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Identification of Endogenous Gibberellins in Inflorescence of *Ornithogalum thyrsoides*

Masaji Koshioka [1] *, Mark Roh [2], Masayoshi Nakayama [1], Tamotsu Hisamatsu [1] and Lewis N. Mander [3]

[1] *Flowering Physiology Laboratory, National Research Institute of Vegetables, Ornamental Plants and Tea, Ministry of Agriculture, Forestry and Fisheries, 360 Kusawa, Ano, Age, Mie 514 - 2392*

[2] *US Dept. Agriculture, Agricultural Research Service, Floral and Nursery Plants Research Units, Beltsville, MD 20705, USA*

[3] *Research School of Chemistry, Australian National University, Canberra ACT 2601, Australia*

Summary

Endogenous gibberellins (GAs) were extracted from inflorescence of *Ornithogalum thyrsoides* and identified by using combined gas - chromatography / mass spectrometry (GC/MS). Three 13 - hydroxylated GAs, GA_{19}, GA_{20} and GA_{53}, and thirteen 13 - non - hydroxylated GAs, GA_1, GA_7, GA_9, GA_{12}, GA_{15}, GA_{24}, GA_{25}, GA_{51}, GA_{61}, GA_{112}, GA_{115}, 1, 2 - didehydro GA_9 (which is a novel GA, and has been assigned as GA_{120}), and GA_{120} - isolactone were detected. The presence of these GAs suggests that both the early - 13 - hydroxylation GA biosynthesis pathway and the early - 13 - non - hydroxylated GA biosynthesis pathway were operating in the inflorescence of *Ornithogalum*. The presence of GA_7, GA_9 and GA_{120} suggests that GA_{120} could be considered as a metabolic intermediate in the conversion of GA_9 into GA_7 in *O. thyrsoides*.

Key Words: gibberellin, *Ornithogalum thyrsoides*.

Introduction

Ornithogalum is a bulbous geophyte and flowers late in the spring or summer, depending on the species. Recently, *O. dubium* seedlings were introduced in European countries as cut flowers and in the USA as pot plants. A new hybrid of *O. thyrsoides* that produces white flowers is now cultivated in Japan, USA, and other countries either as cut flowers or pot plants.

Bulbs become dormant during the dry season (Du Pleissis and Duncan, 1989) and exhibit periodicity in development as described by Rees (1985). Seed germination, dormancy breaking, and flowering in many plants are affected by exogenous gibberellins (GAs), particularly, GA_3 and GA_{1+7}. In *O. thyrsoides* hybrid GA_3 combined with cytokinin promoted flowering (Hisamatsu and Roh, unpublished data). The changes in GA_3 (equivalent by bioassay) levels in response to external temperatures in various parts of *O. arabicum* bulbs could not explain the physiological effect of temperature treatment (Halevy et al., 1971). Therefore, it is necessary to analyze and investigate the pathways of the endogenous GAs. This information could be applied to control growth and flowering during bulb stage and forcing. We report here the results of GA analysis from the inflorescence of *O. thyrsoides* using combined gas chromatography / mass spectrometry (GC/MS).

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*To whom correspondence should be addressed.

Materials and Methods

Inflorescences of *O. thyrsoides* hybrids (100 g fresh mass in total) were harvested when the first two to three florets opened from the plants grown in a greenhouse at 18 ℃ /16 ℃ (day/night) at the Floral and Nursery Plants Research Unit, Beltsville, MD. Samples were lyophilized and GAs were extracted to get an acetic ethyl acetate - soluble (AE) fraction at the Flowering Physiology Laboratory, Japan. The AE fraction was then purified by using ODS - HPLC and $N(CH_3)_2$ - HPLC according to the methods described previously (Koshioka et al., 1996). The GA - like activity on each chromatographic step was analyzed by a rice microdrop bioassay (Nishijima et al., 1992). GAs in fractions after the final $N(CH_3)_2$ - HPLC showing biological activity were derivatized to GA methylesters (GA - Mes) or GA methylestertrimethylsilylesters (GA - MeTMSis) with ethereal diazomethane and *N* - methyl - *N* - (trimethylsilyl)trifluoroacetamide, and finally subjected to GC/MS (HP 5989B MS - system equipped with HP 5890 GC - system) with a capillary column DB - 1 (0.25 mm i.d. × 30m, 0.25 μm film thickness) attached to a retention gap column (0.25 mm i.d. × 5 m) according the methods described by Hisamatsu et al. (1998).

Results and Discussion

Seven apparently biologically active peaks (by the rice microdrop bioassay) showing GA activity from a Develosil ODS - HPLC column were subsequently purified on Nucleosil $N(CH_3)_2$ - HPLC before GC/MS

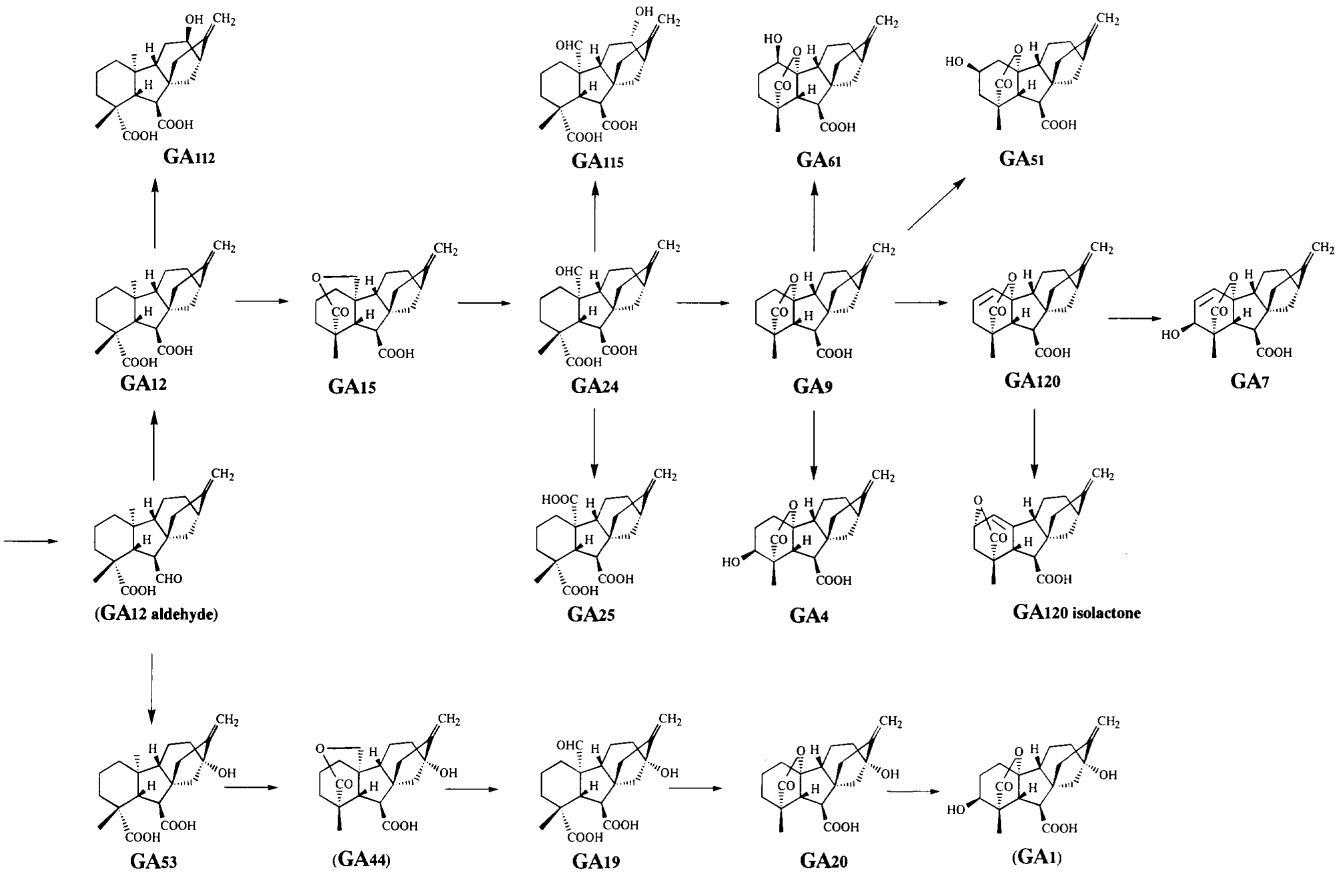

Fig. 1. Possible biosynthetic pathway for endogenous gibberellins identified in the inflorescence of *Ornithogalum thyrsoides*. GAs in the parenthesis are possibly present, but were not detected in the inflorescence.

analysis. Using a full scan mode on mass spectrometry and based on the combined information, including HPLC retention times, Kovats' retention index values (KRI; Kovats, 1958) and mass spectra compared with those of authentic protio GAs or 17,17-dideuterated GAs, fifteen GAs: GA_4, GA_7, GA_9, GA_{12}, GA_{15}, GA_{19}, GA_{20}, GA_{24}, GA_{25}, GA_{51}, GA_{53}, GA_{61}, GA_{112}, GA_{115} and 1,2-didehydro GA_9 were identified. Of these, 1,2-didehydro GA_9 is a novel GA that was first identified in peach immature seeds and has been assigned as GA_{120} (Nakayama et al., presented at the 16th IPGSA meeting, Chiba, 1998, unpublished data). The isolactone form of GA_{120} was identified as well. As GA_{120} does not easily isomerize during extraction and purification procedures, GA_{120}-isolactone was determined as a natural component in *O. thyrsoides*. The occurrence of GA_{12}, GA_{15}, GA_{24}, GA_9 and GA_4 indicates the operation of the early-13-non-hydroxylation pathway (Fig. 1). Both GA_7 (1,2-didehydro-3β-hydroxy GA_9) and GA_{120} (1, 2-didehydro GA_9), identified as endogenous GAs in *O. thyrsoides*, also lack a 13-hydroxyl in their structures. Because GA_3 (1,2-didehydro-3β-hydroxy GA_{20}) is considered to be biosynthesized from GA_5 (2, 3-didehydro GA_{20}) in higher plants and MacMillan et al. (1997) had found that in *Marah macrocarpus*, GA_7 was formed from applied 2, 3-didehydro GA_9, it has been hypothesised that this GA is the biosynthetic precursor of GA_7 in higher plants. However, 2,3-didehydro GA_9, has not yet been shown to be endogenous in any higher plants, however. Having identified GA_{120} (1,2-didehydro GA_9) in *O. thyrsoides*, we now suggest that GA_{120} may well be the natural precursor of GA_7 (Fig. 1). GA_7 is an active endogenous GA in several higher plants and in many bioassay systems and has the characteristic structure of C_{19} GA carrying a 3β-hydroxyl. It can therefore be assumed to be intrinsically biologically active, joining GA_1, GA_3 and GA_4 which have a 3β-hydroxyl in their structures.

Introduction of a 3β-hydroxyl into C_{19} GAs seems to be the final stage in the biosynthesis of active GAs such as GA_1 (3β-hydroxy GA_{20}), GA_3 (1,2-didehydro-3β-hydroxy GA_{20}), GA_4 (3β-hydroxy GA_9) and GA_7 (1,2-didehydro-3β-hydroxy GA_9) in higher plants in all biosynthesis pathways. It has been indicated that GA_5 (2,3-didehydro GA_{20}) is metabolized to GA_3 (1,2-didehydro-3β-hydroxy GA_{20}) in the early-13-hydroxylation pathway accompanied with a rearrangement of 2,3-double bond to the 1,2-location on *Prunus armeniaca* (Bottini et al., 1987), *P. persica* (Koshioka et al., 1988), *Zea mays* (Fujioka et al., 1990), *Marah macrocarpus* (Albone et al., 1990; MacMillan et al., 1997), *Phaseolus vulgaris* (Kobayashi et al., 1991) and *Oryza sativa* (Kobayashi et al., 1991). Thus so far, the

occurrence of C_{20} GAs carrying a 1,2-double bond has not been suggested as natural compounds. It is therefore concluded that GA_7 might be biosynthesized from GA_9 through GA_{120}, an intermediate metabolite in *O. thyrsoides*.

We also identified GAs carrying a 13-hydroxyl (GA_{19}, GA_{20} and GA_{53}), which suggests the presence of the early-13-hydroxylation pathway that leads to the synthesis of GA_1. However, the presence of GA_1 was not confirmed in *Ornithogalum* inflorescence. The identification of GA_{112} and GA_{115} also indicated the occurrence of an enzyme for 12-hydroxylation of C_{20} GAs in the inflorescence of *O. thyrsoides*.

Acknowledgments

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オーニソガラムシルソイデス花穂中の内生ジベレリンの同定

腰岡政二[1]・Mark Roh[2]・中山真義[1]・久松　完[1]・Lewis N. Mander[3]

[1] 野菜・茶業試験場開花制御研究室　514-2392　三重県安芸郡安濃町草生360

[2] US Dept. Agriculture, Agricultural Research, Floral and Nursery Plants Research Units, Beltsville, MD 20705, USA

[3] Research School of Chemistry, Australian National University, Canberra ACT 2601, Australia

摘　要

オーニソガラムシルソイデス (*Ornithogalum thyrsoides*) の花穂から内生ジベレリン (GA) を抽出し，GC/MSを用いて同定した．その結果，13位水酸化ジベレリンとして，GA_{19}，GA_{20}，GA_{53} の3種を，13位非水酸化ジベレリンとして，GA_4，GA_7，GA_9，GA_{12}，GA_{15}，GA_{24}，GA_{25}，GA_{51}，GA_{61}，GA_{112}，GA_{115}，1,2-didehydro GA_9 およびその isolactone 体の13種を検出した．この中で，1,2-didehydro GA_9 は最近 GA_{120} と命名された新規ジベレリンと同一のものであった．これらのジベレリンの存在は，オーニソガラム花穂中において初期13位水酸化経路と13位非水酸化経路の両方が機能していること，GA_{120} が GA_9 から GA_7 への生合成の中間体として存在することを示唆している．