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NONSYMMETRIC INCORPORATION OF CARBOXYL-^{14}C SHIKIMIC ACID INTO ALIZARIN (1,2-DIHYDROXY-ANTHRAQUINONE) IN RUBIA TINCTORUM L.

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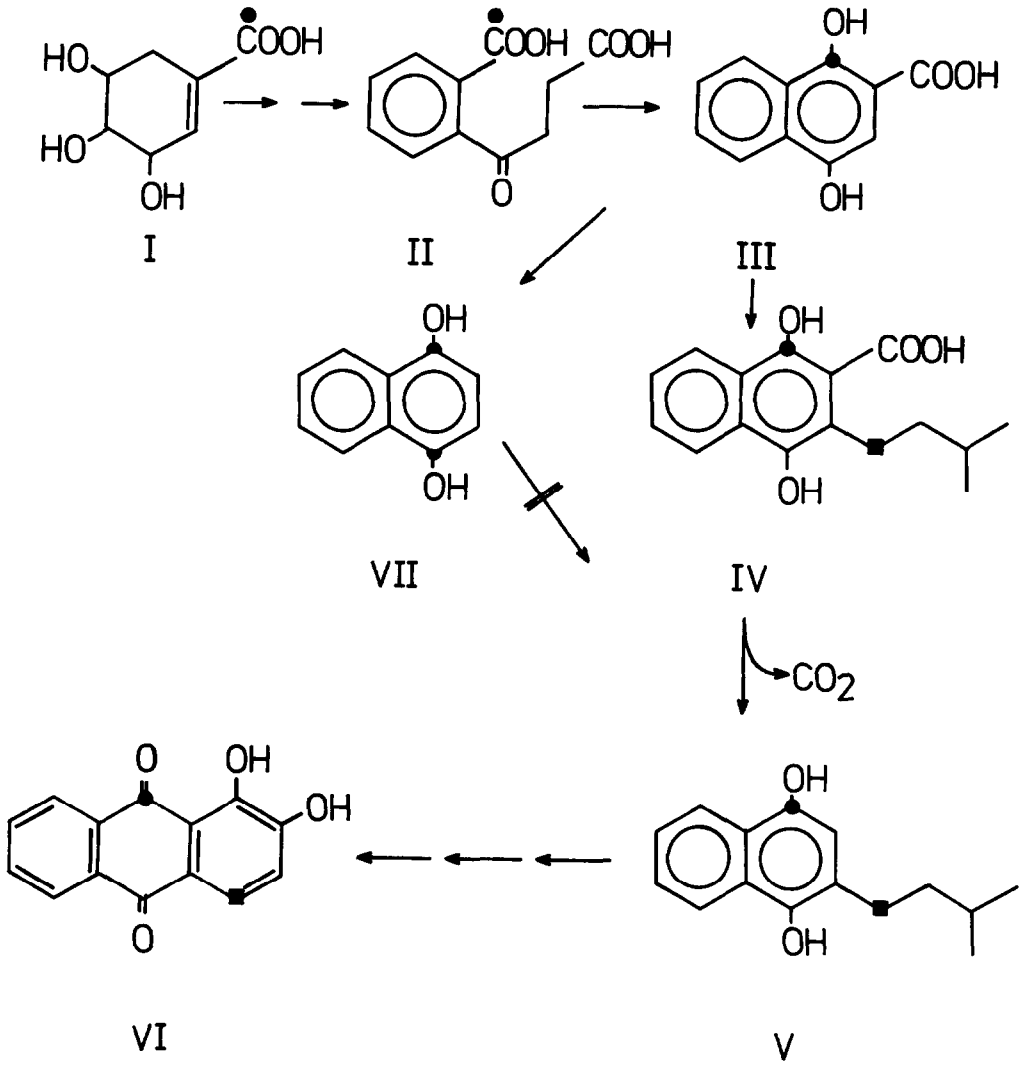
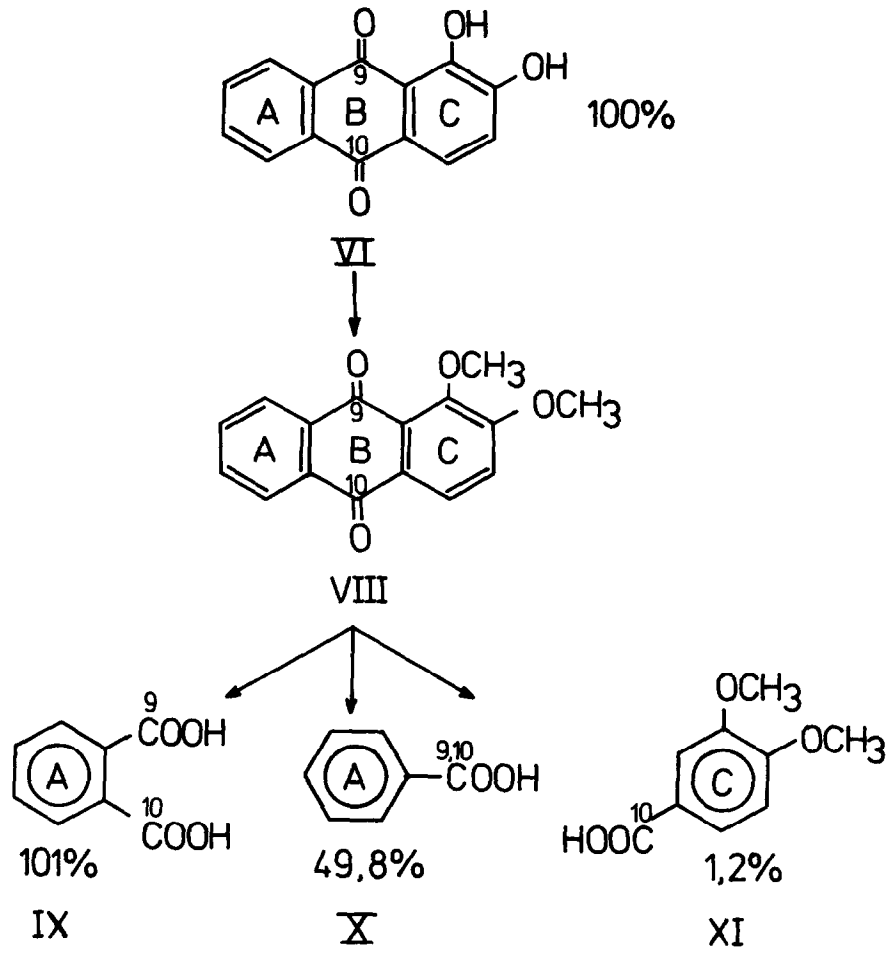
Shikimic acid (I) is known to be incorporated as an intact C_7-unit into the following quinones: vitamin K[1-3], juglone[4], lawsone[5] and alizarin[6](VI). In the case of juglone (5-hydroxy-1,4-naphthoquinone), it has been demonstrated that the carboxyl group of shikimic acid is incorporated into both keto-C-atoms to an equal extent[4]. This observation is consistent with the finding[4] that a symmetrical compound, namely 1,4-naphthoquinone is a precursor[7] of juglone. 1,4-Naphthoquinone is also incorporated into vitamin K[8] and alizarin[9]. Incorporation as such, however, does not establish an intermediate-product relationship. The proposal that 1,4-dihydroxy-2-naphthoic acid (III), a hypothetical product of o-succinylbenzoic acid (II)[10], might be an intermediate in the formation of the naphthoquinone nucleus[11] suggests the possibility that prenylation also can take place at the stage of the naphthoic acid (III). This question has yet to be resolved. In the first case, naphthoquinone (or 1,4-naphthoquinol), a symmetrical compound, would be an intermediate and the carboxyl group of shikimic acid should therefore be incorporated into the keto-C-atoms of vitamin K and alizarin to an equal extent as in the case of juglone. In the second case, however, there would be no symmetrical intermediate and subsequently nonsymmetric incorporation of the carboxyl group of shikimic acid is to be expected.

In order to test these possibilities carboxyl-^{14}C-labelled shikimic acid[12] was administered to roots of Rubia tinctorum for 30 hours. Alizarin was purified, and degraded as outlined in scheme II. After methylation, the alizarindimethyl-ether (VIII) was refluxed with potassium t-butoxide in dimethoxyethane,


HO
HO
OH
COOH
I
COOH
COOH
O
II
OH
COOH
OH
III
OH
OH
VII
OH
COOH
OH
IV
CO2
O
OH
OH
O
VI
OH
OH
V

Scheme I


O
OH
OH
9
A
B
C
10
O
100%
VI
O
OCH3
OCH3
9
A
B
C
10
O
VIII
9
COOH
A
10
COOH
101%
IX
A
9,10
COOH
49,8%
X
OCH3
OCH3
C
10
HOOC
1,2%
XI

Scheme II

yielding phthalic-(IX), benzoic-(X), and veratric acid (XI). The acids were separated and purified by chromatography and their specific activities determined. Phthalic acid was shown to have the same specific activity as the starting-material, benzoic acid contained 49.8 % of the activity of alizarin, whereas veratric acid was almost inactive. Radioactivity in the labelled acids was shown to be restricted to the carboxyl groups. Since the carboxyl group of veratric acid corresponds to keto-C-atom 10 of alizarin (scheme II) the carboxyl group of shikimic acid-carboxyl-^{14}C was incorporated only into C-atom 9 of alizarin. This indicates that, contrary to our earlier assumption[9], a symmetrical compound like 1,4-naphthoquinone (or -quinol) should not be regarded as a true intermediate in alizarin biosynthesis. This compound probably enters the biosynthetic pathway via a non-specific reaction. Additional information regarding the biosynthesis of alizarin was gained in the following way: 5-^{14}C-DL-mevalonic acid was administered to a root of Rubia tinctorum for 30 hours. Alizarin was isolated from the plant material and purified. It was then acetylated, nitrated at the 4-position and submitted to bromopicrin-cleavage[13]. The resulting bromopicrin, representing carbon 4 of alizarin, contained 82.6 % of the activity of the intact alizarin molecule. These results show that:

a) ring C of alizarin is derived from mevalonic acid as shown previously
b) the final hydroxylation of this anthraquinone moiety takes place preferentially at the C-atoms derived from C-atoms 3 and 6 of mevalonic acid, and
c) the dimethylallyl-pyrophosphate is attached in a position meta rather than ortho to the C-atom derived from the carboxyl group of shikimic acid (scheme I). This would be expected if 1,4-dihydroxy-2-naphthoic acid is the compound which undergoes prenylation to give (IV), and not 1,4-naphthoquinol or -quinone.

These results offer further support for the suggestion that 1,4-dihydroxy-2-naphthoic acid is an intermediate in quinone metabolism. We therefore propose the reaction sequence outlined in scheme I. This sequence (compounds I-V) may in principle also account for the biogenesis of vitamin K. The data presented

here, however, do not exclude the possible involvement of α-naphthol[3] and menadione[14] (2-methyl-1,4-naphthoquinone) in vitamin K-biosynthesis.

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