STABLE ISOTOPE STUDIES OF CANTHARIDIN BIOSYNTHESIS BY EPICAUTA PESTIFERA

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ABSTRACT

Stable isotope labeling experiments provide evidence regarding the chemistry of the biosynthesis of cantharidin from farnesol by adult male blister beetles (Epicauta pestifera): (1) Incorporation levels of D3-labeled farnesol, farnesal, and farnesoic acid were similar and indicated that they are natural intermediates in the transformation of farnesol into cantharidin, while the much lower incorporation of D3-methyl farnesoate offers evidence that it is not a normal intermediate in this transformation. (2) Incorporation of 18O from 18O2 into the tetrahydrofuranyl oxygen atom and two of the three anhydride oxygen atoms in cantharidin offers insight into the oxygenation chemistry of farnesol transformation into cantharidin. (3) Incorporation levels of farnesol selectively D3-labeled at one of the terminal farnesyl methyl groups revealed an absence of a kinetic deuterium isotope effect on the initial (and essentially indiscriminate) functionalization, offering evidence that this lack of stereoselectivity is the result of randomization of the methyl groups before presentation of one or the other of the methyl groups to the enzyme-associated chemical agent.

INTRODUCTION

The literature describing research on cantharidin provides a valuable sense of our chemical heritage, particularly regarding the evolution of stereochemical sophistication. Cantharidin was isolated from blister beetles and characterized as a white, crystalline substance in 1810.1 The correct chemical formula was not deduced until 1877, 67 years later.2 Nearly another seven decades passed before the efforts of several laboratories led

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to a structure assignment that seemed secure: In 1941 Woodward and Loftfield published their deductions about the relative stereochemistry of the ether and anhydride bridges.\textsuperscript{1} That structure assignment was confirmed by the first stereorational synthesis ever reported, completed on July 4, 1951, by Stork and three coworkers.\textsuperscript{3}

The biosynthetic pathway that gives rise to cantharidin offered its own puzzle, with respect to both origin of the atoms and latent stereochemistry of this achiral compound. The deceptively simple structure, comprising two enantiomeric halves, arises by a pathway that is without known parallel. The obvious possibility that cantharidin is formed by an overall process involving head-to-head/tail-to-tail coupling of two isopentenyl units, providing identical origins for the mirror image halves, is not the biosynthetic route. Early experiments\textsuperscript{4} also excluded a biochemical equivalent of the conceptually simple Diels-Alder route involving furan and dimethyl maleic anhydride or their equivalents, which also would provide identical origins for the mirror image halves of cantharidin.

**Farnesol as a Cantharidin Precursor**

A series of studies conducted by Schmid's group in Zurich established the origin of the entire carbon skeleton and essentially all of the hydrogen atoms as summarized in Figure 1.\textsuperscript{5} Thus, this apparent monoterpenes has a sesquiterpene parentage and a distinctly dissymmetric derivation of the two symmetric halves: The two enantiomeric portions are derived from the two different ends of farnesol in the specific manner depicted in Figure 1. Experiments established that the ten carbon atoms in a given cantharidin molecule all are derived from the same molecule of farnesol; that is, the six-carbon and four-carbon fragments derived from a molecule of farnesol never have the opportunity to separate in a manner that permits them to mix with others derived from other molecules of farnesol. A stereochemical aspect that presents an unsolved puzzle is the almost complete lack of stereoselectivity inherent in the process that accounts for oxidation of one of the terminal methyl groups, C-11' or C-12, to form, ultimately, one of the two carbonyl groups in the anhydride moiety.

Consideration of the structures of cantharidin and farnesol and the known origins of all ten carbon atoms in cantharidin permits enumeration of a set of required chemical transformations that must occur, as summarized in Figure 1 (atomic numbering of farnesol is used):

- Formation of two carbon-carbon bonds, joining C-4 and C-8 and joining C-3 and C-11

![Figure 1. Schematic illustration of the required chemical transformations involved in the conversion of farnesol into cantharidin.](image-url)