Biosynthesis of Phlebiarubrone in *Phlebia strigosozonata*[1]

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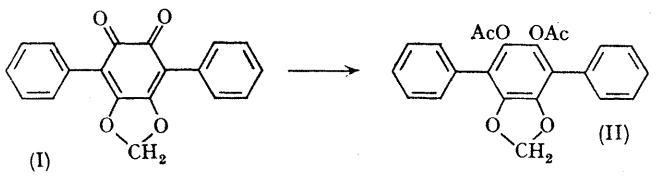
Summary ^{13}C-Labelled precursors and spectral methods show that the terphenyl ring system of phlebiarubrone can be derived from phenylalanine and the methylene group from formate: the relative proportion of doubly-labelled and singly-labelled metabolite reveals the amount of endogenous phenylalanine available for the biosynthesis of phlebiarubrone through the dimerization of a C_6–C_3 unit.

THE feasibility of using ^{13}C-labelled precursors in conjunction with n.m.r. and mass spectroscopic methods for studies on biosynthesis has been demonstrated recently.[1–6] Results from ^{14}C–^{3}H studies may be subject to uncertainty due to the isotope effect for ^{3}H. Furthermore, hydrogen atoms may undergo some exchange with the aqueous medium especially if the incorporation experiment extends over a long time. Precursors that are simultaneously labelled with ^{13}C and ^{14}C are free from the uncertainties of ^{3}H-labelling. The lack of radioactivity of ^{13}C makes it possible to use substrates with a high ^{13}C-level (50—60% is commercially available) whereas the isotope level usual for ^{14}C is very low. One direct consequence of this is important if the metabolite molecule is capable of incorporating the label at more than one site; under these circumstances the use of ^{13}C may provide much more detailed information than ^{14}C about the biosynthetic steps involved.

We report here on the biosynthesis of phlebiarubrone (I),[8] a structure that lends itself readily to studies with ^{13}C-labelled precursors. Labelled substrates were added to a three-week-old shake culture[8] of *Phlebia strigosozonata* and (I) was harvested after 15 d. In a typical experiment, 10 mg. (61 μmoles) of (±)-[3-^{13}C]phenylalanine (^{13}C 50%) containing (±)-[1-^{14}C] phenylalanine (50 μ c) was added to

the substrate and the phlebiarubrone obtained (12 mg.) was converted into the more soluble and more volatile leuco-acetate (II)[8] for n.m.r. and mass spectral studies and liquid scintillation counting.



Since formate is known to serve as a source of a one-carbon fragment, $H^{13}CO_2H$ was examined as a precursor of the methylenedioxy-group in (I). By mass spectral analysis of (II), it was found that ^{13}C-level of 5—11% could be reached in (I) in most experiments. The location of the isotope was ascertained from the ^1H n.m.r. spectra of (II). The area under the ^{13}C-satellites of the methylene signal indicated the ^{13}C-level when compared with the ^{13}C-satellites of the methyl signal of the acetate groups containing the natural abundance of ^{13}C (1·1%). The value so calculated was found to be the same as the total isotope incorporated in (II) as determined by mass spectroscopy. Hence, the formate had labelled efficiently and exclusively the methylenedioxy-group.

In one experiment with phenylalanine as the substrate, the mass spectrum of (II) showed that 5·4% of the molecules were labelled with one ^{13}C atom while 1·8% of the molecules were doubly labelled with ^{13}C. This type of information cannot be obtained from ^{14}C-labelling data as chemical degradation is incapable of discriminating between singly-labelled and doubly-labelled species of a symmetrical compound such as (I) or (II).

If, at the time of the addition of the labelled phenylalanine, the endogenous phenylalanine (or its biochemical equivalent) was present in such a quantity that the mole-fraction of ^{13}C-labelled species became "a" instead of 0·5, then, from the laws of probability it can be shown that the ratio of doubly-labelled dimer† to the singly-labelled dimer must be $a^2/2a(1-a)$ or $a/2(1-a) = 1{\cdot}8$; hence $a = 0{\cdot}4$. This indicates that the maximum amount of phenylalanine (or its equivalent) available endogenously for biosynthesis was 25% of that added or about 15 μmoles. Such quantitative information appears to be unavailable by other methods of analysis.

Of the 12 mg. (40 μmole) of (I) obtained in the above experiment, 5·4%, (2·16 μmole) was doubly labelled. ^{13}C-labelled phenylalanine added as precursor was 0·5 (61 μmole) or 30·5 μmole. Hence, the proportion of precursor transformed to the metabolite (I) was about 12%.

The efficiency of incorporation‡ of ^{13}C is 9·0%. From the radioactivity data it was calculated that the efficiency of ^{14}C incorporation was 7·7%. In a duplicate experiment very similar values were obtained. Since the relative proportion of ^{13}C and ^{14}C in the precursor and the final product were nearly the same within limits of experimental error,§ the three carbon atoms of the side chain of phenylalanine must remain intact during the transformation to (I).

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† Doubly-labelled dimer = a^2; unlabelled dimer = $(1-a)^2$; singly-labelled dimer = $1 - [a^2 + (1-a)^2] = 2a(1-a)$.

‡ Efficiency of incorporation is defined as Sp. activity of product × 100%/Sp. activity of precursor × n where n is the number of sites per molecule that could be labelled.

§ The error in measuring the small peak at $M + 2$ is comparatively large.

[1] Biosynthetic Studies. For previous part see: A. K. Bose, K. S. Khanchandani, R. Tavares, and P. T. Funke, *J. Amer. Chem. Soc.*, 1968, **90**, 3593.

[2] A. K. Bose, P. T. Funke, K. G. Das, and R. J. Suhadolnik, Abstracts, 4th International Symposium on the Chemistry of Natural Products, Stockholm, June 1966, p. 150.

[3] A. K. Bose, Second Natural Products Symposium, University of West Indies, January 1968; A. K. Bose, K. S. Khanchandani, R. Tavares, and P. T. Funke, Abstracts, 5th International Symposium on the Chemistry of Natural Products, London, July 1968, p. 119.

[4] A. K. Bose, K. G. Das, P. T. Funke, I. Kugajevsky, O. P. Shukla, K. S. Khanchandani, and R. J. Suhadolnik, *J. Amer. Chem. Soc.*, 1968, **90**, 1038.

[5] M. Tanabe and G. Detre, *J. Amer. Chem. Soc.*, 1966, **88**, 4515.

[6] (a) D. Sesaty, A. G. McInnes, D. G. Smith, and L. C. Vining, *Canad. J. Biochem.*, 1968, **46**, 1293; (b) A. G. McInnes, D. G. Smith, L. C. Vining, and J. L. C. Wright, *Chem. Comm.*, 1968, 1669.

[7] See for example, G. Guroff, C. A. Reifsnyder, and J. Daly, *Biochem. Biophys. Res. Comm.*, 1966, **24**, 720.

[8] T. C. McMorris and M. Anchel, *Tetrahedron*, 1967, **23**, 3985.