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SYNTHESIS AND REVISED STRUCTURE OF THE O-SUCCINYLBENZOIC ACID COENZYME A ESTER, AN INTERMEDIATE IN MENAQUINONE BIOSYNTHESIS

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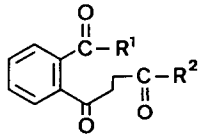
Summary: Synthesis of the two isomers 2, 3 of the mono coenzyme A ester of o-succinylbenzoic acid (1, OSB, i.e. 4-(2-carboxyphenyl)-4-oxobutanoic acid) and enzymic conversion of 3 to 1,4-dihydroxy-2-naphthoic acid 7 shows that as opposed to previous assumptions the "aliphatic" rather than the "aromatic" carboxyl group in o-succinylbenzoic acid 1 is activated during vitamin K_2 biosynthesis in Escherichia coli and Mycobacterium phlei.

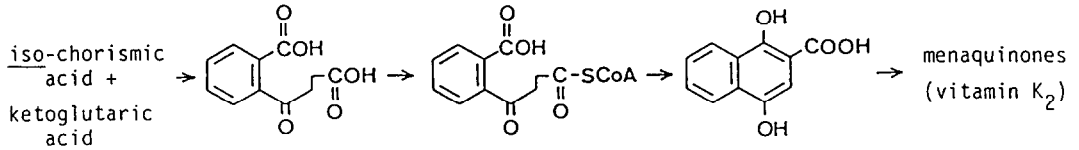
The enzymic conversion of o-succinylbenzoic acid 1 to 1,4-dihydroxy-2-naphthoic acid (DHNA) 7 is an ATP, CoASH and Mg^{2+} dependent reaction[1]. The product DHNA 7 is a precursor of vitamin K_2 (menaquinone). The activated intermediate in the conversion of OSB 1 to DHNA 7 has been isolated, characterized and shown to be a mono coenzyme A ester of OSB[1b].



1 R^1, R^2 = OH
2 R^1 = CoAS, R^2 = OH
3 R^1 = OH, R^2 = CoAS
4 R^1 = imidazole, R^2 = OH
5 R^1 = OH, R^2 = imidazole
6 R^1, R^2 = imidazole
8 $R^1 = OCH_3$, R^2 = OH

Since OSB 1 has two carboxyl groups the question arose which of the two carboxyl groups in OSB 1 is activated. This question was answered by synthesis of both isomers 2, 3 and enzymic conversion[1b] of 3 but not 2 to DHNA 7: OSB 1 was treated with 1,1'-carbonyldiimidazole in a molar ratio of 2,2 to 1. The resulting mixture of OSB monoimidazolides 4, 5 was converted to a mixture of coenzyme A esters[2] 2, 3. The coenzyme A esters 2, 3 were separated by HPLC. On hydrolysis both esters gave OSB 1 and coenzyme A in a molar 1 to 1 ratio. The "aromatic" ester 2 was distinguished from the "aliphatic"

ester 3 by a synthesis which gave the "aromatic" ester 2 alone:
Treatment of OSB 1 with an excess of 1,1'-carbonyldiimidazole gave OSB diimidazolide 6. Mild acid hydrolysis (THF/acetic acid 1:1, pH 3,2, 30°C, 30 min) gave 4 which was characterized (elementary analysis, UV, IR, MS, ^{1}H NMR, derivatisation to the corresponding methylester 8). The imidazolide 4 was also converted[2] to the corresponding coenzyme A ester 2.
When the "aromatic" 2 and the "aliphatic" 3 coenzyme A ester were incubated with the previously used naphthoate synthase fraction[1b], the "aliphatic" coenzyme A ester 3 was converted to DHNA 7 in a 50 - 60% yield without cofactor requirement. The "aromatic" coenzyme A ester 2, however, was not converted. Therefore menaquinone biosynthesis takes place as shown in Scheme I:



1 3 7

Scheme I

This result invalidates previous assumptions[1a,b] and the interpretations of experiments[4,5] which led to the conclusion that 2 rather than 3 is the intermediate in vitamin K[4] and anthraquinone[5] biosynthesis. As will be shown elsewhere[6] this error was eventually traced back to the fact that the enzymically formed[1b] OSB mono coenzyme A ester consisted of both the "aliphatic" 3 (85 %) as well as the "aromatic" 2 (15 %) coenzyme A ester and the apparent instability of 3 as opposed to 2.

Acknowledgement
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