Phytochemistry, 1971, Vol. 10, pp. 2799 to 2802. Pergamon Press. Printed in England.

SHORT COMMUNICATION

GAS–LIQUID CHROMATOGRAPHY OF ENDOGENOUS GIBBERELLINS IN TOMATO, *LYCOPERSICON ESCULENTUM**†

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(*Received* 17 *December* 1970, *in revised form* 29 *March* 1971)

Abstract—Evidence for the identity of several gibberellins present in the trimethylsilylated extracts from both the single gene tomato mutant, *yellow-green* 6, and the normal wild type tomato are presented. Gibberellins A_3, A_8, A_9, A_4 and/or A_7 appear to occur in these two tomatoes. Tentative identification was accomplished by gas–liquid chromatography.

INTRODUCTION

ALTHOUGH different gibberellins have been isolated and identified in many higher plants,[1,2] little information is available about the identity of endogenous gibberellins occurring in the tomato, *Lycopersicon esculentum*. Earlier work on tomato gibberellins involved paper chromatographic studies. Hill and Selman[3] reported the occurrence of two gibberellin-like substances in the acidic fraction of shoot extracts of tomato (cv. 'Potentate') but the identity was not resolved. Pegg[4] found gibberellin-like substances not only in the acidic fractions but also in the basic and neutral fractions of extracts from seed and etiolated seedlings of the same variety of tomato. Several workers[5–7] found a gibberellin-like substance with an R_f which coincided with that for either gibberellin A_1 or A_3 in both shoot and root extracts of tomato of different varieties. Two gibberellin-like substances, the R_f of which corresponded to that of $GA_1 + GA_3$ and $GA_4 + GA_7$, recently were reported occurring in plant tip extracts of a tall variety of tomato, 'Winsall', and a dwarf variety, 'Tiny Tim'.[8]

The feasibility of GLC for the separation and identification of pure gibberellins (GAs) was first reported by Ikekawa and Sumiki.[9] They successfully separated the methyl esters prepared from 9 gibberellins with diazomethane. However, because of the highly explosive nature of diazomethane, its general use for esterification of plant hormones has been limited.[10]

* Portion of the dissertation submitted by A. T. Perez to the University of Massachusetts in partial fulfillment of the requirements for the Ph.D. degree.

† Part III in the series "Physiology of the yg_6 Gene in Tomato".

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[2] A. T. PEREZ, Ph.D. Thesis, "The Role of the Gibberellins in the Physiological Action of the *Yellow–Green* 6 Gene in Tomato, *Lycopersicon esculentum*." University of Massachusetts (1970).

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Sweeley *et al.*[11] reported a safe method of silylating carbohydrates by using trimethylsilyl derivatives (TMS). Davis *et al.*[10] successfully applied GLC techniques to TMS ether derivatives of abscisic acid, gibberellic acid and indoleacetic acid which were prepared by reacting with bis-(trimethylsilyl) acetamide.

We now report the presence of gibberellins A_3, A_8, A_9, A_4 and/or A_7 in both the single gene tomato mutant, *yellow-green* 6, and the normal wild type tomato, *L. esculentum* var. 'cerasiforme' Line 018. The tentative identification was undertaken using GLC of TMS derivatives from acidic extracts of tomato. This paper also reports the retention times of TMS derivatives of 7 standard gibberellins on 3 chromatographic columns, namely 5% SE-30, 5% SE-32 and 5% OV-22.

RESULTS AND DISCUSSIONS

In our previous paper[12] we reported that the single mutant gene yg_6 appears to be responsible for the greater amount of gibberellins found in the mutant tomato as compared to that of the normal wild type tomato. It was also found that the yg_6 mutant gene did not cause any qualitative change, only a quantitative change in the gibberellins occurring in the tomato mutant when compared to that of the normal tomato. Since the extracts of both the yg_6 and the normal wild type tomato seedlings were available, both extracts were used in the identification studies.

The first technique employed in the identification of tomato gibberellins was the comparison of retention times between those of the standard GAs and of the compounds present

TABLE 1. RETENTION TIMES OF TMS DERIVATIVES OF STANDARD AND TOMATO GIBBERELLINS*

Gibberellins	Retention time (min)		
	SE-30†	SE-52‡	OV-22§
Standard GAs			
A_1	27·8	31·0	16·3
A_3	27·9	30·2	16·5
A_4	26·8	28·8	16·2
A_5	26·5	28·2	16·1
A_7	26·8	28·6	16·2
A_8	28·7	32·2	16·4
A_9	25·5	26·1	15·8
Tomato GAs			
(A_8)	28·8	(A_8) 32·4	—
(A_1/A_3)‖	27·8	(A_3) 30·1	(A_3) 16·6
(A_4/A_7)‖	26·8	—	(A_4/A_7)‖ 16·2
(A_9)	25·6	—	(A_9) 15·8
	—	(A_5) 28·1	—

* Perkin–Elmer Model 900 gas chromatograph with dual columns and dual flame ionization detectors was employed. Carrier gas was N_2, used at a flow rate of 40 ml/min.

† Initial temp. of 70° maintained for 6 min followed by programming at 10°/min to a final temp. of 290°.

‡ Initial temp. of 150° maintained for 6 min followed by programming at 5°/min to a final temp. of 290°.

§ Initial temp. of 150° maintained for 6 min followed by programming at 20°/min to a final temp. of 290°.

‖ Not resolved.

[11] C. C. SWEELEY, R. BENTLEY, M. MAKITA and W. W. WELLS, *J. Am. Chem. Soc.* **85**, 2497 (1963).

[12] A. T. PEREZ, H. V. MARSH, JR. and W. H. LACHMAN (in preparation.)

in the tomato extracts. The retention times of 7 standard GAs and of the tomato gibberellins on 3 different chromatographic columns employing a Perkin–Elmer Model 900 gas chromatograph are shown in Table 1. Based on the data in Table 1, gibberellins A_8, A_3, A_9, A_4 and/or A_7 appear to be present in the TMS extracts of the tomato. No attempt was made to identify the other nine GLC peaks obtained from the tomato because of a lack of standard

Previously, two chromatographic columns were found to satisfactorily separate standards. GAs. Ikekawa and Sumiki[9] reported complete separation and identification of the methyl esters of 9 gibberellins (A_1–A_9) using 1·5% SE-30 and 2% QF-1-0065. Similarly, MacMillan and Pryce[13] reported satisfactory separation of methyl esters and in addition, TMS ethers of methyl esters of 23 gibberellins (A_1–A_{23}) using 2% SE-33 and 2% QF-1. The feasibility of separating several TMS derivatives of gibberellins using OV-22 column, in addition to SE-30 and SE-52 columns was determined. As shown in Table 1, among the three columns studied under our chromatographic condition, OV-22 appears to be unsuitable for separating the TMS gibberellins.

Another technique employed to identify the tomato gibberellins was co-chromatography. One or more TMS standard gibberellins were added to a sample of tomato extract which was then injected into a Varian Aerograph HY-FI Model 600-D, equipped with a flame ionization detector and 5% SE-30 column and was supplied with N_2 as the carrier gas at a flow rate of 25 ml/min. It was found that four tomato GLC peaks co-chromatographed with 4 of the standard gibberellins: one with GA_3 (retention time of 22·5 min), one with GA_4 and/or GA_7 (21·0 min), one with GA_8 (23·6 min) and one with GA_9 (18·4 min). Gibberellins A_1 and A_5 did not co-chromatograph with any of the tomato GLC peaks.

EXPERIMENTAL

The single gene tomato mutant, *yellow-green* 6 (*yg*$_6$), and the normal tomato, *Lycopersicon esculentum* var. 'cerasiforme' Line 018 from which the mutant was derived by seed irradiation[14] were used in this study. The tomato mutant differs from the normal wild type by a single recessive gene *yg*$_6$, located on chromosome 11 at map position 50 between the genes hairless and anthocyaninless.[15]

Preparation of tomato extract. Tomato seedlings were grown in the greenhouse in soil. The shoots and cotyledons of 6-week-old plants were harvested and extracted in MeOH (1:2, w/v). Following a 24-hr soaking in MeOH, each sample was macerated, and filtered through 4 layers of cheesecloth. The filtrate was passed twice through 2 layers of No. 1 Whatman filter paper using vacuum. The MeOH was evaporated *in vacuo* and the aqueous residue was first extracted 4× with light petroleum (b.p. 30–60°) at pH 5·0 followed by 3× with $CHCl_3$ at pH 7·0.[16] The final aqueous extract resulting from the last extraction was adjusted to pH 3·0. A portion of this aqueous acidic extract was dried thoroughly.

Preparation of TMS derivatives. The method of Davis *et al.*[10] was followed in preparing the trimethylsilylated derivatives using the reagent bis-(trimethylsilyl) acetamide (BSA). The thoroughly dried acidic extracts, either from the *yg*$_6$ or the wild type tomato, were treated with 1 ml of the reagent BSA (Pierce Chemical Co., Rockford, Illinois, U.S.A.) shaken vigorously and allowed to stand for at least 30 min to ensure satisfactory silylation. Standards of gibberellins A_1, A_3, A_4, A_5, A_7, A_8 and A_9 were silylated with the BSA reagent in an identical manner.

GLC. Two types of gas chromatographic instruments, a Perkin–Elmer Model 900 and a Varian Aerograph HY-FI Model 600-D, were used in this study. The Perkin–Elmer Model 900 with dual columns and dual flame ionization detectors was employed using the following parameters: (a) 5% SE-30 column, 152·4 × 0·32 cm, acid washed and DMCS treated Chromosorb W, initial temp. of 70° maintained for 6 min followed by programming at 10°/min to a final temp. of 290°; (b) 5% SE-52 column, 274·3 × 0·32 cm, acid washed and DMCS treated Chromosorb W, initial temp. of 150° maintained for 6 min followed by programming at 5°/min to a final temp. of 290°; or (c) 5% OV-22 column, 121·9 × 0·32 cm, acid washed and DMCS treated Chromosorb W, initial temp. of 150° maintained for 6 min followed by programming at 20°/min

[13] J. MACMILLAN and R. J. PRYCE, in *Society of Chemical Industry Monograph*, No. 31, 36 (1968).
[14] A. B. BURDICK, *Tomato Genetics Coop.* **10**, 8 (1960).
[15] I. A. DE LA ROCHE and W. H. LACHMAN, *J. Hereditary.* **58**, 147 (1967).
[16] D. KOHLER and A. LANG, *Plant Physiol.* **38**, 555 (1963).

to a final temp. of 290°. The carrier gas used with the Perkin–Elmer instrument was N_2 at a flow rate of 40 ml/min.

The Varian Aerograph was equipped with a flame ionization detector and isothermal temperature control. GLC was carried out using 5% SE-30 column coated on 60/80 mesh, 152·4 × 0·32 cm, acid washed, DMCS treated Chromosorb W. Carrier gas was N_2, at a flow rate of 25 ml/min. A column temp. of 70° for the first 6 min was employed followed by programming at 10°/min to a final temp. of 290°. The injector and detector temperatures were both kept at approximately 200°. In all instances, a total sample of 3 μl was injected into the gas chromatograph.

Identification of gibberellins by GLC. The following techniques were employed to identify the endogenous gibberellins from the yg_6 and wild type tomatoes: (1) comparison of the retention times of the various GLC peaks of the tomato extracts with the retention times of 7 standard GAs on 3 different chromatographic columns, and (2) co-chromatography. Co-chromatography was carried out by adding one or more trimethylsilylated standard GAs to a sample of silylated tomato extract which was then injected into the GLC. The added standard GA caused an increase in the size of the particular tomato GLC peak with which they co-chromatographed, indicating that the standard GA and the unknown tomato peak in fact are identical.

Acknowledgements—We are indebted to Drs. I. S. Fagerson and R. J. Croteau, Department of Food Science, and to Dr. J. H. Baker, Department of Plant and Soil Sciences for their assistance. One of us (A.T.P.) would like to thank the East-West Center, Honolulu, Hawaii for the scholarship (1965–1967) awarded to him.