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 15*B*.

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 15*B*, 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 15*B*, 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
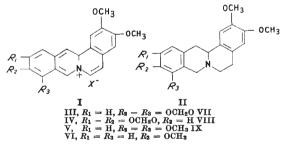
Canada Agriculture Research Station,
Lethbridge, Alberta.

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Berberine Alkaloids via 6,7-Dimethoxyisoquinoline-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 = $ OCH₂O VII
IV, $R_1 - R_2 = $ OCH₂O, $R_3 = $ H VIII
V, $R_1 = $ H, $R_2 = R_3 = $ OCH₃ IX
VI, $R_1 = R_3 = $ H, $R_2 = $ OCH₃

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*).