On the Biosynthesis of Cantharidin

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Abstract □ Feeding experiments have eliminated two obvious possible routes of cantharidin biosynthesis: the tail-to-tail condensation of two isoprene units and the head-to-tail condensation of two isoprene units followed by a methyl shift.

Keyphrases □ Cantharidin—*Mylabris* biosynthesis □ Mevalonate, acetate, radioactive—cantharidin biosynthesis □ Isoprenoids—cantharidin biosynthesis

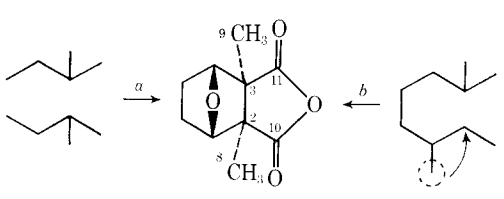
Schlatter *et al.* (1) recently reported results relating to the biosynthesis of cantharidin (I), the well-known active principle of "Spanish flies" (*Lytta vesicatoria*) and other cantharids. The authors wish to communicate some data which have been obtained in a similar study and which confirm the results of the Swiss group.

METHODS

In 1962, one of the authors (E.R.) had the opportunity to carry out a number of radioactive feeding experiments with "blistering flies" (*Mylabris* sp.) collected in Taiwan. For each experiment four insects were starved for 4 days and then fed the labeled precursor on small pieces of lettuce which were consumed. After 2 days, the flies were killed by exposure to chloroform vapors, dried in a vacuum over calcium oxide, ground to a powder, and extracted twice with chloroform with shaking for 24 hr. The residue from the filtered extract was washed repeatedly with petroleum ether and then dissolved in chloroform and enough 1 *N* NaOH to form a creamy mixture into which an excess of a 5% permanganate solution was stirred. After heating for 10 min. on a waterbath, the mixture was acidified with sulfuric acid, excess permanganate was destroyed with ferrous sulfate, and the cantharidin was extracted with chloroform and sublimed in a vacuum. Later, these samples of cantharidin were further purified to constant specific radioactivity by co-crystallization with carrier from chloroform/petroleum ether and from formic acid/water followed by another sublimation. Kuhn-Roth oxidation (2) gave acetic acid from carbon atoms 2, 3, 8, and 9, an aliquot of which was subjected to the Schmidt degradation (2) to give CO_2 (C-2 + C-3) and methylamine (C-8 + C-9). All samples and backgrounds were counted to at least 2% statistical error in a liquid scintillation spectrometer (Beckman LS 100), and counting efficiencies were determined using internal standards.

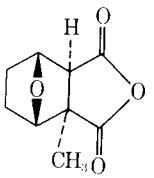
RESULTS AND DISCUSSION

The structure of cantharidin suggests an isoprenoid origin. Two possible modes of formation are immediately obvious (Fig. 1), one involving the tail-to-tail condensation of two isoprene units (3) (*a*) and the other the modification of a molecule of geranyl pyrophosphate, including a 1,2 shift of a methyl group (*b*). Pathway *a* requires that acetate-1-^{14}C gives rise to cantharidin carrying 50% of its radioactivity in carbon atoms 2 + 3, whereas according to Pathway *b* these carbon atoms should contain 25% of the radioactivity. Cantharidin obtained from feeding acetate-1-^{14}C (49 μc., 11.8 μc./μm.) after dilution had a specific activity corresponding to an incorporation of at least 0.007%. Upon Kuhn-Roth oxidation it gave acetic acid containing 68% of the total radioactivity, which was shown to be labeled only in the carboxyl group (less than 1% of ^{14}C in methylamine). This result clearly rules out Pathway *b*, and is also not in agreement with Pathway *a*. If mevalonate-2-^{14}C is fed, carbon atoms 8 + 9 according to Pathway *a* could carry 0, 50, or 100% of the total radioactivity. A feeding experiment with D,L-mevalonate-2-^{14}C (40 μc.) gave 0.005% incorporation and the acetic acid representing carbon atoms 2, 3, 8, and 9 of the cantharidin contained 21% of the radioactivity.



I

Figure 1.—*Two ways in which the carbon skeleton of cantharidin could arise from isoprenoid units.*



II

Figure 2.—*Desmethylcantharidin, a compound obtained from seeds of* Butea frondosa.

The results of this study show that neither of the two hypotheses mentioned above can account for the biosynthesis of cantharidin.[1] They suggest that cantharidin formation may be a more complicated process and seem to even cast some doubt on the mevalonoid origin of this compound. It may be of interest in this connection that a compound isolated from *Butea frondosa* seeds has recently been identified (Fig. 2) as desmethylcantharidin (II) (4).

REFERENCES

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[1] An experiment with methionine-methyl-^{14}C gave no detectable incorporation of ^{14}C into cantharidin.