Biosynthesis of some Amino-Acids from Sucrose by Germinating Uredospores of Wheat Stem Rust, Race 15B

RUST development may depend on the availability of specific nutrients, such as certain amino-acids, which are supplied by the susceptible, but not by the resistant, host plant[1]. Demonstrations of nutritional requirements by the classical deletion procedure necessitate growing the organism on a chemically defined medium. Although some plant rusts have been grown on artificial media[2], little is known about their nutritional requirements. A method permitting the determination of at least some of these requirements without the use of chemically defined diets would be useful. An indirect procedure which utilized metabolites labelled with carbon-14 when applied to the mouse[3] and blowfly[4] gave results for amino-acid requirements which agreed with the classical deletion procedure. The present report gives results of the application of this indirect method to germinating uredospores of *Puccinia graminis tritici*, race *15B*.

Leaves and stems of the wheat plant at different stages of growth contain sucrose[5]. In addition, Shu *et al.*[6] demonstrated that uredospores of wheat stem rust, race *15B*, during at least the initial stages of germination oxidized this sugar. Consequently uniformly labelled sucrose-^{14}C was chosen as the substrate in the present study.

About 10 mgm. of rust spores produced on Rescue wheat were evenly distributed over the surface of 5 ml. of sucrose-^{14}C solution in each of 18 Petri dishes, 100 mm. in diameter. Each dish contained about 0·05 mgm. of sucrose-^{14}C (specific activity 100 μc./mgm.) (Atomic Energy of Canada, Ltd.). The Petri dishes containing the spores were covered and held in a closed desiccator at 20° C. for 24 hr. A small container of 0·4 *M* sodium hydroxide was included in each Petri dish to absorb any carbon dioxide evolved by the germinating spores. Radioactive carbon dioxide was produced during the incubation period showing that, in these conditions and in agreement with the work of others[6], sucrose was metabolized by the germinating rust spores.

After the 24-hr. incubation period, about 75 per cent of the spores had germinated. The spores and mycelium were then collected by centrifugation, washed with water, and hydrolysed with 6 *M* hydrochloric acid for 24 hr. under reflux. The acid was removed *in vacuo* and the resulting residue was redissolved in water. The amino-acids in this solution were separated on an ion-exchange column and further purified by band paper chromatography. Final purification and subsequent quantification of the amino-acids were accomplished as described earlier[4] except that another general ninhydrin method, with slight modifications, was used[7]. Infinitely thin samples of the amino-acids plated on copper planchets were assayed for radioactivity in a windowless gas flow detector to give a probable counting error of 2 per cent. After the amino-acids were separated on paper chromatograms in *n*-butanol/acetic acid/water (4 : 1 : 5) the bands were eluted and 5 μ*M* of the authentic compound were added to each eluate. The samples, diluted with carrier amino-acid, were afterwards band chromatographed in a series of other solvents until constant specific activity was obtained. After development in each solvent the band was eluted and the specific activity of the compound was determined.

The following amino-acids were radioactive after purification to constant specific activity: α-alanine, glutamic acid, glycine, leucine, phenylalanine, proline, tyrosine, and valine. In addition, autoradiographs of chromatograms, after *n*-butanol/acetic acid/water (4 : 1 : 5) chromatography of the appropriate fractions from the ion-exchange column, showed that radioactivity was present in isoleucine, serine, and threonine. These results demonstrate that the germinating uredospores of *P. graminis tritici*, race *15B*, can synthesize at least 11 amino-acids from sucrose. Thus, assuming that the organism continues to synthesize these amino-acids during subsequent development on the host, it seems unlikely that they are limiting factors in resistant wheat varieties[8].

R. KASTING
A. J. MCGINNIS
W. C. BROADFOOT

Canada Agriculture Research Station,
Lethbridge, Alberta.

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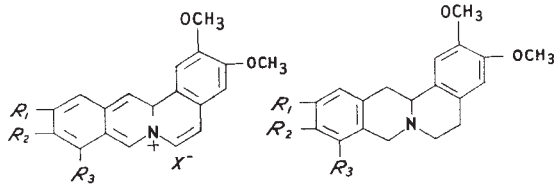
Berberine Alkaloids via 6,7-Dimethoxy*iso*quinoline-1-aldoxime

FOR the past two years[1], it has been clear that the development of a new and convenient method for the synthesis of the berberine alkaloids hinged on the preparation of 6,7-methylenedioxy (I)- and 6,7-dimethoxyisoquinoline aldehydes (II). The use of the first of these in the synthesis of tetrahydroberberine and related alkaloids has been reported[2]. The present communication describes the preparation and use of the dimethoxyisoquinoline aldehyde (II).



I II

III, R_1 = H, $R_2 - R_3$ = OCH_2O VII
IV, $R_1 - R_2$ = OCH_2O, R_3 = H VIII
V, R_1 = H, R_2 = R_3 = OCH_3 IX
VI, R_1 = R_3 = H, R_2 = OCH_3

Oxidation of 1-methyl-6,7-dimethoxyisoquinoline[3] afforded the 1-aldehyde (II), m.p. 176°, in 35 per cent yield (found: C, 66·18; H, 5·28; N, 6·85. $C_{12}H_{11}NO_3$ requires: C, 66·40; H, 5·07; N, 6·45 per cent). The oxime, m.p. 248° of II was quaternized with 2,3-methylenedioxy-, 3,4-methylenedioxy-, 2,3-dimethoxy-, and 3-methoxybenzyl bromides, and each of the crude salts cyclized in hydrochloric acid to afford the expected benz[*a*]acridizinium salts (III–VI) in yields of the order of 80 per cent.

The chloride of III (dihydrate) melted at 275° (*d*), while the perchlorate melted at 315–316°. The chloride of IV melted at 278–280° (*d*). The bromide of V melted at 250° (*d*), while the perchlorate melted at 312° (*d*). The 2,3,10-trimethoxybenz[*a*]acridizinium (VI) chloride (methanol-water solvate) melted at 242° (*d*), while the perchlorate (methanol solvate) melted at 310–312° (*d*).

Catalytic reduction of the first three benz(*a*)acridizinium salts (III–V) over platinum oxide yielded the expected tetrahydroberberine alkaloids. From III, tetrahydroepiberberine (± sinactine, VII) was produced in 50 per cent yield, m.p. 167–168° (refs. 4 and 5) 169–170°, 168° (found: C, 70·55; H, 6·16; N, 4·22. $C_{20}H_{21}NO_4$ requires C, 70·78; H, 6·24; N, 4·13 per cent). The base gives the reported [4,5] colour reaction in a sulphuric-acetic acid mixture. The hydrochloride decomposed at 246° (refs. 5 and 6, dec. about 286°; dec. 285–290°; found: C, 63·95; H, 5·81. $C_{20}H_{21}NO_4$·HCl requires C, 63·91; H, 5·85 per cent).

The reduction of IV yielded tetrahydropseudoepiberberine, m.p. 160 (lit.[7] m.p. 160–161; found: C, 70·85; H, 6·37; N, 4·20. $C_{20}H_{21}O_4N$ requires: C, 70·78; H, 6·24; N, 4·13 per cent). The picrate melted at 150° (d) (ref. 7. m.p. 149–150°). The reduction of V afforded ± tetrahydropalmatine (m.p. 147°) in 53 per cent yield. This material was identical in melting point, mixed melting point and infra-red absorption spectrum with an authentic sample (found: C, 71·08; H, 7·04; N, 4·24. $C_{21}H_{25}O_4N$ requires C, 70·99; H, 7·04; N, 3·94 per cent). The hydrochloride melted at 215–216° (ref. 8, 215°).

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C. K. Bradsher
N. L. Dutta

Department of Chemistry,
Duke University,
Durham,
North Carolina.

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Formation of Allantoin and Allantoic Acid from Adenine in Leaves of *Acer saccharinum* L.

Allantoin and allantoic acid have been shown to be major constituents of the xylem sap of many species of trees[1], and have been proposed as important translocatory forms of nitrogen[1,2]. Most workers in ureide metabolism in plants are of the opinion that allantoin and allantoic acid are not formed primarily by purine catabolism, as in the case in animals, but are synthesized from simpler molecules such as urea, glycine, or glyoxylate[2–4]. This communication presents evidence that allantoin and allantoic acid are readily formed from adenine in leaves of silver maple (*Acer saccharinum* L.).

Adenine-8-^{14}C was fed to two young leaves, through the petioles, in the light. After all the radioactive solution had been absorbed, the leaves were put in 1/10 strength Hoagland's solution. After 24 hr. the leaves were washed with water and extracted with 80 per cent ethanol. The ethanol extract was concentrated and chromatographed two-dimensionally in phenol/water and butanol/propionic acid/water solvents. Radioactive compounds were located by radioautography, and activities of the spots were counted directly on the paper with a Geiger–Müller tube.

The majority of the radioactivity from adenine-8-^{14}C appeared in allantoin, allantoic acid, and urea. The radioactivity expressed as percentages of total activity excluding that remaining in adenine, 26 per cent, are given in Table 1.

Table 1

Compound in leaves	Per cent radioactivity
Hypoxanthine	13
Xanthine	14
Uric acid	3
Allantoin	17
Allantoic acid	27
Urea	20
Ribosides	2
Ribotides	3
Unkonwn	1

Some of the urea may, however, have been formed from allantoic acid during the extraction. It is significant that 94 per cent of the total activity on the chromatogram was in compounds previously shown to be involved in purine catabolism in animals. The reaction sequence in maple leaves was therefore indicated to be the same as in animals, namely: adenine → hypoxanthine → xanthine → uric acid → allantoin → allantoic acid → urea plus glyoxylate. All these compounds, save glyoxylate, would be expected to acquire the carbon-14 label from adenine labelled in position 8.

The results indicate that purine catabolism is one process leading to ureide formation in higher plants. The hypothesis that ureides might also be formed directly from simpler molecules has not been investigated by me. An analysis of the xylem sap from a silver maple branch showed that allantoic acid was the major nitrogenous constituent, with glutamine second in abundance. Considerable allantoin, allantoic acid, and urea were present in the foliage. These findings indicate that allantoic acid is indeed an important translocatory and metabolic form of nitrogen in this species. Further investigations of ureide metabolism in several species of forest trees are presently under way.

Robert L. Barnes

Southeastern Forest Experiment Station
Forest Service,
U.S. Department of Agriculture,
Duke University,
Durham, North Carolina.
Aug. 6.

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ANIMAL PHYSIOLOGY

Pathophysiological Effects of Circulating Ferritin

Ferritin is an iron-containing protein which is stored mainly in the spleen, liver and marrow. The richest source of ferritin is the reticulo-endothelial system of horse spleen ; but there is no ferritin in the blood of a healthy horse.

In previous studies[1,2], a large quantity of ferritin was proved, by the precipitin test and the complement fixation test[1] to be circulating in the blood of horses suffering from infectious anæmia.

On the other hand, several workers have obtained results which suggest that a small quantity of ferritin (nitrogen 0·0005 μgm./0·5 ml.) has an effect on the vasodepressor mechanism[3,4] which inhibits the constrictor response of the muscle capillaries in the