Phytochemistry, 1965, Vol. 4, pp. 311 to 315. Pergamon Press Ltd. Printed in England

A RELATION OF ADENINE TO SERINE BIOSYNTHESIS IN CHLOROPHYLL-DEFICIENT LEAVES OF *PELARGONIUM ZONALE*

D. SCHLEE and H. REINBOTHE

University of Halle, Institute for General Botany, and German Academy of Sciences, Institute for Plant Biochemistry, Halle/Saale, Germany (DDR)

(*Received* 27 *July* 1964)

Abstract—The main route of adenine degradation in chlorophyll-deficient and variegated leaves of *Pelargonium zonale* (Geraniaceae) may be the over-all reaction path of oxidative purine catabolism. Only part of the supplied adenine is transformed into free guanine. Part of the 8-^{14}C-adenine supplied gives rise to serine-^{14}C. It is suggested that the imidazole ring of adenine or a metabolically related adenine derivative is opened and C-8 is lost as a single carbon unit which is used in serine biosynthesis, giving carbon-3 atom of serine.

INTRODUCTION

ADENINE is a component both of nucleic acids and of metabolically important nucleotides. In animals the over-all pathway of adenine catabolism is via hypoxanthine and xanthine to uric acid, allantoin, allantoic acid, or urea and glyoxylic acid.[1] The distribution of purine-catabolizing enzymes among animals is very erratic, and therefore the end products of the degradative pathway of purines vary between different animals.[2-4]

Relatively little work has been done on adenine metabolism in tissues of higher plants. Evidence has been presented using 8-^{14}C-adenine[5] that adenine degradation in excised leaves of silver maple (*Acer saccharinum*) may follow the same general pathway as in animals. Complete confirmation of such a metabolic pattern has been presented in the case of ureide plants.[6] Additional studies on adenine catabolism have been reported by Barnes, using embryos and tissue cultures of pine. In those tissues degradation of the adenine beyond hypoxanthine appeared to occur to a far less extent than in plants characterized by the accumulation of allantoin and allantoic acid.[7] We have obtained analogous results using leaves of *Nicotiana rustica* and *Coffea arabica*.[8] 8-^{14}C-Hypoxanthine fed both to leaf discs and sterile root cultures of *Symphytum uplandicum* (ureide plant), was, however, largely converted to allantoin.[9] The determination of radiocarbon distribution in the allantoin molecule indicated that the carbon-14 was equally distributed between the ureido carbons. This is in accordance with the view that in the course of uricolysis a symmetrical compound is intermediary.

[1] J. N. DAVIDSON, *The Biochemistry of the Nucleic Acids* (4th Ed.), Methuen, London (1960).
[2] M. FLORKIN, *L'évolution du métabolism des substances azotées chez les animaux.* Actualités biochimiques No. 3, Liège (1945).
[3] M. FLORKIN, *Biochemical Evolution*, New York (1949).
[4] J. KEILIN, *Biol. Rev.* **34**, 265 (1959).
[5] R. L. BARNES, *Nature* **184**, 1944 (1959).
[6] H. REINBOTHE, *Flora* **151**, 315 (1961).
[7] R. L. BARNES, *Botan. Gaz.* **123**, 141 (1962).
[8] H. REINBOTHE, Unpublished.
[9] G. W. BUTLER, J. D. FERGUSON and R. M. ALLISON, *Physiol. Plant.* **14**, 310 (1961).

In plants, any glyoxylate which may be formed by aerobic purine breakdown can be readily re-introduced into the purine and ureide biosynthetic pathway *de novo*, whereas urea (the second degradation product) may be split by the action of urease thus furnishing carbon dioxide and ammonia which are only partly reassimilated.[10] The first step in adenine breakdown may proceed at either the free purine level (i.e., by hydrolytic deamination to hypoxanthine by means of adenase), or by deamination of the corresponding nucleoside (adenosine to inosine) or nucleotide (adenylic acid to inosinic acid).

Studying the catabolism of adenine and some other purine derivatives in ureide plants, we observed an unexpected high incorporation of radiocarbon from 8-^{14}C-adenine into serine, using chlorophyll-deficient leaves of *Pelargonium zonale*. The present communication deals with the relation between the imidazole carbon atom of adenine and the β-carbon atom of serine.

RESULTS AND DISCUSSION

Chlorophyll-deficient (i.e., pure yellow) leaves of *Pelargonium zonale* were supplied with 8-^{14}C-adenine in the light. The incorporation of radioactivity into components of the 70% ethanol extracts was determined after several different periods of time (Table 1).

TABLE 1. CATABOLISM OF 8-^{14}C-ADENINE IN CHLOROPHYLL-DEFICIENT LEAVES OF *Pelorgonium zonale*

Time	Total† radio-activity cpm × 10^{-3}	% of radioactivity in 70% EtOH extract*								
		adn	hyp	xan	gua	all	all.ac.	urea	ser	unknowns
15 min	0·8	90	10	—	—	—	—	—	—	—
30 min.	1·5	60	26		—	2	4	-	4	4
2 hr	1·6	32	17	—	—	13	14	9	10	4
8 hr	5·0	23	7	5	4	10	28	14	7	1
24 hr	8·0	20	9	15	4	13	18	6	7	8

* Abbreviations used: adn = adenine, hyp = hypoxanthine, xan = xanthine, gua = guanine, all = allantoin, all.ac. = allantoic acid, ser = serine; the unknown compounds could be purine derivatives at either the nucleoside or nucleotide level.

† 0·5-mM of 8-^{14}C adenine added (20 mC/mM).

It can be seen that radioactivity from 8-^{14}C-adenine is preferentially introduced into the same compounds which are the products of aerobic purine breakdown in animal tissues, indicating that the same over-all pathway of oxidative purine catabolism may be operative. Surprisingly, however, serine is also highly labelled. This might be the result of randomization of radiocarbon via the route of oxidative purine breakdown followed by a reutilization of liberated $^{14}CO_2$ in glycine/serine formation. If this view is correct, compounds such as 8-^{14}C-guanine, 2-^{14}C-uric acid, or ^{14}C-urea should also be precursors of serine. However, radioactivity from guanine labelled in position 8 was not introduced in the serine of chlorophyll-deficient leaves of *Pelargonium zonale* (Table 2).

Variegated (i.e., only partly chlorophyll-deficient) leaves of *P. zonale* were fed with 8-^{14}C-adenine, 8-^{14}C-hypoxanthine, or 2-^{14}C-uric acid in the light (Table 3). The results obtained

[10] D. SCHLEE and H. REINBOTHE, *Phytochem.* 2, 231 (1963).

indicate that 2-^{14}C-uric acid, which is readily decomposed to ^{14}C-urea (and glyoxylate), is not used in serine biosynthesis. However, in this plant material, serine labelling from 8-^{14}C-adenine is only weak, and this is also the case with 8-^{14}C-hypoxanthine. In experiments using ^{14}C-urea, there was a small amount of incorporation of radioactivity into the soluble

TABLE 2. CATABOLISM OF 8-^{14}C-GUANINE IN CHLOROPHYLL-DEFICIENT LEAVES OF *Pelargonium zonale* IN THE LIGHT

Time (hr)	Total† radio-activity cpm × 10^{-3}	% of radioactivity in the 70% EtOH extract*						
		gua	xan	all	all.ac.	urea	ser	unknowns
1	1·65	77	—	2	2	4	—	15
2	2·10	75	—	5	2	6	—	12
8	4·35	70	4	5	3	8	—	9
24	7·40	52	3	5	5	22	—	12

* Abbreviations as in Table 1.
† 8·7 mM of 8-^{14}C guanine added (1·2 mC/mM).

TABLE 3. CATABOLISM OF ^{14}C-LABELLED PURINES IN VARIEGATED LEAVES OF *Pelargonium zonale*

^{14}C detected in:	% of radioactivity in the 70% EtOH extract					
	8-^{14}C-adenine*		8-^{14}C-hypoxanthine†		2-^{14}C-uric acid‡	
	2 hr	8 hr	4 hr	8 hr	2 hr	8 hr
Total radioactivity, cpm × 10^{-3}	1·0	2·95	3·0	4·75	1·0	3·1
Adenine	39	36	—	—	—	—
Hypoxanthine	22	9	12	7	—	—
Xanthine	3	7	2	2	—	—
Guanine	—	4	—	—	—	—
Uric acid	—	—	—	—	9	5
Allantoin	8	4	15	8	28	18
Allantoic acid	9	5	11	24	28	20
Urea	7	22	55	56	31	47
Serine	1	1	2	1	—	—
Asparagine	2	2	1	Tr.	—	—
Glutamine	1	3	—	—	—	—
Unknowns	8	7	2	1	4	10

* 0·5 mM of 20 mC/mM.
† 0·8 mM of 12·3 mC/mM.
‡ 1·7 mM of 5·9 mC/mM.

amino acid fraction, but even after 18 hr, no labelling in serine.[11] The main part of the supplied ^{14}C-urea was not metabolized suggesting weak urease activity both in chlorophyll-deficient and variegated leaves of *P. zonale*.

The ^{14}C-serine formed in chlorophyll-deficient *Pelargonium* leaves after 8-^{14}C-adenine feedings, was eluted from papers, pooled and chemically degraded, and it was found that

[11] H. REINBOTHE, *Flora* **150**, 474 (1961).

most of the radiocarbon from 8-^{14}C-adenine was incorporated into the β-carbon atom of serine (Table 4).

Thus, taken together, it seems very unlikely that serine labelling from 8-^{14}C-adenine is the result of randomization of radioactivity. We therefore suppose that the imidazole nucleus of adenine (or an adenine derivative) undergoes ring opening with loss of carbon-8 which is subsequently utilized in serine synthesis presumably by serine aldolase action, thus constituting the carbon-3 atom of serine. Further studies are in progress to elucidate the reaction.

Loss of carbon-8 atom of the purine skeleton has been reported in riboflavin and pteridine synthesis. McNutt has found that "the ureido carbon in the imidazole ring of adenine is incorporated into riboflavin to only a very slight extent, from which it may be surmised that the principal precursor of riboflavin to which adenine gives rise, is lacking in this carbon atom."[12] In experiments in which growing *Corynebacterium* cultures were supplied with 2-^{14}C-adenine or with 8-^{14}C-adenine, the teropterin (pteroyltriglutamic acid) formed was found to be highly radioactive when the purine labelled in position 2, but not when the one labelled in position 8, was given.[13] Smith *et al.* reported that the imidazole carbon atom of adenine was not incorporated in the aromatic ring of the riboflavin produced by *Eremothecium*

TABLE 4. CHEMICAL DEGRADATION OF ^{14}C-SERINE FORMED AFTER 8-^{14}C-ADENINE FEEDINGS TO CHLOROPHYLL-DEFICIENT *Pelargonium* LEAVES

Feeding time (hr)	% of activity in whole molecule	
	Serine-C-1 + 2	Serine-C-3
2	15	85
8	22	78

ashbyii, but did appear in position 2. Thus, it was postulated that the carbon-8 atom lost from the adenine serves as "active formate" for the closure of the purine ring in the synthesis of additional purine molecules.[14] It seems possible that in our system (chlorophyll-deficient leaves of *Pelargonium zonale*) serine labelling from 8-^{14}C-adenine is correlated with some mode of riboflavin or pteridin synthesis, but this requires further proof.

EXPERIMENTAL

Radioactive purines were purchased from The Radiochemical Centre Amersham (England). Some batches of 8-^{14}C-adenine were also products of Reanal (Hungary).

Excised chlorophyll-deficient (pure yellowish) and variegated (with green parts) leaves of *Pelargonium zonale* (grown in the greenhouse) were placed with their petioles in solutions of 8-^{14}C-adenine, 8-^{14}C-guanine, 8-^{14}C-hypoxanthine, or 2-^{14}C-uric acid. After different feeding times, the plant material was washed with water, cut in pieces and dropped into boiling 70% ethanol. Extraction was carried out three times further with 70% ethanol. The alcohol extract was concentrated, and chromatographed two-dimensionally on Schleicher

[12] W. S. MCNUTT, *J. Am. Chem. Soc.* **83**, 2303 (1961).
[13] E. VIEIRA and E. SHAW, *J. Biol. Chem.* **236**, 2507 (1961).
[14] C. G. SMITH, C. CONNELLY and M. SMEAD, *J. Biol. Chem.* **237**, 3207 (1962).

and Schüll 2043 b paper, using propanol:water (azeotropic, app 3:1) in the first direction and phenol:water (3:1, g/v) in the second. Autoradiograms of the chromatograms were prepared using Diavidox X-ray film (Fotochemische Werke Berlin). Radioactivities in compounds were determined directly by counting on the paper with a thin end-window Geiger-Müller tube. Data are expressed as a percentage of the total radioactivity of all the areas detected by radioautography. Carbon-14 labelled compounds were identified by elution and co-chromatography with inactive known standards. ^{14}C-serine was eluted from two-dimensionally run chromatograms and rechromatographed in phenol: 12·0 pH phosphate buffer (1:1) using the unidimensional descending technique. The pooled ^{14}C-serine was freed from "overlapping" glycine, and was degraded with periodate as described by Nicolet and Shinn.[15] Radioactivities of the degradation products were counted in infinite thinness, using a gas flow counter (Frieseke and Hoepfner, Erlangen-Bruck).

Acknowledgement—The authors are greatly indebted to Prof. Dr. K. Mothes for encouraging this investigation and for helpful discussions.

[15] B. H. Nicolet and L. A. Shinn, *J. Biol. Chem.* **139**, 687 (1941).