Phytochemistry, 1968, Vol. 7, pp. 83 to 84. Pergamon Press. Printed in England

SHORT COMMUNICATION

AN ATTEMPT TO FIND EVIDENCE FOR THE EXISTENCE OF (+)-GIBBERELLIC ACID

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(*Received* 5 *July* 1967)

Abstract—[17-^{14}C](+)-Kaurene has been prepared and added to a fermentation of *Gibberella fujikuroi*; it was not incorporated into gibberellic acid.

INTRODUCTION

The gibberellins are a class of endogenous plant hormones which regulate various aspects of plant growth and development.[1,2] Their occurrence is so widespread that it is possible that they are to be found in all higher plants.[1] Many of the gibberellins are also produced by the fungus *Gibberella fujikuroi*[3] and it has been shown[4] that (−)-gibberellic acid (I), the most readily available gibberellin, can be biosynthesized by the fungus from the diterpene (−)-kaurene (III) which is known to occur in several trees.[5] Furthermore with *G. fujikuroi*, the closely related (−)-kauren-19-oic acid (IV) has also been shown[6] to act as a precursor of gibberellins A_1 (VI), A_4 (VII) and A_7 (II), whilst (−)-kauren-19-ol (V) is transformed[7] into gibberellin A_{13} (VIII). It is generally believed that the biosynthesis of the gibberellins in plants follows a similar pathway.[3,8,9]

All the known gibberellins are believed to belong to the same stereochemical series, i.e. to the diterpenes with an antipodal A/B ring fusion. However the widespread occurrence of diterpenes of the enantiomeric series ('normal' A/B ring fusion) in higher plants and in particular the isolation of (+)-kaurene from *Podocarpus spicatus*[10] and *P. ferrugineus*[11] suggests that some plants may contain gibberellins which are the enantiomers of those so far reported. Since the isolation of gibberellins from a tree such as *P. spicatus* would almost certainly be long and tedious and the amount of gibberellin obtained might be inadequate for complete characterization we tried another approach to the search for (+)-gibberellins. The possibility that the enzyme systems of *G. fujikuroi* would utilize (+)-kaurene as a precursor of

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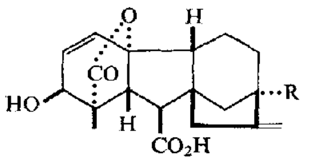
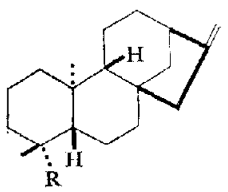
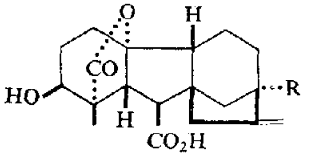


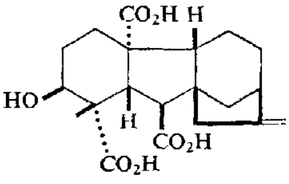
(I) R=OH
(II) R=H



(III) R=Me
(IV) R=CO_2H
(V) R=CH_2OH



(VI) R=OH
(VII) R=H



(VIII)

(+)-gibberellic acid was clearly very remote, however the consequences of success would be very important and [17-^{14}C](+)-kaurene is readily prepared. Labelled (+)-kaurene was therefore added to a fermentation of the fungus in the usual way.[4,7] The gibberellic acid produced was isolated and purified as its methyl ester but it was not radioactive. Consequently in order to determine whether gibberellins of the (+)-series exist it would appear to be necessary to isolate the gibberellins from a (+)-kaurene producing plant.

EXPERIMENTAL

(General experimental methods have been previously described.[7])

[17-^{14}C](+)-*Kaurene*

(+)-Kaurene (m.p. 47–48°, $[\alpha]_D$ +80° (*c* 0·56); 150 mg) was converted into [17-^{14}C](+)-kaurene (48 mg) by the method used[4] to prepare [17-^{14}C](−)-kaurene.

Addition of [17-^{14}C](+)-*kaurene to a* G. fujikuroi *fermentation*

G. fujikuroi ACC 917 was grown on a Cerelose-ammonium tartrate medium until the ammonium nitrogen was exhausted. [17-^{14}C](+)-Kaurene (18 mg; 4·06 μc) in EtOH (20 ml) was added and the fermentation continued for 112 hr. The fermentation was worked-up as previously described[7] to give crude gibberellic acid (540 mg) which, after methylation with ethereal diazomethane and purification,[7] gave methyl gibberellate (296 mg; 58 counts/100 sec/m-mole) which was identified by its i.r. spectrum. TLC of the crude gibberellic acid on silica gel G in di-isopropyl ether-AcOH (95:5) followed by autoradiography[4] failed to reveal any radioactive spots.

Acknowledgements—We thank Mr. E. G. Jefferys [I.C.I. Ltd. (Pharmaceuticals Division)] for the fermentation and the Science Research Council for a Research Studentship (to K.N.).