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ON THE SYNTHESIS OF GIBBERELLINS IN ROOTS

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Abstract—The biosynthesis of gibberellins in sunflower roots was examined. Exudate from roots of decapitated sunflower (*Helianthus annuus*) plants continued to show gibberellin-like activity four days after decapitation. Also, incubation of root apices in 2-^{14}C mevalonate yielded labeled (−)-kauren-19-ol, an intermediate in gibberellin synthesis. The findings are consistent with the premise that roots synthesize gibberellins.

INTRODUCTION

GIBBERELLIN translocation takes place so readily throughout the plant that it is difficult to establish the sites of gibberellin synthesis. There is good evidence, however, that gibberellins are present in roots. Butcher[1] isolated a material possessing gibberellin-like activity in extracts of tomato roots that were grown in isolated cultures for 5 yr. Phillips and Jones[2] detected gibberellin-like activity in the bleeding sap of the root system of *Helianthus annuus*. Carr *et al.*[3] thought that the root system synthesized gibberellins but did not provide any direct evidence for their suggestion. Earlier work in our laboratory[4] inidcated that gibberellins may be synthesized in the sunflower root. This possibility is examined here.

While this research was being completed Jones and Phillips,[5] collecting diffusates of gibberellin on agar blocks, showed convincing evidence that biosynthesis of gibberellin in root tips continued throughout the diffusion period. The data presented in this paper augments the thesis that the root apex produces gibberellins.

RESULTS AND DISCUSSION

Initially, mature sunflower plants were decapitated, the root exudate was collected, and after partial purification was tested for gibberellin-like activity.

If the root itself was not the source of gibberellins found in root exudate,[2] gibberellin-like activity in the root exudate would have disappeared shortly after decapitation. Table 1 shows that this is not so. Two biologically active gibberellin-like fractions were distinguished in the root exudate. The contents of both fractions decreased up to the third day after decapitation but increased on the fourth day, the increase in fraction I being quite marked. Similar results were obtained in other experiments. The fact that gibberellins were still present in the root exudate three days after decapitation and even increased on the fourth day is consistent with the hypothesis that gibberellins may be synthesized in the roots.

[1] D. N. BUTCHER, *J. Exptl. Botany* **41**, 272 (1963).
[2] I. D. J. PHILLIPS and R. L. JONES, *Planta* **63**, 269 (1964).
[3] D. J. CARR, D. M. REID and K. G. M. SKEENE, *Planta* **63**, 382 (1964).
[4] H. KENDE and D. SITTON, *Ann. N.Y. Acad. Sci.* (In press).
[5] R. L. JONES and I. D. J. PHILLIPS, *Plant Physiol.* **41**, 1381 (1966).

TABLE 1. GIBBERELLIC ACID EQUIVALENTS IN ROOT EXUDATE OF DECAPITATED SUNFLOWER PLANTS

Days after decapitation of top	1	2	3	4
Total sap collected* (l.)	3·90	3·50	3·22	3·07
1st Fraction†	0·3340	0·1450	0·0393	0·2330
2nd Fraction†	0·0513	0·0211	0·0018	0·0025

* Sap collected from 700 plants.
† μg Gibberellic acid equivalents per litre sap.

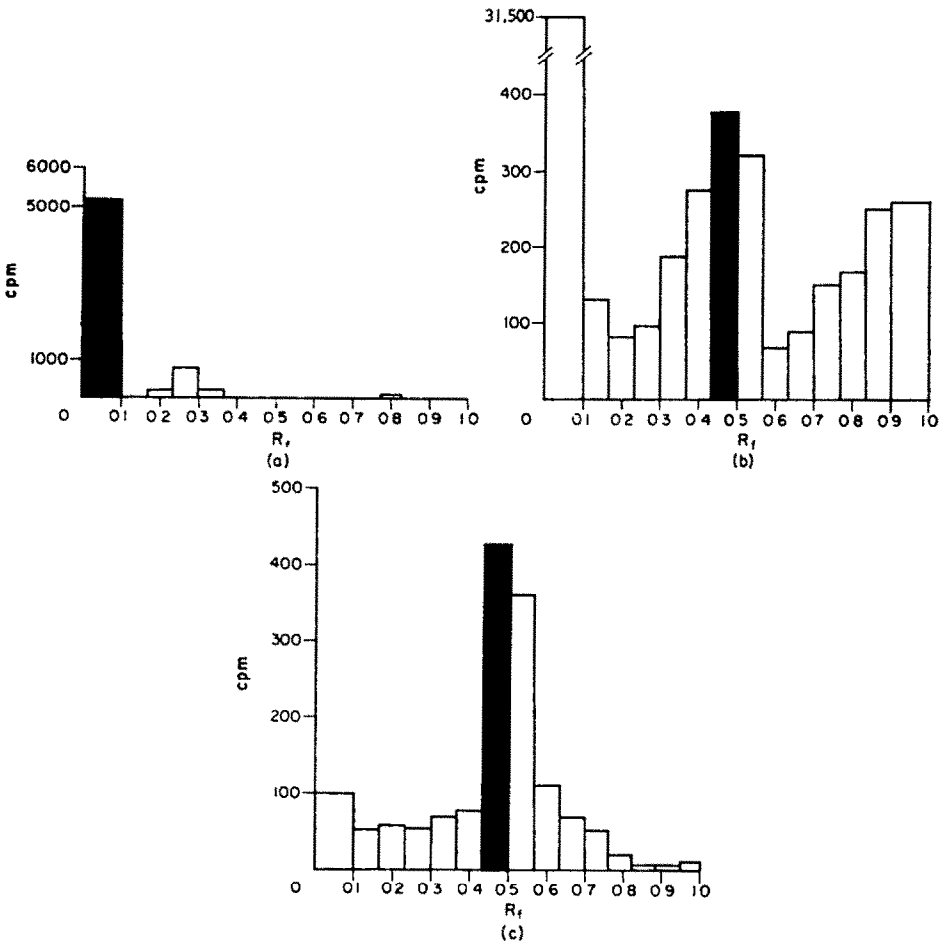

FIG. 1. THIN-LAYER CHROMATOGRAPHY OF AN ACETONE EXTRACT OF ROOT APICES INCUBATED IN 2-^{14}C MEVALONATE.

(a) Acetone root extract developed in hexane; (b) Material from plate (a) (see Experimental) redeveloped in benzene-ethylacetate 9:1 v/v; (c) Material from plate (b) (see Experimental) redeveloped in benzene-ethylacetate-propanol 82:15:3 v/v.
The darkened areas indicate the R_f values for the kaurenol standard.

The work of Cross *et al.*[6] and Graebe *et al.*[7] facilitated an analytical elucidation of this hypothesis. Cross' group showed that carbon from mevalonate 2-^{14}C was readily incorporated into gibberellic acid in *Fusarium moniliforme* and that (−)-kauren-19-ol were intermediates in gibberellin biosynthesis. Graebe *et al.*[7] reported that 2-^{14}C mevalonate was incorporated into various diterpenes in cell-free homogenates of the endosperm of *Echinocystis macrocarpa*. The labelled kauren and kaurenol thus obtained were then synthesized to gibberellic

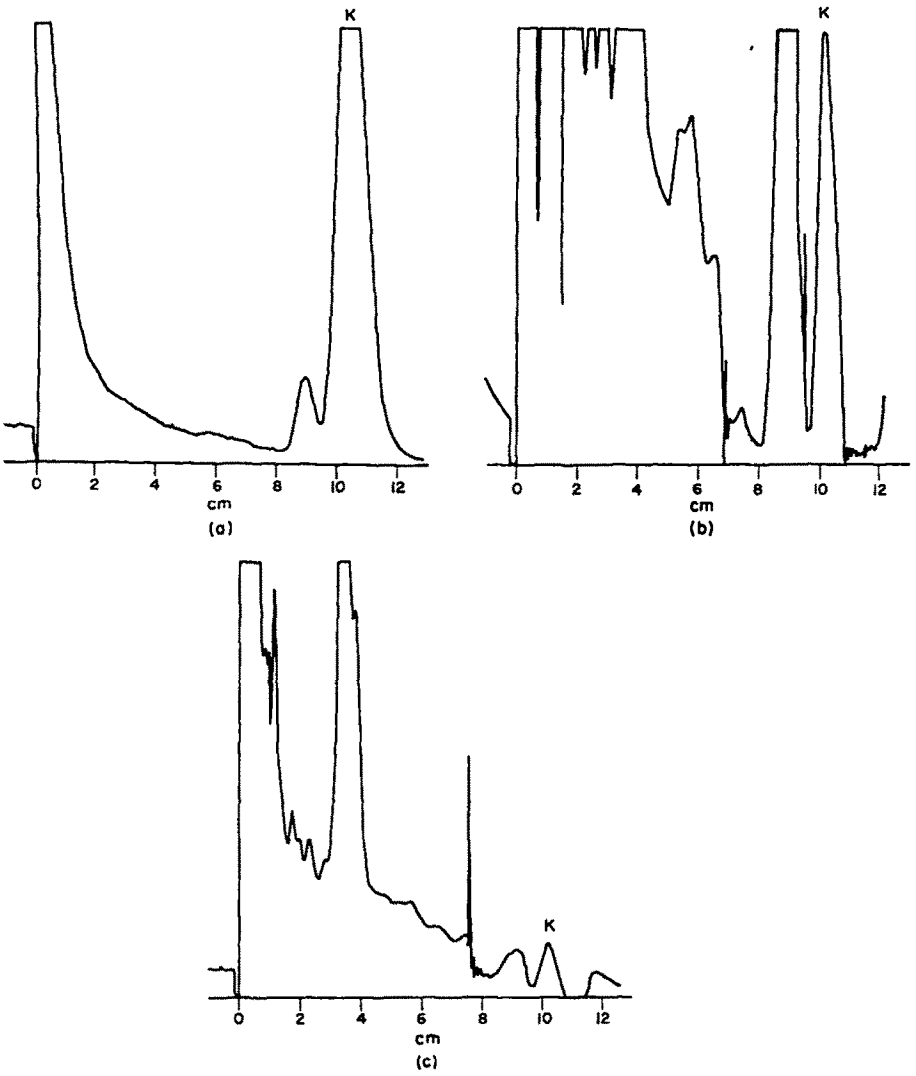

FIG. 2. GAS CHROMATOGRAMS OF TLC FRACTIONS.

(a) Chromatography of the TLC (−)-kauren-19-ol standard; (b) Chromatography of the fraction of Fig. 1(b) showing identical retention time to the standard K; (c) Chromatography of the fraction shown in Fig. 1(c).

[6] B. E. CROSS, R. H. B. GALT and J. R. HANSON, *J. Chem. Soc.* 295, (1964).
[7] J. E. GRAEBE, D. T. DENNIS, C. D. UPPER and C. A. WEST, *J. Biol. Chem.* **240**, 1847 (1965).

acid in a suspension of *Fusarium monilforme*. Hence our experimental approach was to verify if kaurenol could be detected, labelled, after incubation of root apices in ^{14}C mevalonate.

Figure 1(a) shows the radiochromatograph of an acetone extract of root apices incubated in 2-^{14}C mevalonate and developed on TLC with solvent 1 (see Experimental). The standard as well as most of the radioactivity remained at the origin. Figure 1(b) shows the radiochromatograph of this fraction after the latter was scraped off the plate, re-extracted with acetone, and developed in solvent 2. Most of the radioactivity still remained at the origin. However, a second radioactive peak with an R_f of 0·43–0·50 was detected at a spot similar to that of (−)-kauren-19-ol. When this fraction was reapplied on TLC and developed in solvent 3, peak radioactivity once again coincided with the R_f of the standard (Fig. 1(c)). (−)-Kauren-19-ol was further identified in root apices with the aid of gas chromatography. Figure 2(a) shows that the sample of (−)-kauren-19-ol that served for a standard in our various chromatography procedures was homogeneous, except for a minor contamination which exhibited a lower retention time. Prior to injection into the column, the acetone root extract was purified on TLC with various developers. Fig. 2(b) shows the gas-chromatogram of a fraction obtained from TLC run in solvent 1 followed by solvent 2 (see Experimental). It was evident that the sample was quite heterogenous. Nevertheless, a peak with a retention time identical to that of (−)-kauren-19-ol was clearly present. This peak was still evident in a fraction obtained from TLC which was developed consecutively in solvents 1, 2, and 3. (Fig. 2(c)). We concluded that (−)-kauren-19-ol, a precursor of gibberellic acid, was synthesized in root apices of *Helianthus annuus*. This evidence, together with the discovery that gibberellin-like activity was still present in root exudate 4 days after decapitation, supports the thesis that at least some gibberellins found in higher plants are synthesized in the root apex. There is thus an indication of the role played by the root in hormonal regulation of plant growth and development.

EXPERIMENTAL

Assay of Gibberellin-like Activity in Sunflower Root Exudate

Seven hundred sunflowers (*Helianthus annuus*) of a local cultivar were grown in half strength Hoagland solution for 60 days. The plants were then decapitated close to the cotyledons (residual cotyledons were removed) and latex tubes were connected to the stubs. The root exudate was collected twice a day for 4 days. The exudate was frozen and stored at −15°, and thereafter lyophilized. The dry material was extracted in absolute methanol and an aliquot centrifuged. The supernatant was collected and dried in vacuum at 30° and re-extracted in 1·0 M phosphate buffer at pH 8·4. This was agitated with an equal vol. of ethyl acetate. The aqueous layer was re-extracted twice and conc HCl added to pH 2·5. A resulting brown precipitate was removed by centrifugation and the acidified extract was agitated three times against equal volumes of ethyl acetate. The ethyl acetate was dried (Na_2SO_4 in vacuum at 30°), and the residue was dissolved in a small volume of ethyl acetate and methanol (1:1 v/v) and was streaked on Whatman No. 3 paper. Descending chromatography was carried out in isopropanol:ammonia:water. (10:1:1 v/v). The chromatogram was cut into 10 uniform strips which were eluted with methanol:ethyl acetate (1:1 v/v). The eluate of each strip was dried with a stream of air, and dissolved in 0·5 cc of 0·05% Tween 20. Dwarf corn mutant (d_2), kindly supplied by Professor B. O. Phinney, was used for the bioassay[8] which was found to be sensitive and reproduceable.

Incubation of Root Apices with 2-^{14}C-Mevalonate

Sunflower (*Helianthus annuus*) seed of a local cultivar were soaked in water and sown in vermiculite. 3–5 mm-long root apices were removed from 3-day-old seedlings and washed in ice-cold water. Some 2800 root apices weighing 1·12 g were incubated in 4 ml of 0·5% sucrose and 0·02 M Tris, pH 7·0. The incubation media contained 1·3 μmole ($2·04 + 10^6$ cpm) of mevalonic acid 2-^{14}C-lactone (obtained from Calbiochem, Los Angeles) and the incubation was carried out in a shaker for 4 hr at 26°. To exchange the label from the free space, the root apices were immersed in 0·01 M unlabelled mevalonate (dibenzylethylene diamine salt, from Calbiochem), and then lightly dried on filter paper.

[8] M. KATSUMI, B. O. PHINNEY, P. R. JEFFERIES and C. A. HENRICK, *Science* **144**, 849 (1964).

Extraction of (−)-Kauren-19-ol from the Root

Root apices were handhomogenized in 4 ml acetone, the homogenate centrifuged 35,000 *g* for 20 min and the supernatant collected. The pellet was washed twice with acetone and the washings and supernatant combined. The radioactivity in the supernatant, which represented approximately the uptake of mevalonate ^{14}C, was some 20 per cent of the original amount in the incubation medium. The acetone was evaporated by air and the aqueous residue lyophilized.

Thin-layer Chromatography (TLC)

The lyophilate was dissolved in a small volume of acetone and streaked on a 20 × 20 cm, 0·25 mm thick silica gel GF_{254} plate (according to Stahl, E. Merk, Darmstadt). In all the TLC systems, a sample of (−)-kauren-19-ol, kindly supplied by Drs. Cross and Jefferies, was developed on a separate plate alongside the root extract. The standard was identified on the plate by u.v. scanning, following a bromine spray.

The plate was developed to a distance of 5 cm with hexane (solvent 1). The gel was scraped up to 2·5 cm from the origin and the scraped material was re-extracted with acetone. It was restreaked and redeveloped with benzene: ethyl acetate, 9:1 v/v (solvent 2). This plate was scraped from the 6th cm on to the 10th and the material extracted with acetone and then rechromatographed in hexane: ethylacetate: propanol. 82:15:3 v/v (solvent 3).

Gas Chromatography

Acetone extract of the material scraped from the TCL plate was injected into a 180 × 0·4 cm column, with 5% S.E. 30 on 80/100 Gaschrom P in a Packard Model 7521 with a flame-ionization detector. The inlet temperature was 300°, column temperature 215° and the detector and outlet temperature 250°. Sensitivity 3×10^{-12} ampere full scale. Carrier nitrogen flow was 60 ml/min, hydrogen flow 60 cm/min and air flow 700 ml/min. Root acetone extracts of two states of purity were chromatographed. One consisted of material developed on TLC in solvent 1 following with solvent 2. The other sample was obtained after an additional run on TLC with solvent 3 as described above.

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