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Short Communication

Gradient C_{18} high-performance liquid chromatography of gibberellins

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ABSTRACT

Retention times of 66 gibberellins (GAs) in gradient C_{18} high-performance liquid chromatography (HPLC) are reported. These include the retention times of 21 new GAs added to our previously reported 24 GAs. Retention times of the other 21 GAs are the ranges estimated from previously reported C_{18} HPLC. The polarity order (elution order) of GAs with hydroxyl groups at different locations and orientations is 12a > 13 > 11β > 16a > la > 2a > 15β > 1β > 2β > 3a > 3β.

INTRODUCTION

High-performance liquid chromatography (HPLC) is now a routine procedure for the purification and separation of gibberellins (GAs), a group of plant hormones. We have previously reported both the reversed-phase C_{18} HPLC and the normal-phase silica HPLC of GAs and their methyl esters [1]. These four HPLC systems can be sequentially used for purification and identification of radioactive GAs in metabolism studies using a radioactive flow detector. Reversed-phase C_{18} HPLC of free GAs has been the most frequently used and reported HPLC system [1–6]. For the positive identification of endogenous GAs in plants, gradient C_{18} HPLC of free GAs is usually used before bioassay and gas chromatography (GC)–mass spectrometry (MS) or GC-selected ion monitoring (SIM). We reported previously the retention times of 24 GAs in a gradient C_{18} HPLC system. Jensen *et al.* [2] reported the retention times of 42

GAs in isocratic C_{18} HPLC systems. Since gradient C_{18} HPLC can separate all the free GAs in one HPLC run, and isocratic C_{18} HPLC cannot, gradient C_{18} HPLC is the method of choice for the analysis of endogenous GAs in plants. We report here the retention times of additional GAs in our gradient C_{18} HPLC system, and incorporate some data of Jensen *et al.* [2] and Koshioka *et al.* [3] into this HPLC system to assist the analysis of GAs of plant origin.

EXPERIMENTAL

A Waters Assoc. liquid chromatograph was used which consisted of two pumps (M510), a multiwavelength detector (M490) and a data and chromatography control station (M840). The injector was a Rheodyne Model 7125. The column was a reversed-phase C_{18} column (25 cm x 4.6 mm I.D., 5 μm, Ultrasphere ODS; Beckman, San Ramon, CA, U.S.A.). The GA standards (about l-5 μg each) dissolved in less than 25 μl of methanol were chromatographed on the column. Eluent, linear gradient from 35% methanol in water (containing 0.05% acetic acid) to 100% methanol (containing 0.05% acetic acid) in 40 min; flow-rate, 1 ml/min; initial pressure, 120 bar; final pressure, 55 bar. ent-Kaurenoic acid and *ent*-kaurene (dissolved in ethanol) were eluted by the gradient followed by 100% methanol (containing 0.05% acetic acid) isocratically.

RESULTS AND DISCUSSION

Retention times of GAs in the gradient C_{18} HPLC are given in Table I in the order of elution. We have previously reported the retention times of 24 GAs in this gradient C_{18} HPLC system [1]. Since that report we can add to this list the retention times of 16 GA standard and 5 endogenous GAs from immature apple seeds [7] which were identified by GC-SIM in HPLC fractions. The retention times of 21 other GAs in Table I were estimated as ranges based on previous publications by Jensen *et al.* [2] and Koshioka *et al.* [3]. The HPLC columns and eluents used by Jensen *et al.* [2] and Koshioka *et al.* [3] were different from those of this work. However, the orders of elutions of GAs shown were the same in general. When listing the retention ranges estimated in Table I we assumed that the elution orders of GAs including the GAs not available to us were the same. There are in total 66 GAs listed in Table I. Some GAs such as GA_{48}, GA_{58}, GA_{69} and GA_{71} not listed in Table I have been purified previously by gradient C_{18} HPLC [8,9], however, several HPLC fractions were combined for the identification by GC-MS and the ranges of the estimated retention times are too large to be listed here.

Retention times [2,4] and HPLC fractions [3,5] of GAs in isocratic [2,4,6] and gradient [3,5,6] C_{18} HPLC have been reported. The GAs for which new retention data were measured in this work are as follows with references of previous works: GA_8 [2,3,5], GA_{29} [2,5], GA_{39} [2], GA_{33} [2], gibberellenic acid [3], GA_{30} [2], GA_{23} [2,5], GA_{28} [2], GA_{38} [2], GA_{41} [2], GA_{26} [2], GA_3 [2–6], 3-epi-GA_1 [3], GA_1 [2–6], GA_{29} catabolite [3], $\Delta^{1(10)}GA_1$ counterpart [3], GA_6 [2], GA_{18} [2,3,5], GA_{35} [2], GA_1 methyl ester [3], $\Delta^{1(10)}GA_1$ counterpart methyl half ester [3], GA_{22} [2], GA_{21} [2], GA_{31} [2], GA_{43} [3], GA_5 [2–6], GA_{10} [2], GA_{16} [2], GA_{20} [2–6], GA_{27} [2], GA_{47} [2], GA_{36} [2,5], GA_{13} [2–5], GA_{40} [2], allogibberic acid [3], GA_{44} [2,5], GA_{19} [2,3,5], GA_{34} [2,3], GA_{17}

TABLE I

RETENTION TIMES OF GIBBERELLINS IN GRADIENT C_{18} HPLC

For HPLC conditions, see Experimental.

Gibberellins[a]	Retention times (min)	Gibberellins	Retention times (min)
GA_{55}	5.03	GA_{27}	19.61
GA_8	5.08	GA_{47}	19.6–20.4[b]
GA_{29}	6.0–6.5[b]	GA_{36}	20.44
GA_{39}	6.0–6.5[b]	GA_{13}	20.47
iso-GA_3	6.36	GA_{68}	20.5'
GA_{32}	6.50	GA_{40}	20.5–22.0[b]
GA_{33}	6.98	Allogibberic acid	20.5–22.5[b]
Gibberellenic acid	7.42	GA_{44}	21.4'
GA_{30}	7.46	GA_{63}	21.5'
GA_{23}	7.7–8.2[b]	GA_{19}	22.41
GA_{28}	8.0–8.4[b]	GA_{54}	22.58
GA_{38}	8.2–8.6[b]	GA_{34}	22.83
GA_{41}	9.1–9.3[b]	GA_{62}	23.5
GA_{26}	9.1–9.3[b]	GA,,	23.74
GA_3	9.38	epi-Allogibberic acid	23.5–24.0[b]
3-epi-GA_1	9.41	GA,,	24.07
GA_1	10.51	GA_{61}	24.5'
GA_{29} catabolite	10.5–11.0[b]	GA_{51}	24.5'
$\Delta^{1(10)}GA_1$ counterpart	11.32	3-epi-GA_4	24.42
GA_6	11.5–12.3[b]	GA,	24.92
GA_{18}	12.0–12.5[b]	iso-GA_7	25.0–26.0[b]
GA_{35}	12.89	GA4	26.07
GA_1 methyl ester	13.40	GA_{53}	27.81
$\Delta^{1(10)}GA_1$ counterpart methyl half ester	13.2–13.6[b]	GA_{14}	28.17
GA_{22}	13.61	GA_{24}	28.86
GA_{21}	14.25	GA_9	29.36
GA_2	14.96	GA_{25}	29.54
GA_{31}	16.07	GA_{15}	29.76
GA_{43}	14.5–17.5[b]	GA_4 methyl ester	29.0–31.0[b]
GA_5	17.86	GA_{12}	32.0–38.0[b]
GA_{10}	17.8–18.4[b]	GA_{12}-ald	38.0–44.0[b]
GA_{16}	18.42	*ent*-Kaurenoic acid	44.82
GA_{20}	18.97	*ent*-Kaurene	60.72

[a] For structures of GAs, see refs. 3 and 10.

[b] The ranges of elution times were estimated from the data of Jensen et *al.* [2] and Koshioka et *al.* [3] (fraction/min).

[c] Detected by GC-SIM in HPLC fractions (fraction/min) from immature apple seeds [7]. The ranges are ± 0.5 min.

[2,5], epi-allogibberic acid [3], GA_{37} [2,5], GA_{51} [2], 3-epi-GA_4 [3], GA_7 [2–6], iso-GA_7 [3], GA_4 [2–6], GA_{53} [2,3], GA_{14} L-51, GA_{24} [2], GA_9 [2–6], GA_{25} [3–5], GA_{15} [2], GA_4 methyl ester [3], GA_{12} [2,3,5], GA_{12}-ald [2,3], *ent*-kaurenoic acid [3] and *ent*-kaurene [3].

The more polar compounds elute sooner from reversed-phase C_{18} HPLC. We have previously given the polarity order (elution order) of GAs with hydroxyl groups at different locations and orientations as $12\alpha > 16a > 13 > 11\beta > 1a > 2a > 1\beta >$

$2\beta > 3\alpha > 3\beta$. Based on the retention times of additional GAs reported in Table I, 15β-hydroxyl GAs can be included in this order and the location of 16α-hydroxyl GAs in this polarity order is modified. The new polarity order is $12\alpha > 13 > 11\beta > 16\alpha >$ la $> 2\alpha > 15\beta > 1\beta > 2\beta >$ 3a $> 3\beta$. Alloftheexamples we can find in Table I are as follows: $12\alpha >$ 13; $GA_{31} > GA_5$, $GA_{30} > GA_3$. $GA_{39} > GA_{28}$. 13 $> 11\beta$; $GA_1 > GA_{35}$. $11\beta >$ 16a; $GA_{35} > GA_2$. 16a > la; $GA_2 > GA_{16}$. la $> 2\alpha$; $GA_{16} > GA_{47}$. 2α > 15β; $GA_{47} > GA_{63}$. 15β > 1β; GA_{63} > GA_{54}. $1\beta > 2\beta$; $GA_{55} > GA_8$, $GA_{54} > GA_{34}$. $2\beta >$ 3a; GA$_{29}$ > 3-epi-GA,. $3\alpha > 3\beta$; 3-epi-$GA_1 >$ GA_1, 3-epi-$GA_4 >$ GA,+. All of the examples given here are without any exception in the polarity order, while the examples in the polarity order we gave previously [1] had one exception. Gibberellins with a double bond are more polar than those without the double bond in the C_{18} HPLC system and examples are as follows: $GA_3 > GA_1$, $GA_7 > GA_4$, GA_5 > GA_{20}, $GA_{62} > GA_{61}$, $GA_{68} > GA_{63}$.

The approximate retention times of GAs not listed in Table I can be predicted using the retention times in Table I and the retention properties given here. The HPLC of $GA_{51,61,62,63,68}$ have not been previously reported and the standards are not presently available to us. We successfully predicted the retention times of these GAs in immature apple seeds [7] as demonstrated by GC-SIM of the correct HPLC fractions using the retention times in Table I and retention properties given here. Since 1-min fractions were collected, the retention times given in Table I are in the range of $\pm$ 0.5 min. The retention time of GA_{60} is not given in Table I, but it is eluted between GA_8 and GA_1 [11], which agrees with the retention properties presented.

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