Tetrahedron Letters No. 25, pp. 32-36, 1960. Pergamon Press Ltd. Printed in Great Britain

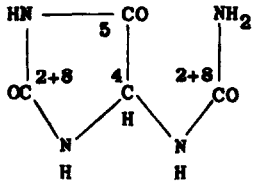
# UREIDE BIOSYNTHESIS IN HIGHER PLANTS

H. Reinbothe and K. Mothes

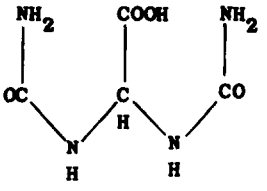
Institut für Allgemeine Botanik der Universität Halle/S., and Institut für Biochemie der Pflanzen der Deutschen Akademie der Wissenschaften in Halle/S.

(Received 9 November 1960)

THE origin and fate of allantoin and allantoic acid in plant tissue is at present not quite clear.



allantoin



allantoic acid

As a result of the work of Fosse et al[1] and of Mothes and Engelbrecht[2] we know that these glyoxylic acid ureides are major constituents of members of some plant families. The ureides play an important role in the nitrogen economy of such plants. Thus allantoin and allantoic acid function for the storage and translocation of nitrogen[2,3].

In the animal kingdom allantoin arises from oxidative purine

---

1 Listed by Bollard, E.G., Symp. Soc. exp. Biol. 13, 304 (1959).

2 K. Mothes, Encyclopedia of Plant Physiology, Vol. VIII, p. 716, Heidelberg, (1958).

3 K. Mothes and L. Engelbrecht, Flora 139, 586 (1952); 141, 356 (1954)

degradations. In the course of the hydrolysis of nucleoprotein, ureides are formed as intermediates of the catabolic sequence of reactions. In plants, however, the high level of nitrogen stored as allantoin and allantoic acid cannot be derived from nucleic acid breakdown alone. Ureide accumulation is related to protein metabolism. Allantoin and allantoic acid appear as special products of ammonia detoxication in some plants.

Certain indications of the metabolic role and origin of ureides in wheat emerge from the results of Krupka and Towers[4]. Glycine is a more immediate precursor for allantoin biosynthesis than glyoxylic acid which presumably enters ureide formation via glycine. The result of feeding experiments with xanthine and uric acid are consistent with the view that the biogenesis of allantoin involves purine synthesis and breakdown. Barnes[5] demonstrated with the aid of adenine-8-$C^{14}$ that the breakdown of purines in leaves of Acer saccharinum is concerned in ureide biosynthesis. There is some evidence that ureides can also be formed directly from simpler molecules. When extracts of certain higher fungi were incubated with a mixture of glyoxylate and urea, glyoxylic acid disappeared and allantoic acid was formed[6]. But it is as yet questionable whether allantoin can be derived from allantoic acid by dehydration.

Material and Methods.

Ureide biosynthesis was studied in plants which have a high content of allantoin or allantoic acid. "Allantoin plants" like Boraginaceae contain only traces of allantoic acid. "Allantoic acid plants", e.g. Acer negundo contain allantoic acid as the predominant glyoxylic acid

---

4 R. M. Krupka and G. H. N. Towers, Canad. J. Bot. 36, 165, 179 (1958); 37, 539 (1959).

5 R. L. Barnes, Nature 184, 1944 (1959).

6 M. A. Brunel and G. Brunel-Capelle, C. R. Acad. Sci. 232, 1130 (1951).

ureide. But allantoin can also be present in even greater amounts and in such plants free urea is found. Different $C^{14}$-labelled compounds were fed alone or in combination with hypothetical precursors or inhibitors of ureide synthesis. Plant material was extracted twice with hot 70% alcohol and the extracts submitted to paper chromatographic analysis directly or after fractionation on columns of Dowex 50 ($H^+$). Propanol-water ( aceotr. ) and phenol-water (3:1, g/v) were used as solvent systems for the two-dimensional separation of amino acids and ureides. Autoradiographs were prepared using Agfa-Duro-safety-X-ray film. Amino acids were detected with ninhydrin reagent, urea and urea derivatives with Ehrlich's reagent. Allantoin and allantoic acid were estimated as glyoxylic acid by colorimetry in an Eppendorf-photometer at 546 m [7]. Radioactive measurements were made by means of a thin end-window Geiger-Müller tube. Specific activities and incorporation rates were determined after isolation of allantoin-$C^{14}$ by a combined procedure of column chromatography and double repeated one-dimensional paper chromatography. Radioactivity of allantoin-$C^{14}$ was measured on alumina discs in infinite sample thickness. The respective quantity was estimated in an aliquot part. The position of $C^{14}$ in allantoin was cleared up after hydrolysis to glyoxylic acid and urea which were separated from each other by column chromatography on Dowex 1 (formate). The specific activities of allantoin and urea released by hydrolysis were determined. From these data the distribution of radioactivity over the sum of carbon atoms 4 and 5, also 2 and 8 was calculated.

Allantoin Plants.

Glycine-1-$C^{14}$, glycine-2-$C^{14}$ and glyoxylic acid-1,2-$C^{14}$ were fed to

---

7 G. E. Young and C. F. Conway, J. Biol. Chem. 142, 839 (1942).

detached leaves of Platanus orientalis, Cynoglossum officinale and Symphytum officinale, seedlings of Borago officinalis, and storage roots (root sections) of comfrey. In each case radioactivity was mainly incorporated into serine and allantoin. Glyoxylic acid gives rise to the formation of glycine and serine. Presumably glyoxylate participates in allantoin synthesis via glycine. Glycine and serine are interconvertible in roots of Symphytum. Glycine carbon enters allantoin in position 4 and 5. The methylene grouping also forms carbon atoms 2 and 8 presumably via binding to tetrahydrofolic acid derivatives. Serine synthesis is understood as a C-1-transfer to glycine as acceptor. Folic acid ($1{,}6 \times 10^{-6}$ m) stimulated allantoin formation from glycine. Sulphonamides as e.g. sulphanilamide ($10^{-3}$ m), sulphathiazole ($10^{-3}$ m), and sulphadiazine ($10^{-4}$ m) had little or no effect upon allantoin synthesis from glycine. Added purines as e.g. adenine, hypoxanthine, xanthine, and uric acid were effective in decreasing allantoin-$C^{14}$ production from labelled glycine in an order which is in accordance with their relative positions in aerobic purine breakdown. Purine antagonists as e.g. 8-azaxanthine and benzimidazole stimulated allantoin formation from glycine slightly. Possibly these purine analogues inhibit the incorporation of purines formed by the de novo purine synthesis into nucleic acids in a competitive manner. Thus purines will be transformed more rapidly to allantoin by degradation. Urea-$C^{14}$ alone or in combination with glyoxylic, mesoxalic or tartronic acids was not transferred either to allantoin or to allantoic acid. Urea is broken down to ammonia and carbon dioxide. Ammonia raises the level of glutamine, carbon dioxide is incorporated into different nitrogen compounds by carboxylations. In the presence of inactive glycine or glyoxylate $C^{14}O_2$ is very readily incorporated into glycine, serine and

allantoin by an unknown fixation mechanism. This effect is still under investigation.

Allantoic Acid Plants.

In plants such as Acer negundo, Aesculus hippocastanum and Pelargonium zonale glycine-$C^{14}$ is predominantly incorporated into serine, allantoic acid and allantoin. In chlorophyll-deficient leaves of these plants the methylene-C-atom of glycine enters urea formation via the ureide groupings of allantoin and allantoic acid. Added purines and allantoin are decomposed to allantoic acid, glyoxylic acid and urea in white leaves of Acer negundo. The first products of glyoxylate metabolism in chlorophyll-deficient leaves of Pelargonium zonale were glycine and serine. Urea is broken down by the action of urease to ammonia and carbon dioxide in allantoic acid plants. Urease activity could be inhibited in the presence of alloxanic acid[8] or mesoxalic acid. But urea is also transferred as a molecule to allantoic acid (urease activity is not very effective). In the presence of glyoxylic acid the production of allantoic acid from urea was stimulated; allantoin was also formed to a small extent. But glyoxylic acid and urea react already spontaneously at quite low concentration to form allantoic acid at room temperatures. Spontaneous allantoin formation involving urea and glyoxylate is achieved only under more extreme conditions and even then only to a small extent. If the reaction partners are available a resynthesis of ureides appears to be possible. Further studies are needed in order to clear up the mechanism and physiological role of such reaction paths in allantoic acid plants.*

* A more detailed account and discussion of the results will be published in Flora (Jena).

8 C. T. Gray, M. S. Brooke and J. C. Gerhart, Nature 184, 1936 (1959).