Tetrahedron Letters No. 46, pp 4685 - 4686, 1972. Pergamon Press. Printed in Great Britain.

THE ROLE OF 2-KETOGLUTARATE IN LAWSONE BIOSYNTHESIS IN IMPATIENS BALSAMINA

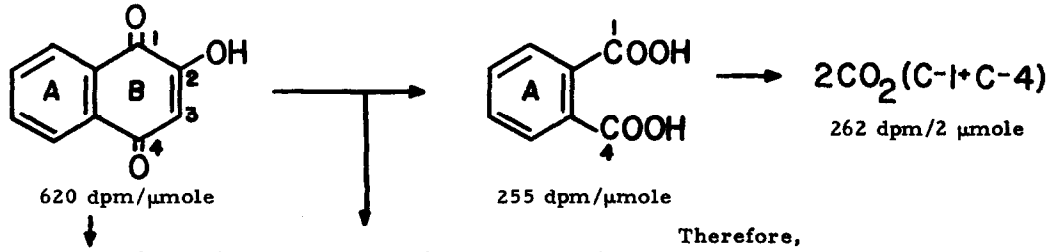
Eric Grotzinger and Iain M. Campbell

Department of Biochemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania, 15213

(Received in USA 1 September 1972; received in UK for publication 10 October 1972)

We have proposed (1) that the unit which together with shikimic acid (I) or derivative thereof (2), forms the naphthoquinone nucleus of lawsone (II), phylloquinone (IIIa) and the menaquinones (IIIb) is the thiamine pyrophosphate complex of succinyl semialdehyde (IV). This can be formed from 2-ketoglutarate (V) by the action of the citric acid cycle enzyme, 2-ketoglutarate dehydrogenase (3,4). Incorporation data in support of this proposal has come from feeding experiments with glutamate in I. balsamina (5), and in E. coli and Mycobacterium phlei (6). Glutamate (VI) can be expected to transaminate to 2-ketoglutarate. Direct evidence for the utilization of 2-ketoglutarate in menaquinone biosynthesis in E. coli has been obtained by Robins and Bentley (7). The finding of Dansette and Azerad (8) that 4-(2'-carboxyphenyl)-4-oxobutyrate (VII) was an intermediate in lawsone and menaquinone biosynthesis, provided additional support to our postulate. In this note, we not only demonstrate that 2-ketoglutarate is used in lawsone biosynthesis, we also corroborate that the observed labeling pattern precludes the involvement of symmetrical intermediates in the pathway.

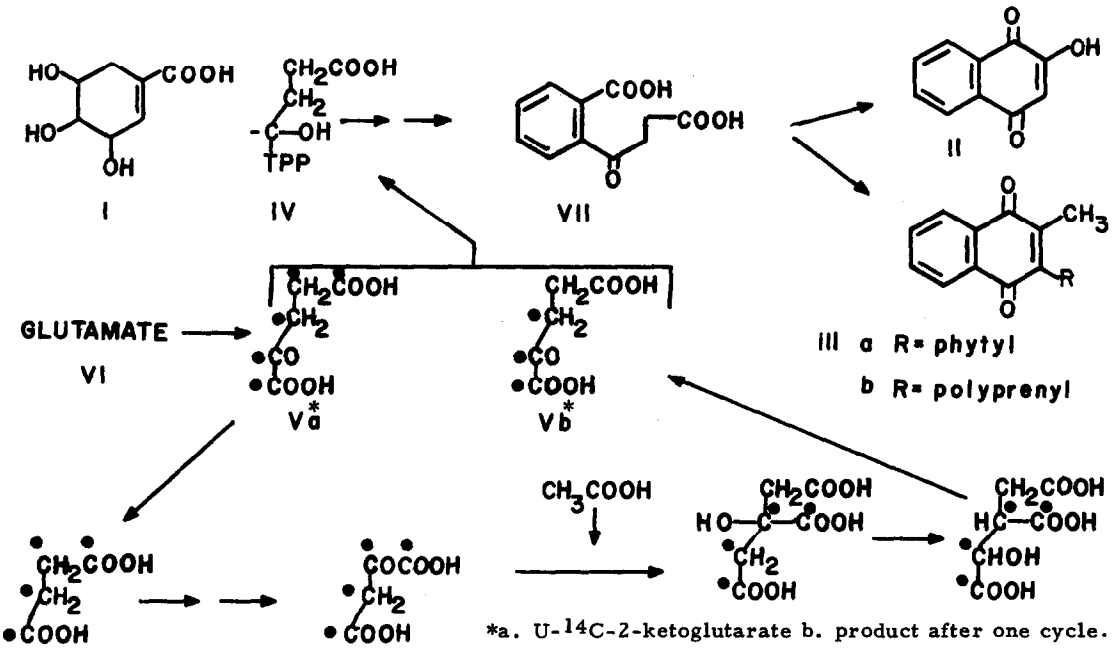
U-^{14}C-2-Ketoglutarate (100 μCi, 154 μg) was fed to two 5-week old cuttings of I. balsamina over a period of twelve hours by the methods previously described (5). The isolated lawsone had a specific activity of 11,163 dpm/μmole; the incorporation level was 0.2%; the dilution value 1.8×10^4. Degradation results are shown in the diagram below (for degradation methods, see references 1 and 5):



Therefore,

C-2 (620-255-250) = 120 dpm/μmole
C-3 = 250 dpm/μmole
C-4 (C-1 comes from shikimic acid) = 262 dpm/μmole

The label distribution is wholly in keeping with the utilization of the non-carboxyl carbon atoms of 2-ketoglutarate in the construction of ring B of lawsone. The relatively lower specific activity of C-2 vis-a-vis C-3 and C-4 is a simple ramification of the operation of the citric acid



*a. U-^{14}C-2-ketoglutarate b. product after one cycle.

cycle. As shown above every time the cycle turns, and assuming a pro-S citrate synthase is involved (9), cold acetate serves to dilute the activity present in C-4 (and C-5) of 2-ketoglutarate. This dilution phenomenon with cold endogenous acetate is a corollary of our previous observation (5) that radioactive acetate, when fed to I. balsamina, is preferentially accumulated in C-2. Taken together these two facts establish unequivocally that no symmetrical intermediate can occur between 2-ketoglutarate and lawsone.

Acknowledgements. The authors are grateful to Dr. R. Bentley for helpful discussion. This work was supported in part by Grant RR00273 of the U.S. Public Health Service.

References and Footnotes

1. I. M. Campbell, Tetrahedron Letts, 4777 (1969).
2. The exact nature of the shikimate derived C_7 unit is presently uncertain as are the details of the coupling. Mechanisms have been proposed which involve shikimate itself (reference 1), chorismate (reference 8), and prephenate (Grotzinger and Campbell, ACS Central Regional Meeting, Pittsburgh, 1972, Abstract #118).
3. D. R. Sanadi in Methods in Enzymology, Volume XIII, (Eds. S. P. Colowick and N. O. Kaplan) Academic Press, New York, 1969, p. 52.
4. L. J. Reed and B. B. Mukherjee, Reference 3, p. 55.
5. E. Grotzinger and I. M. Campbell, Phytochemistry, 11, 675 (1972).
6. D. J. Robins, I. M. Campbell, and R. Bentley, Biochem. Biophys. Res. Commun. 39, 1081 (1970).
7. D. J. Robins and R. Bentley, J. C. S. Chem. Commun., 232 (1972).
8. P. Dansette and R. Azerad, Biochem. Biophys. Res. Commun., 40, 1090 (1970).
9. R. Bentley, Molecular Asymmetry in Biology, Volume 2, Academic Press, New York, 1970, p. 96.