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ISOLATION AND IDENTIFICATION OF GA_{112} (12β-HYDROXY-GA_{12}) IN *MATTHIOLA INCANA*

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Key Word Index—*Matthiola incana*; Cruciferae; stock; gibberellins; 12β-OH-GA_{12}; GA_{112}.

Abstract—Shoots and flower buds of stock (*Matthiola incana* (L.) R. Br. cv. Sourei) were analysed for gibberellin (GA). After purifying the acidic ethylacetate-soluble fraction by several chromatographic procedures, GA fractions were surveyed by GC-MS and Kovats retention indices (R_I). Consequently, GA_1, GA_4, 3-*epi*-GA_4, GA_8, GA_{19}, GA_{20}, GA_{24}, GA_{34}, GA_{37} and GA_{53} were identified. A further GA-like substance was detected and shown to be 12β-OH-GA_{12} (GA_{112}) by comparison with an authentic compound prepared by synthesis. © 1997 Elsevier Science Ltd

INTRODUCTION

Stock (*Matthiola incana* (L.) R. Br.), a member of Cruciferae family, is an important ornamental plant as a cut flower in Japan. This species is a cold-requiring long-day plant. Differences in flowering times associated with genetic variations of stock is attributed to differential sensitivity to low temperature treatment and to day length and/or varying lengths of the juvenile phase [1–3]. Low temperature requirements for flowering, in general, are more stringent for late-flowering cultivars than for early-flowering cultivars. The observation that exogenous gibberellins (GAs) accelerate flowering in stock [2–4] is suggestive of an involvement of endogenous GAs in the flowering process, as has been noted for other photoperiodic or cold-requiring plants [5]. However, the endogenous GAs of stock have not been studied. Accordingly, we have investigated the endogenous GAs in shoot and flower buds of stock and report the results in this paper.

RESULTS AND DISCUSSION

The fractions from a Develosil ODS HPLC were combined to give seven groupings according to their biological activity. These fractions were subsequently purified by Nucleosil $N(CH_3)_2$ HPLC columns before GC-MS. Consequently, five 13-hydroxy GAs, GA_1, GA_8, GA_{19}, GA_{20} and GA_{53}, and five further GAs, GA_4, 3-*epi*-GA_4, GA_{24}, GA_{34} and GA_{37}, were identified by comparison of mass spectra and R_I with those of authentic GAs (Table 1). Additionally, one GA-like compound was detected. The Me-TMSi derivative of the compound gave a mass spectrum with a molecular ion at *m/z* 448 and several characteristic ions at *m/z* 416 $[M-32]^+$, 388 $[M-60]^+$, 356 $[M-92]^+$, 298 $[M-150]^+$, and 207. Of these ions, the *m/z* 356 ion was consistent with the presence of a di-carboxy C_{20}-GA Me ester, while the *m/z* 207 ion in the absence of ions at *m/z* 208, 193, 180 and 167, indicated a 12-hydroxy-GA [6]. Moreover, the fragmentation pattern was the same as that reported for the putative 12α-hydroxy-GA_{12} isolated from the seeds of pumpkin (*Cucurbita maxima*) [7] and the sporophytes of several tree ferns, namely, *Cibotium glaucum* and *Dicksonia antarctica* [8], and for the putative 12β-hydroxy-GA_{12} obtained from *D. antarctica* [8]. These structures were confirmed by comparison with metabolites and derived from *ent*-12α-hydroxykaurenoic acid by incubation with *Gibberella fujikuroi* (mutant B1-41a). With the recent availability of synthetic samples of the 12-hydroxy-GA_{12} derivatives [9], it has been possible to confirm the original assignments to the hydroxy-GA_{12} derivatives isolated from *C. maxima* and the tree ferns, and to determine the identity of the new GA from stock as 12β-hydroxy-GA_{12}. While the mass spectra (Fig. 1) of the 12α- and 12β-epimers are essentially identical, the R_I values (Table 2) are quite distinct.

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Table 1. Gibberellins identified by GC-MS as methyl ester trimethylsilyl ether derivatives in the acidic EtOAc extract of stock (*Matthiola incana* (L.) R. Br. cv. Sourei)

ODS-HPLC fraction	$N(CH_3)_2$-HPLC fraction	Relative intensities of major ions: *m/z* (%)						R_t	Identity
3–6	16–18	506 (100)	492 (14.0)	448 (31.7)	376 (19.4)	313 (13.1)	207 (42.0)	2665	GA_1
3–6	20–23	594 (100)	579 (25.3)	504 (31.6)	488 (30.4)	448 (32.9)	281 (50.6)	2809	GA_8
10–12	19–22	418 (100)	403 (15.5)	375 (64.9)	359 (9.7)	301 (21.8)	207 (11.0)	2485	GA_{20}
15–17	11–13	432 (5.8)	417 (2.7)	400 (2.6)	342 (10.7)	310 (9.6)	129 (100)	2759	GA_{37}
19–20	15–17	506 (100)	459 (3.5)	431 (3.4)	416 (8.6)	387 (4.7)	372 (3.4)	2660	GA_{34}
19–20	29–31	462 (2.8)	434 (100)	402 (51.3)	374 (90.4)	345 (61.6)	315 (49.1)	2591	GA_{19}
22	21–22	418 (24.5)	386 (29.4)	371 (40.0)	343 (35.9)	328 (18.0)	289 (100)	2616	3-*epi*-GA_4
24–27	9–10	448 (17.3)	416 (6.7)	389 (15.4)	373 (6.7)	251 (39.3)	207 (100)	2495	GA_{53}
24–27	14–16	418 (11.7)	386 (18.3)	358 (18.3)	328 (19.2)	289 (63.2)	284 (100)	2500	GA_4
24–27	26–28	374 (0.8)	342 (6.8)	314 (31.6)	286 (28.7)	254 (27.4)	226 (100)	2446	GA_{24}*

*: Me derivative.

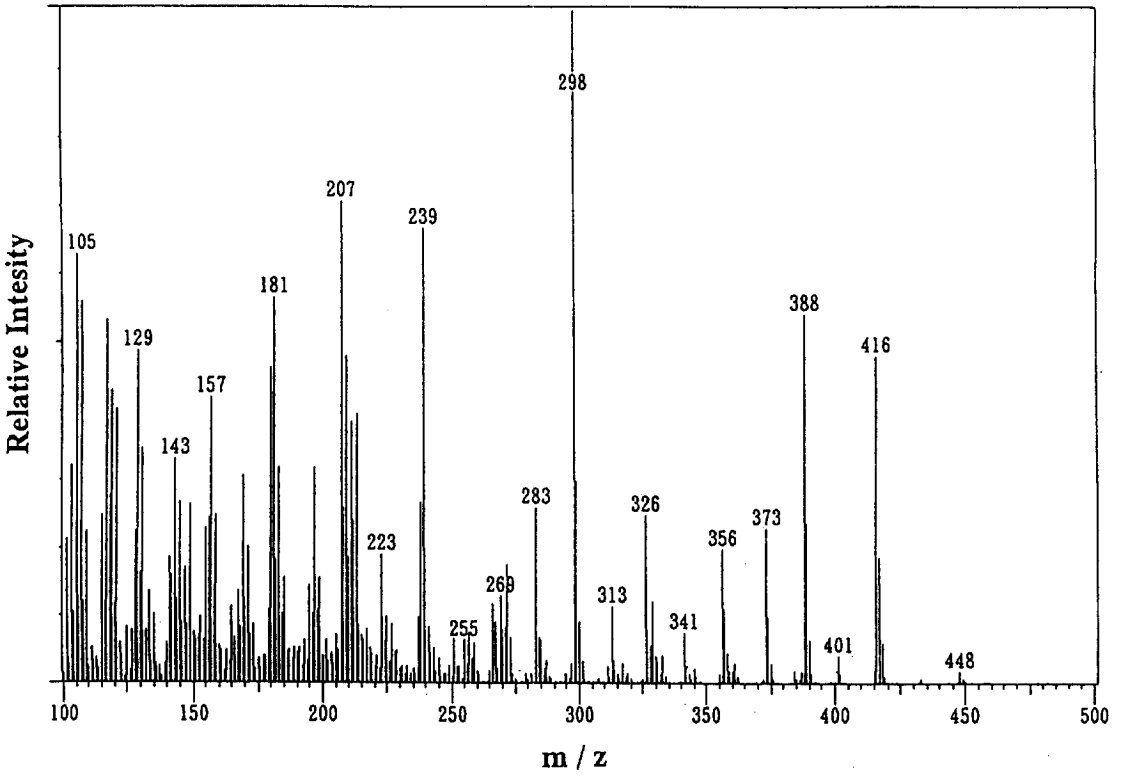

Fig. 1. Mass spectrum of 12β-OH-GA_{12}(GA_{112}) Me-TMSi derivative.

Table 2. 12β-OH-GA_{12} identified by GC-MS as methyl ester trimethylsilyl ether derivatives in the acidic EtOAc extract of stock (*Matthiola incana* (L.) R. Br.)

	ODS-HPLC fraction	$N(CH_3)_2$-HPLC fraction	Relative intensities of major ions: *m/z* (%)							R_t
Sample	10–12	10–11	448 (4.4)	416 (61.6)	388 (54.2)	373 (17.1)	356 (15.7)	326 (24.0)	298 (100)	2552
12β-OH-GA_{12}(GA_{112})			448 (1.7)	416 (53.0)	388 (59.8)	373 (23.9)	356 (23.1)	326 (29.9)	298 (100)	2552*

*: Reported R_t values [6]: 12β-OH-GA_{12} 2533; 12α-OH-GA_{12} 2538.

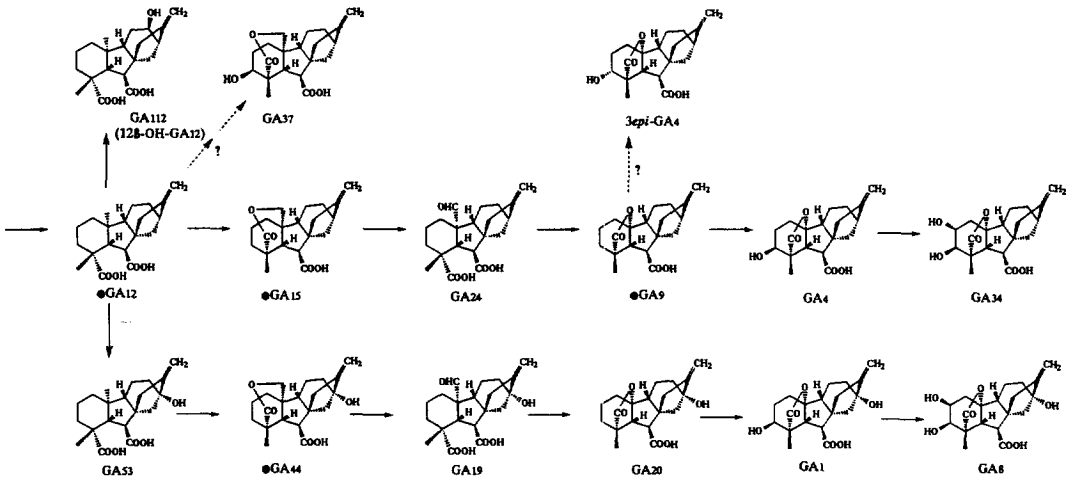

Fig. 2. Possible biosynthetic pathways of endogenous gibberellins in the shoot and flower buds of stock (*Matthiola incana* (L.) R. Br. cv. Sourei). ●: presence not shown in this experiment.

Any possible ambiguity, however, was eliminated by co-injection of the synthetic samples with the new stock GA. According to convention [10], 12α-hydroxy-GA_{12} is now identified as GA_{111} and its 12β-epimer as GA_{112}. Recent studies carried out concurrently with the present research have led to the identification of 2β-hydroxy-GA_{12} (GA_{110}) in spinach and oil palm (Mander, L. N. unpublished data), thus bringing the total of confirmed monohydroxy GA_{12} derivatives (with GA_{14} and GA_{53}) to five.

The distribution of GAs in the other cruciferous plants, namely *Thlaspi arvense* L. [11], *Arabidopsis thaliana* [12], *Brassica napus* L. [13, 14] and *Raphanus sativus* L. [15–17] are consistent with both the early-non-hydroxylation ($GA_{12} \rightarrow GA_{15} \rightarrow GA_{24} \rightarrow GA_9 \rightarrow GA_4 \rightarrow GA_{34}$) and early-13-hydroxylation ($GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1 \rightarrow GA_8$) GA biosynthetic pathways. The GA profile for the flower buds and shoots of stock (Fig. 2) is consistent with the operation of both the biosynthetic pathways. The occurrence of the new gibberellin, GA_{112}, in stock and of putative 12β-hydroxy-GA_{15} and 12β-hydroxy-GA_{24} analogues in Japanese radish (*R. sativus* L.) [15–17], encourages the speculation that an early 12β-hydroxylation pathway could be operative in the Cruciferae family.

EXPERIMENTAL

Plant material. Seeds of stock (*Matthiola incana* (L.) R. Br. cv. Sourei) were sown on 30 August, 1995, and were grown in plug trays (cell size was 3.0 cm (L) × 3.0 cm (W) × 5.5 cm (D)) containing Metoro Mix 350 (Scotts, OH, USA) in a greenhouse. When the seedlings grew into five unfolded leaves stage, they were transplanted into a plastic-film greenhouse. They were harvested on 20 November, 1995, when the plants grew into the flower bud emergence stage.

Extraction and purification of gibberellins. The harvested shoots and flower buds (500 g fr. wt) were extracted with 90% aq. MeOH. After filtration, MeOH was removed *in vacuo* at 45°. The aq. residue was adjusted to pH to 2.5 with 1 N HCl and partitioned against hexane (3 × 40 ml), then followed EtOAc (3 × 40 ml). The combined EtOAc phase was then partitioned against 0.5 M K-Pi buffer, pH 8.3 (3 × 40 ml). The combined aq. phase was mixed with PVP (5 g) then filtered. The aq. phase was adjusted to pH to 2.5 with 5 N HCl and partitioned against EtOAc (3 × 60 ml). The combined EtOAc phase was dried over Na_2SO_4. After filtration, the EtOAc fr. was evapd *in vacuo* and then dissolved in a small amount of 100% MeOH. The soln was pre-purified through a Bondesil DEA (5 g) column (packed with 100% MeOH). After sample loading, the column was washed with 100% MeOH (100 ml), and the GAs eluted with MeOH containing 0.75% HOAc (100 ml). The eluate was then reduced to dryness *in vacuo* and the residue dissolved in a small amount of 45% aq. MeOH containing 0.1% HOAc (referred to as acidic MeOH). The soln was chromatographed by HPLC on a Develosil ODS column (15 × 1 cm i.d.), eluting with a linear gradient of H_2O–MeOH (both containing 0.1% HOAc). The linear gradient elution conditions were as follows: 45 to 50% acidic MeOH for 25 min; followed 25 min from 50 to 80% acidic MeOH; 10 min with 80% acidic MeOH; and finally 20 min with 100% acidic MeOH. The total elution time was 80 min, with a flow rate of 2 ml min^{-1}, and 2 min frs were collected. The frs were dried *in vacuo* and bioassayed by a dwarf rice (cv. Tan-ginbozu) microdrop procedure [18]. The biologically active frs were further chromatographed by HPLC on Nucleosil $N(CH_3)_2$ columns (15 × 1 cm i.d.), eluted with 100% acidic MeOH at a flow rate of 2 ml min^{-1}, and 2 min frs were collected, dried and bioassayed, as already described.

Identification of gibberellins. The GAs present in shoots and flower buds of stock were identified using

GC-MS. After purification on Nucleosil $N(CH_3)_2$ columns, the frs showing gibberellin-like activity were dissolved in MeOH (20 μl) and methylated (Me) with ethereal CH_2N_2 (100 μl) at room temp. They were then dried and trimethylsilylated in glass tubes with bis-(trimethylsiyl)-trifluoroacetamide (BSTFA, 20 μl) at 75°. The derivatized samples were analysed using a JEOL Automass 20 equipped with HP 5890 GC equipment. The samples (1 μl) were injected into a fused-silica cross-linked 5% phenylmethylsilicone capillary column (15 m × 0.25 mm i.d., 0.33 μm film thickness, WCOT DB-1-15N). The oven temp. programme started at 120° and after 2 min was increased at 16° min^{-1} to 216°, then after a further 5 min was increased at 8° min^{-1} to 280°. The electron energy was 70 eV and the source temp. was 200°. The GAs were identified from mass spectra and R_ts in comparison with authentic GAs or published data [6].

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