–40 min and R_f 40–44 min were methylated with ethereal CH_2N_2 in preparation for analysis by GC-MS.

Purification of shoots extract

The shoots (1180 g) were extracted with MeOH and the extract was red. to the aq. phase *in vacuo*. The aq. residue was partitioned using the same method as described above to obtain AE fraction. The AE fraction was loaded on a PVP column and eluted with 0.1

M phosphate buffer (pH 8.3). The eluate was adjusted to pH 3 and passed through an ODS column. The column was washed with 0.1% HOAc and eluted with 30% aq. MeOH (0.1% HOAc) and 60% aq. MeOH (0.1% HOAc), successively. The 60% aq. MeOH (0.1% HOAc) fr. was dissolved in MeOH and loaded onto a Bondesil DEA column. The column was eluted with MeOH and 0.5% HOAc in MeOH, successively. One half of the fr. eluted with 0.5% HOAc in MeOH was dissolved in 30% MeOH (0.1% HOAc) and injected onto a Develosil ODS column (15 × 1 cm i.d.) attached to a Guard-pak in a Hewlett Packard 1100 HPLC system. The eluting and fractionating conditions were the same as those described above. The frs were dried *in vacuo* and bioassayed by a dwarf rice (cv. Tan-ginbozu) microdrop procedure [19]. The combined frs of R_t 30–34 min and of R_t 34–38 min were methylated with ethereal CH_2N_2 and subjected to TLC using chloroform–EtOAc (1:2) as the solvent system. The regions of R_f 0.31–0.50 and R_f 0.60–0.74 of the HPLC fr. of R_t 30–34 min and the region of R_f 0.20–0.39 of the HPLC fr. of R_t 34–38 min were scraped off and extracted with H_2O-satd EtOAc. Each fr. was dissolved in MeOH and passed through a Bondesil NH_2 column and analyzed by GC-MS.

The other half of the DEA column fraction eluted with 0.5% HOAc in MeOH was methylated with ethereal CH_2N_2 and subjected to TLC using chloroform–EtOAc (1:2) as the solvent system. The regions of R_f 0.33–0.52 and R_f 0.64–0.76 were scraped off and extracted with H_2O-satd EtOAc. Each fr. was dissolved in 40% MeOH and purified using a Develosil ODS column (15 × 1 cm i.d.) attached to a Guard-pak in a Hewlett Packard 1100 HPLC system. The eluting conditions were as follows: solvent, 0–10 min, 0% of solvent A (MeOH) in solvent B (40% MeOH); 10–40 min, 0–100% of solvent A; 40–50 min, 100% of solvent A; flow rate, 2 mil min^{-1}; column temperature, 40°. Frs were collected every 1 min to give 50 frs. HPLC fr. of R_t 25–36 min of TLC fr. of R_f 0.33–0.52 and HPLC fr. of R_t 35–36 min of TLC fr. of R_f 0.64–0.76 were analyzed by GC-MS.

GC-MS *and* GC-SIM *analysis*

The methylated fractions to be analyzed were trimethylsilyated with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) at 80° for 30 min. The derivatized samples were analyzed using a HP 5989B MS-system equipped with HP 5890 GC-system fitted with a capillary column HP-5 (30 m × 0.25 mm i.d., 0.25 μm film thickness) at an ionization voltage of 70 eV. To identify 12α-hydroxy GA_{24} and 12β-hydroxy GA_{24}, column temp. program 2 was used; otherwise, column temp. program 1 was used. Program 1 was started at 60.0° for 2 min, at 30.0° min^{-1} to 240.0°, at 2.0° min^{-1} to 280.0° and finally, isothermally held at 280.0° for 5 min. Program 2 was started at 60.0° for 2 min, at 30.0° min^{-1} to 240.0°, at 0.5° min^{-1} to 250.0°, at 30.0° min^{-1} to 280.0° and finally, isothermally held at 280.0° for 5 min. Helium carrier gas flow rate was 1 ml min^{-1} by using the constant flow mode. Temp. of the injection port, the separator, the ion source and the analyzer were 220.0°, 280.0°, 200.0° and 100.0°, respectively. KRIs were obtained according to Kovats [20]. For GC-SIM analysis to identify GA_{111}, the following ions were selected: 448, 416, 388, 373, 356, 326, 298, 283 and 239.

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