Phytochemistry, Vol. 21, No. 2, pp. 391–393, 1982.
Printed in Great Britain.

0031-9442/82/020391-03$03.00/0

HORMONES OF YOUNG TASSELS OF *ZEA MAYS*

PETER HEDDEN,* BERNARD O. PHINNEY, RICK HEUPEL, DWIGHT FUJII, HOWARD COHEN, PAUL GASKIN,† JAKE MACMILLAN† and JAN E. GRAEBE‡

Department of Biology, University of California, Los Angeles, CA 90024, U.S.A.; †School of Chemistry, The University, Bristol BS8 ITS, U.K.; ‡Pflanzenphysiologisches Institut der Universität, Göttingen, West Germany

(*Received* 31 *July* 1980)

Key Word Index—*Zea mays*; Gramineae; tassels; GC/MS and selected ion monitoring; GA_1; GA_8; GA_{17}; GA_{19}; GA_{20}; GA_{29}; GA_{44}; GA_{53}; ABA; phaseic acid; dihydrophaseic acid.

Abstract—The ethyl acetate-soluble acids from an aqueous methanolic extract of young tassels from *Zea mays* plants were fractionated by treatment with PVP, then by chromatography on a column of celite–charcoal. Methylated and trimethylsilylated fractions were analysed by GC/MS and the following compounds were identified by comparison with reference spectra: GA_{17}, GA_{19}, GA_{20}, GA_{44}, GA_{53}, ABA, phaseic acid and dihydrophaseic acid. Evidence is also presented for the presence of metabolite C of ABA and of a 16,17-dihydro-17-hydroxy-derivative of GA_{53}. In addition, the presence of small amounts of GA_1, GA_8 and GA_{29} was indicated from a derivatized fraction analysed by capillary GC/SICM.

INTRODUCTION

We are presently examining GA-biosynthesis and its control of growth and development in normal and GA-deficient dwarf mutants of *Zea mays*. This program is dependent on the identification of the native GAs in maize. To obtain such information we initiated preliminary studies on the presence of GA-like substances in ears and tassels of maize. Relatively high levels of GA-like substances were obtained by bioassay from extracts of young tassels and these extracts were studied further by GC/MS. (The choice of material was based on the observation by Kaufman *et al.* [1] that young inflorescences of oats (*Avena sativa*) have relatively high levels of these substances.) Neutral–basic, acidic EtOAc and acidic *n*-butanol fractions were obtained from an aqueous MeOH extract of the tassels. Of these the acidic EtOAc extracts were found to contain most of the GA-like activity.

RESULTS AND DISCUSSION

An aliquot of the acidic EtOAc fraction was chromatographed on a celite–charcoal column which was eluted with Me_2CO–H_2O. The fractions from the column were methylated and trimethylsilylated and examined by GC/MS using a 2% SE-32 column. The following compounds were identified by comparison of their mass spectra with reference spectra: GA_{17}(**1**), GA_{19}(**2**), GA_{20}(**3**), GA_{44}(**4**), GA_{53}(**5**), ABA(**6**), phaseic acid (PA)(**7**) and dihydrophaseic acid (DPA)(**8**). In addition to the identified compounds, the derivatized material from the first column fraction contained a partially resolved GC-peak which, on GC/MS, gave a mixed mass spectrum indicating the presence of three methyl ester TMSi derivatives with M^+ *m/z* 506, 506 and 594. Using a 2% QF-1 column the derivative with M^+ 594 had a much shorter R_t and may be the methyl ester TMSi derivative of a GA-derived dicarboxylic acid. The derivatives with M^+ 506 were not resolved on this column; the mass spectrum contained the prominent ions of GA_1 and GA_{29} methyl ester TMSi but was too weak and was contaminated with background to such an extent that the relative intensities of the distinguishing ions *m/z* 303 (GA_{29}) and 448 (GA_1) were unreliable for identification.

A new compound was also detected and is tentatively assigned the structure **12**, (16,17-dihydro-17-hydroxy GA_{53}) from the mass spectrum of the methyl ester TMSi derivative. Fragment ions at *m/z* 407 ($M^+ - 131$), 131, and 375 (407–32) with the possible structures **14**, **15** and **16**, respectively, indicated the ring D structure, shown in (**12**). Ions at *m/z* 103, from fission of the 16,17-bond, and at *m/z* 147, from the adjacent TMSi ethers, also support structure **12**.

Finally GC/MS of the derivatized first column fraction indicated the presence of a compound identical to, or isomeric with, the ABA metabolite C (**13**) reported by Milborrow[2]. No reference spectrum was available for direct comparison and the structure was deduced from mass spectral features alone. However, some confirmation of the presence of **13** was obtained from the following observation. When the methylated fraction was heated under vacuum for some time, then trimethylsilylated, GC/MS revealed the absence of the compound, while the previously detected small mass peak of phaseic acid had increased in intensity to *ca* that of the original metabolite C. This result is consistent with the conversion of the methyl ester of **13** into phaseic acid (**7**) by heating.

A second aliquot from the same acidic fraction was examined by capillary GC/MS with selected ion monitoring (GC/SICM). After purification on the cel-

*Present address: Pflanzenphysiologisches Institut der Universität, Untere Karspüle 2, D-3400 Göttingen, West Germany.

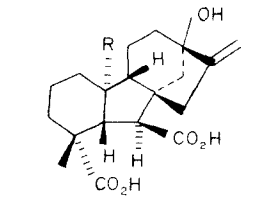
ite–charcoal column, the first fraction to be eluted was derivatized to methyl esters TMSi ethers and analysed using a SP-2100 WCOT column. Ions at *m/z* 506, 491, 377 and 375 were monitored. The two components with M^+ 506 were resolved and present in the ratio of *ca* 1:2, assuming equal relative intensities for the M^+ ions. The minor component also showed the other ions monitored with the same relative intensities as for GA_1 methyl esters TMSi. Furthermore, this component co-chromatographed with authentic GA_1 methyl ester TMSi. The major component showed the ions monitored with the relative intensities expected for GA_{29} methyl ester TMSi (*m/z* 377 was absent). No authentic GA_{29} was available for co-chromatography but the R_f of this component was similar to that observed in an extract from pea seedlings containing GA_{29}. A third component with M^+ 594 was found to be present by mass fragmentography. Ions at *m/z* 594, 579, 536 and 488 (ions present in the mass spectrum of GA_8 methyl ester TMSi) were monitored. The ions at *m/z* 579 and 536 were not observed, but the other two were present at the R_f expected for GA_8 methyl ester TMSi. The coincidence of R_f for this component and GA_8 methyl ester TMSi was confirmed by co-injection. Thus, the presence of GA_1(**9**), GA_8(**10**) and GA_{29}(**11**) in the maize tassels is also indicated although at concentrations much lower than the other GAs.

It is now common practice, especially with microorganisms, to use mutants as a tool in physiological and biochemical studies. Through this approach, an apparently complicated series of biochemical reactions can be separated into individual components, each mutant of which controls a specific step in the biosynthetic series. It is the availability of mutants that usually limits the approach. *Zea mays* is particularly useful in this respect because there are available a number of dwarf mutants (e.g. d_1, d_2, d_3, d_5, an) which apparently control GA biosynthesis [3–7]. GA-like activity is small or absent in these mutants and it has been shown that the d_5 mutant controls the cyclization step leading to the GA precursor, *ent*-kaurene [8].

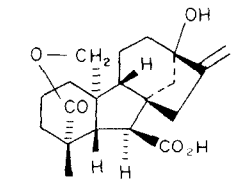
The identification of the eight GAs from maize tassels provides a qualitative basis for the quantification of endogenous levels of GAs in organs of both normal and dwarf types. This identification is also a starting point for biosynthetic studies since the identified C_{20} GAs in maize differ only in the oxidation state of C-20. They may thus represent a biosynthetic sequence from GA_{53} leading to the C_{19} GAs, GA_{20}, GA_{29}, GA_1 and GA_8. We are presently investigating this possibility in maize by feeding [^{3}H]GAs to the d_5 mutant and following the fate of the label in terms of GA-metabolites. It is interesting that the combination of GAs present in maize has recently been identified by GC/MS from broad bean (*Vicia faba*)[9], spinach (*Spinacia oleracea*)[10] and corn cockle (*Agrostemma githago*)[11].

EXPERIMENTAL

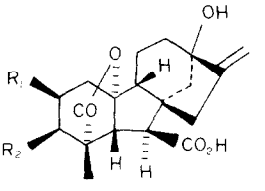
Young tassels were harvested from *Zea mays* cv. Burpee's Hybrid grown at the USDA Cotton Field Station in Shafter, California. Plants *ca* 100 cm in ht were collected and the young tassels (5–10 cm length) were dissected from the enclosed leaves and immediately frozen in dry ice. The frozen tassels (1.03 kg) were homogenized at 5° in 75% MeOH–H_2O (8 l.) using a Waring Blender. The homogenate was stirred for 48 hr at 5°, filtered through PVP (80 g) on a bed of celite on filter paper, and the residue and PVP washed with cold MeOH (1 l.). The MeOH was removed from the combined filtrate and washings, *in vacuo*, at 30° and the residual aq. extract (1.8 l.) stirred with PVP (70 g) overnight at 5°. PVP was removed by filtration through celite and washed with M K–Pi buffer at pH 8 (800 ml), after which the combined filtrate and washings were concd *in vacuo* at 30°. The aq. extract (1.1 l.) was buffered at pH 8 and partitioned against EtOAc (6 × 300 ml). The pH of the aq. phase was then adjusted to 3.5 with conc HCl and the



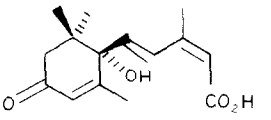
1 R = CO_2H
2 R = CHO
5 R = Me



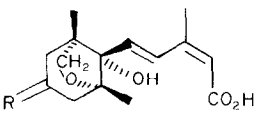
4



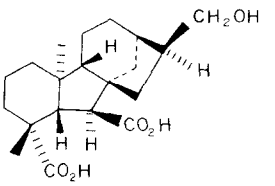
3 R_1 = H, R_2 = H
9 R_1 = H, R_2 = OH
10 R_1 = OH, R_2 = OH
11 R_1 = OH, R_2 = H



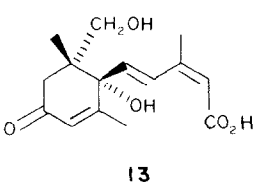
6



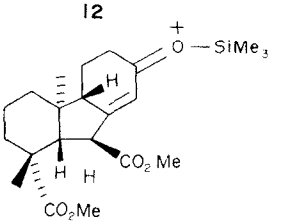
7 R = O
8 R = H, βOH



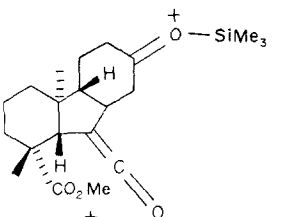
12



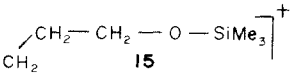
13



14



16



15

extract again partitioned against EtOAc (6 × 300 ml). The latter EtOAc phases were combined, washed with H_2O (2 × 1 l.), and concd to dryness *in vacuo* to give the acidic fraction (189 mg). 2% and 0.2% of this fraction were tested for GA-like activity in the d_5 maize seedling assay. Activity was calculated to be 1.5 μg GA_3-equivalents/mg acidic fraction. 15% of the sample was dissolved in Me_2CO–H_2O (4 : 1), applied to a column [1 cm (i.d.) × 27 cm] of celite–charcoal (3 g: 1 g) and eluted with the same solvent mixture. Seven 25-ml fractions were collected and evaporated to dryness to give (in order of elution) the following weights: 7.2, 2.4, 0.4, 0.4, 0.2, 0.3, 0.25 and 0.5 mg. These fractions were methylated (CH_2N_2) and trimethylsilylated [Me_3SiCl–$(Me_3Si)_2NH$–pyridine] and subjected to GC/MS using a silicone membrane separator (University of Bristol). MS were obtained at 24 eV with a source temp. of 210° and a separator temp. of 190°. The spectra were recorded at 6.5 sec/mass decade and processed on-line by computer. The GC conditions were: (A) 2% SE-33 on 80–100 mesh Gas Chrom Q in a glass column of 170 × 0.3 cm (o.d.), held at 180° for 5 min then temp. programmed to 280° at 2°/min; (B) 2% QF-1 on 80–100 mesh Gas Chrom Q in a glass column of 170 × 0.2 cm (o.d.) held at 170° for 5 min, then temp. programmed to 250° at 2°/min. In both cases a He flow-rate of 25 ml/min was used.

The following compounds were identified in the methyl ester TMSi-derivatized fractions by comparison of their mass spectra with reference spectra: fraction 1 (7.2 mg) ABA, PA, DPA, GA_{20}; fraction 2 (2.4 mg) GA_{20}, GA_{53}, GA_{17}, GA_{19}, GA_{44}; fraction 3 (0.4 mg) GA_{20}, GA_{53}, GA_{17}, GA_{19}, GA_{44}; fraction 4 (0.4 mg) GA_{20}, GA_{53}, GA_{44}; fraction 5 (0.2 mg) GA_{44}. The underlined compounds are the major components in each fraction.

The following compounds were tentatively identified from the mass spectra of their methyl ester TMSi derivatives: (a) metabolite C (**13**); *m/z* (%) 366 (M^+, 1.4), 351 ($M^+ - 15$, 1.4), 334 ($M^+ - 32$, 4.5), 263 ($M^+ - 103$, 33), 221 (23), 190 (100), 161 (32), 134 (13), 125 (46), 112 (15), 103 (26), 75 (46) and 73 (93); (b) 16,17-dihydro-17-hydroxy GA_{53} (**12**): *m/z* (%), 538 (M^+, 8), 523 ($M^+ - 15$, 3.5), 509 ($M^+ - 29$, 3), 448 ($M^+ - 90$, 13), 407 ($M^+ - 131$, 52), 375 ($M^+ - 131 - 32$, 87), 297 (21), 259 (34), 181 (24), 147 (17.5), 131 (88), 103 (10), 75 (23), and 73 (100).

A second aliquot (15 mg) from the acidic fraction was purified on charcoal–celite, as described above, for examination by capillary GC/SICM. The first 25 ml fraction eluted was methylated (CH_2N_2) and trimethylsilylated (MSTFA) and subjected to GC/SICM (University of Göttingen). GC conditions: samples (1 μl) were applied to a SP-2100 30 m × 0.24 mm WCOT column using the Grob splitless injection method. The column, which was coupled directly to the source, was held at 50° for 1 min, programmed to 230° at 15°/min and then to 280° at 3°/min. The split (50:1) was opened after 0.5 min. A He flow-rate of 2 ml/min was used. Mass spectrometer conditions were: electron energy 50 eV, electron emission current 0.25 mA and source temp. 250°. Data were collected from 255°; ions for GA_1 or GA_{29} (375.2, 377.2, 491.2, 506.3) were monitored for 5.8 min and thereafter ions for GA_8 (448.2, 536.3, 579.3, 594.3) with an integration time of 60 msec/ion and a scan time of 1 sec.

REFERENCES

1. Kaufman, P. B., Ghosheh, N. S., Nakosteen, L., Pharis, R., Durley, R. and Morf, W. (1976) *Plant Physiol.* **58**, 131.
2. Milborrow, B. V. (1964) *J. Chem. Soc. Chem. Commun.* 966.
3. Emerson, R. A., Beadle, G. W. and Fraser, A. C. (1935) *N. Y. State Agric. Col. Expt. Sta.* **39**, 1.
4. Neuffer, M. G., Jones, L. M. and Zuber, M. S. (1968) *Crop Sci. Soc. Am.* Madison, WI.
5. Neuffer, M. G. and Coe, E. H., Jr. (1974) *Handbook of Genetics* (King, R. C., ed.) Vol. 2. Plenum Press, New York.
6. Coe, E. H., Jr. and Neuffer, M. G. (1977) *Corn and Corn Improvement* (Sprague, G. F., ed.) 2nd edn. American Society of Agronomy.
7. Phinney, B. O. and West, C. A. (1960) *Symp. Soc. Dev. Growth* **18**, 71.
8. Hedden, P. and Phinney, B. O. (1979) *Phytochemistry* **18**, 1475.
9. Sponsel, V. M., Gaskin, P. and MacMillan, J. (1979) *Planta* **146**, 101.
10. Metzger, J. D. and Zeevaart, J. A. D. (1980) *Plant Physiol.* **65**, 623.
11. Jones, M. G. and Zeevaart, J. A. D. (1980) *Planta* **149**, 274.