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FORMATION OF *o*-SUCCINYLBENZOIC ACID FROM *ISO*-CHORISMIC ACID IN PROTEIN EXTRACTS FROM ANTHRAQUINONE-PRODUCING PLANT CELL SUSPENSION CULTURES

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Key Word Index—*Galium* spp.; Rubiaceae; cell suspension culture; biosynthesis; *iso*-chorismic acid, *o*-succinylbenzoic acid; anthraquinones.

Abstract—Enzyme preparations from cell cultures of three *Galium* species were shown to convert α-ketoglutaric acid and *iso*-chorismic acid (but not chorismic acid) to *o*-succinylbenzoic acid, a precursor of anthraquinones.

INTRODUCTION

o-Succinylbenzoic acid (OSB) (4-(2'-carboxyphenyl)-4-oxobutyrate) is a precursor of menaquinones in bacteria, and phylloquinone and anthraquinones in plants [1–3] (Fig. 1). The aromatization leading to *o*-succinylbenzoic acid is likely to be a multistep reaction sequence. An intermediate has been isolated [4].

Although work with bacterial mutants [5] and bacterial enzyme preparations [6] seemed to indicate that *o*-succinylbenzoic acid is derived from chorismic acid, these mutants proved to be leaky [7]. On the other hand, bacterial enzyme preparations ('*o*-succinylbenzoic acid synthase') converted *iso*-chorismic acid to *o*-succinylbenzoic acid with 88% yield [7, 8] while chorismic acid did not serve as a substrate.

It remained to be determined whether chorismic or *iso*-chorismic acid was the precursor of *o*-succinylbenzoic acid during anthraquinone synthesis in plant tissues. For this comparison, cells of suspension cultures raised from *Galium mollugo*, *G. verum* and *G. uliginosum* plants were used as source of enzyme preparations. These cell suspension cultures produce anthraquinones abundantly [9, 10]. By using an extraction protocol which largely eliminated the problems that high anthraquinone levels cause in protein extracts from anthraquinone-containing cell cultures [11], active enzyme preparations could be obtained.

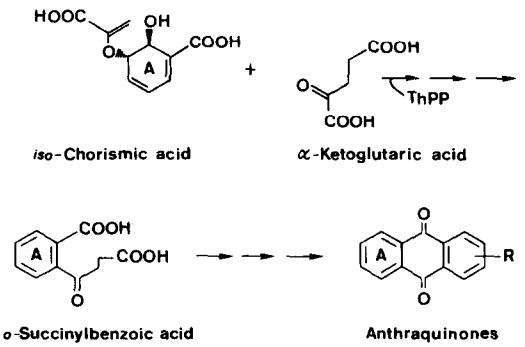

Fig. 1. Biosynthetic pathway leading from *iso*-chorismic and α-ketoglutaric acid to OSB and anthraquinones.

RESULTS AND DISCUSSION

The *o*-succinylbenzoic acid (OSB) formed (radiochemical yield 4%) was isolated (HPLC) and identified as described [7]. When thiamine pyrophosphate was omitted from the incubation mixture the yield dropped to 2%.

When α-keto[U-^{14}C]glutaric acid was replaced by α-keto[1-^{14}C]glutaric acid the OSB formed was inactive. This is in agreement with expectations because C-1 of α-ketoglutaric acid is removed during biosynthesis of OSB [1, 3, 4, 6–8]. When concentrated protein solutions were employed ($(NH_4)_2SO_4$, 30–70% saturation) and the incubation extended to 30 hr the yield of OSB increased to more than 80% with respect to *iso*-chorismic acid. Formation of OSB was not observed in heat-denatured protein solutions.

When *iso*-chorismic acid was replaced by chorismic acid no formation of OSB was observed. This indicates (i) that *iso*-chorismic acid is the immediate precursor of OSB in plant cells which produce anthraquinones and (ii) that an active *iso*-chorismate synthase was not present in these extracts. These results were observed with enzyme extracts from all three suspension cultures tested. It is concluded that not only menaquinones but also anthraquinones are ultimately derived from *iso*-chorismic acid.

EXPERIMENTAL

The freshly collected cells (5 g, medium A [10]) were homogenized (Branson sonifier) in Pi buffer (pH 7.0, 0.1 M) containing dithiothreitol (0.02 mM), EDTA (10 nM) and bovine serum albumin (10 g/l). After centrifugation (10 min, 20 000 rpm, 4°), the supernatant was freed from low-M_r compounds (Sephadex G 25) and incubated (pH 7.8, 30°, 180 min) in the presence of isochorismic acid (or chorismic acid) (10 nmol), α-keto[U-^{14}C]glutaric acid (1 nmol, 10.8 KBq), thiamine pyrophosphate

(120 nmol), Tris–HCl (pH 8.1, 50 μmol), $MnCl_2$ (2 μmol) and protein (3 mg) in a final vol. of 395 μl. The reaction was terminated (50 μl conc. HCl) and the protein centrifuged off.

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