Phytochemistry, 1971, Vol. 10, pp. 215 to 217. Pergamon Press. Printed in England.

SHORT COMMUNICATION

POSSIBLE IDENTIFICATION OF GIBBERELLINS IN *TULIPA GESNERIANA* BY GAS–LIQUID CHROMATOGRAPHY

L. H. AUNG

Virginia Polytechnic Inst., Blacksburg, Va., U.S.A.

and

A. A. DE HERTOGH and G. L. STABY

Michigan State University, East Lansing, Michigan, U.S.A.

(*Received* 19 *February* 1970, *in revised form* 31 *March* 1970)

Abstract—Gas–liquid chromatography of trimethylsilyl ethers of methyl esters (TMS) of partially purified extracts of tulip bulb organs on 3% OV-1 column suggested the presence of gibberellins A_1, A_5, A_8, A_9 and A_{13}. Gibberellins A_1, A_5, A_8 and A_9 in the fleshy scales, gibberellin A_1 in the shoot, and gibberellins A_5, A_9 and A_{13} in the roots. Many of the TMS derivatives did not, however, correspond with any of the gibberellin samples available. The TMS derivatives were found to be biologically active in the dwarf pea bioassay.

INTRODUCTION

PREVIOUS STUDIES,[1–3] using bioassay procedures, have revealed the presence of gibberellin-like substances in tulip bulbs. It was shown that the quantitative and qualitative nature of these substances were altered by subjecting the bulbs to either varying periods of cold[2] or by different temperature regimes.[3] At present, no evidence is available as to the identity of these growth hormones in any bulbous plant. In this study, the nature of some of the gibberellins in various organs of tulip was examined in order to provide a basis for understanding the physiological responses of the tulip during its developmental cycle.

RESULTS

The GLC results of trimethylsilyl ethers of the methyl esters (TMS) of authentic gibberellins and partially purified extracts of 3 tulip bulb organs are summarized in Table 1. When the retention times of the derivatives of various extracts are compared with the authentic gibberellins, a tentative identification of several gibberellins can be made. In the roots, we tentatively identified gibberellins A_5 and A_{13} in the free fraction and gibberellin A_9 in the bound fraction. The only identification of gibberellins which could be made in the shoots was gibberellin A_1 in the free fraction. The scales appear to have gibberellins A_1, A_5, A_8 and A_9 in the free fraction, and gibberellin A_9 in the bound fraction. While the results must remain tentative in view of the fact that only a single type of column (3% OV-1) was used, the data on retention times of the TMS derivatives were quite reproducible.

It is quite apparent from Table 1 that a large number of the derivatives in the various extracts did not correspond to any of the samples which were available for this study. It may be of interest to note, however, that common peaks with retention time of 5·4 min, 8·8 min and approximately 40 min were seen in the three organs studied. The possible significance of this observation is not known.

[1] L. H. AUNG and A. A. DE HERTOGH, *Plant Cell Physiol.* **8**, 201 (1967).
[2] L. H. AUNG and A. A. DE HERTOGH, *Biochemistry Physiology Plant Growth Substances*, p. 943, Runge Press, Ottawa (1968).
[3] L. H. AUNG, A. A. DE HERTOGH and G. STABY, *Plant Physiol.* **44**, 403, (1969).

TABLE 1. RETENTION TIME (min) OF TRIMETHYLSILYL ETHERS OF METHYL ESTERS OF KNOWN GIBBERELLINS AND PARTIALLY PURIFIED EXTRACTS FROM FLESHY SCALES, SHOOTS AND ROOTS OF *Tulipa gesneriana* CV. *Elmus*

Gibberellin standards*	Organ and fraction assayed†					
	Roots		Shoots		Scales	
	Free	Bound	Free	Bound	Free	Bound
	4·8	5·0	—	—	—	—
	5·4	—	—	5·5	—	5·4
A_9 (6·5)	—	6·5	—	—	6·5	6·5
	—	—	—	—	8·0	—
	8·8	9·1	—	8·6	—	8·9
A_5 (10·9)	10·9	—	—	—	11·0	—
	—	—	—	—	—	11·3
A_4, A_6, A_7 (11·8)	—	—	—	—	—	—
	—	12·5	—	—	—	—
A_3 (13·0, 14·5, 16·5)	13·9	—	14·0	13·3	—	—
A_{13} (15·5)	15·8	—	—	—	—	—
	—	—	—	—	—	17·8
A_1 (18·8)	—	—	18·3	—	18·5	—
	20·5	—	—	—	21·0	—
	—	—	21·8	—	—	—
	—	—	26·4	—	—	—
A_2 (27·9)	—	—	—	—	—	—
A_8 (29·8)	—	—	—	—	29·4	—
	31·4	—	—	—	—	—
	—	—	37·0	—	—	—
	40·3	—	40·5	—	—	39·5

* Only the methyl ester of gibberellin A_9 was chromatographed. The major peak of gibberellin A_3 was located at 14·5 min and two minor peaks at 13·0 min and 16·5 min.

† Free and bound fractions refer to the extracts obtained before and after acid hydrolysis respectively (see Ref. 3).

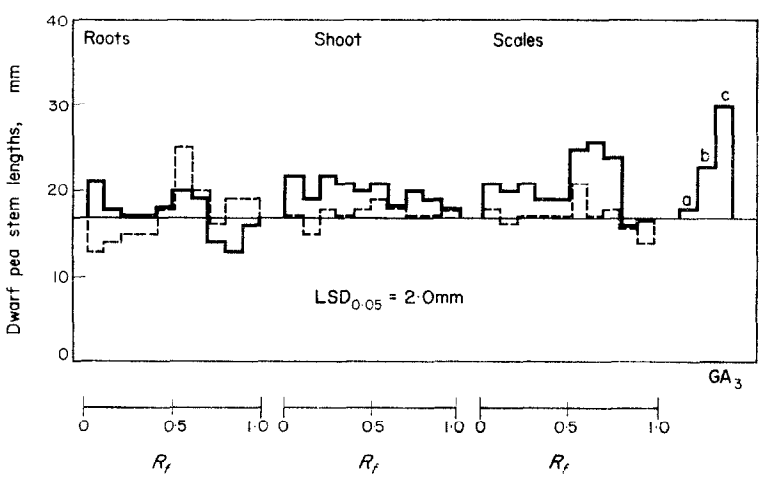

FIG. 1. BIOLOGICAL ACTIVITY OF TRIMETHYLSILYL ETHERS OF METHYL ESTERS OF ELMUS (14 weeks AT 9°) TULIP BULB EXTRACTS AFTER PAPER CHROMATOGRAPHY (SOLVENT, ISOPROPANOL–AMMONIUM HYDROXIDE–WATER, 10:1:1, by vol.) IN THE DWARF PEA ASSAY

Solid line represents the free fraction and dotted line the bound fraction. Symbols a, b and c indicate 10^{-4}, 10^{-3} and 10^{-2} μg gibberellin A_3/ml respectively.

A test was also made to determine if the methylation and trimethylsilylation procedures destroyed the biological activity of the extracts. The results (Fig. 1) show that these procedures did not destroy the biological activity of the extracts. This suggests that either the derivatives *per se* were active or that the pea plants used for bioassay possess the ability to hydrolyze the derivatives to form active gibberellins.

DISCUSSION

The complete identification of the gibberellins in the bulb extracts was prevented by a lack of a complete set of authentic gibberellin standards. Therefore, a more complete identification of the bulb gibberellins must await the availability of known gibberellin standards or the use of GLC-mass spectrometry technique.[4]

The compound corresponding to gibberellin A_9 is distributed in the bound fraction of the roots and in the free and bound fractions of the scales, but absent from the shoot fractions (Table 1). This suggests the possibility that gibberellin A_9 may be native to or have some special function in the roots and scales. Gibberellin A_9 may serve as an intermediate in the biosynthesis of other gibberellins,[5] or may directly affect plant function in its own right.[6,7]

Davis *et al.*[8] have shown that the TMS derivatives obtained by direct trimethylsilylation of abscisic acid and gibberellin A_3 are active in the cotton explant bioassay. Our data (Fig. 1), on the other hand, show that derivatives prepared by methylation and trimethylsilylation of extracts from the tulip bulbs are also active in the dwarf pea bioassay.[3] Thus, in future work it should be possible to collect the various GLC effluents of plant extracts and if present in sufficient quantities, test them for biological activity.

EXPERIMENTAL

Plant material. Elmus bulbs of the 1967 harvest were used. A sample of 600 bulbs, with differentiated flower primordia, were planted in wooden boxes containing a medium of loam, sand and peat-moss (1:1:1, v/v). The bulbs were kept watered at 9° in a dark storage room for 14 weeks (14 December 1967–2 April 1968). On completion of the treatment the rooted bulbs with an average sprout length of 12 cm were cleansed in cold tap-water and separated into fleshy scales, shoots and adventitious roots for extraction.

Extraction and purification. 4 kg of shoots, 3·4 kg of healthy fleshy scales and 0·9 kg of roots were used for extraction. The samples were frozen in liquid N_2 and homogenized with cold (5°) absolute MeOH in a Waring blender. The homogenates were shaken for 48 hr at 25°. The subsequent steps involving filtration, evaporation of the organic solvent, partitioning and final ethyl acetate extraction were identical to procedures already reported.[3]

Preparation of Samples and GLC. The partially purified bulb extracts and known gibberellin standards in absolute MeOH (1–2 ml) were treated with CH_2N_2.[9] The dried methylated samples were dissolved in pyridine and treated with pyridine, hexamethyldisilizane and trimethyl silyl chloride (10:2:2, by vol.) as reported by Cavell *et al.*[10] The methyl ester of gibberellin A_9 and trimethylsilyl ethers of the methyl esters of authentic gibberellins (A_1–A_8, A_{13}) and plant extracts were chromatographed by injecting a 0·5–2 μl of the entire sample mixture into a gas chromatograph (Hewlett-Packard Model 402 equipped with a hydrogen flame ionization detector). The glass column, 1·8 m × 3 mm i.d., was packed with 3% OV-1 on 100/120 mesh chromosorb W. The column temperature was 220° isothermal and the carrier gas (A) was at a flow rate of 60 ml/min^{-1}.

Acknowledgements—We thank Dr. W. W. Wells of the MSU Biochemistry Department for his assistance and use of the GLC facilities, and Abbott Laboratories for providing reference samples of gibberellins A_4, A_7 and A_{13}. Research supported in part by a grant from the Netherlands Flower-Bulb Institute, N.Y. and the Ornamental Marketing Board, The Hague.

[4] R. Binks, J. MacMillan and R. J. Pryce, *Phytochem.* **8**, 27 (1969).
[5] B. O. Phinney and C. Spector, *Ann. N.Y. Acad. Sci.* **144**, 204 (1967).
[6] P. W. Brian, H. G. Hemming and D. Lowe, *Ann. Botany London* **28**, 369 (1964).
[7] L. Paleg, D. Aspinall, B. Coombe and P. Nicholls, *Plant Physiol.* **39**, 286 (1964).
[8] L. A. Davis, B. E. Heinz and F. T. Addicott, *Plant Physiol.* **43**, 1389 (1968).
[9] H. Schlenk and J. L. Gellerman, *Anal. Chem.* **32**, 1412 (1960).
[10] B. D. Cavell, J. MacMillan, R. J. Pryce and A. C. Sheppard, *Phytochem.* **6**, 867 (1967).