Biosynthesis of Benzofurans

By J. D. BU'LOCK,* A. T. HUDSON, and B. KAYE

(*Microbial Chemistry Laboratory, Department of Chemistry, University of Manchester, Manchester* 13)

IN terms of our present knowledge, the biosynthetic origin of the fungal metabolite[1] 5-methoxybenzofuran (I) is not immediately obvious. Here we present a preliminary account of the somewhat surprising biogenetic complexity of this substance and of a series of new benzofurans from the same source, which give some new insight into the secondary metabolism of shikimic acid derivatives in fungi.

Since the carbon–oxygen skeleton of (I) is also present in homogentisic (2,5-dihydroxyphenylacetic) acid, a tyrosine metabolite in some organisms, the incorporation of [2-^{14}C]phenylalanine and -tyrosine into (I) was first examined. Reduction of (I) with Na–NH_3 and Kuhn–Roth oxidation of the resulting 3-ethyl-4-methoxyphenol (as the phenylurethane) to acetic and propionic acids gave a degradative route to C(2), C(3), and the adjacent C(3a), of (I). The incorporations observed were not good ($<0{\cdot}1\%$) and with both precursors only 30% of the total activity of (I) was in the expected position at C(2); C(3) and C(3a) were virtually inactive (6% and 2%, respectively). Even more disturbing was the result of incorporating [1-^{14}C]-phenylalanine (incorporation $0{\cdot}2\%$); 80% of the activity of (I) was at C(3) (!) and C(2) and C(3a) were inactive.

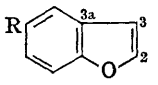
It was next found that use of [1-^{14}C]- and [2-^{14}C]-acetate gave virtually the same distributions of activity in (I) as [1-^{14}C]- and [2-^{14}C]-phenylalanine respectively, but with higher efficiencies ([1-^{14}C]acetate, $0{\cdot}4\%$; [2-^{14}C]acetate, $0{\cdot}6\%$). From this we concluded first, that C(2) and C(3) of (I) represent, respectively, C(2) and C(1) of an "acetate unit" (which might originate either from acetate itself or from a mevalonate-derived isoprene unit) while the remainder of the carbon skeleton has an unknown non-acetate origin; second that in the basidiomycete (*Stereum subpileatum* Berk and Curt) from which (I) is obtained, aromatic amino-acids can be degraded so as to give C(1) and C(2) as C(1) and C(2) of an acetyl derivative. Some indication of the origin of the benzene ring of (I), and confirmation that it is not "acetate-derived", was given by the relatively efficient ($0{\cdot}2\%$) incorporation of [6-^{14}C]glucose. This incorporation was specifically into *part* of the benzene ring, since C(3a) was inactive as well as C(2) and C(3).

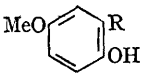
At this stage we decided to re-examine the co-metabolites of (I), reported originally as accompanied by cinnamaldehyde and cinnamic acid. By omitting the over-selective step of steam distillation used by Birkinshaw *et al.*,[1] we have now isolated and characterised the further substances (II)—(VIII) and also detected (by gas chromatography after methylation) (IX)—(XII) [for the products (II)—(VII), which are new, fully adequate analytical, spectroscopic, and degradative data were obtained]. Of these products the diol (IV), 5-(1′,2′-dihydroxypropyl)benzofuran, is the major co-metabolite. These structures themselves throw some light on the biosynthetic origins of (I) and (IV); we continued by investigating the incorporation of (uniformly-labelled) [U-^{14}C]phenylalanine into (IV), since this now offers a larger carbon–oxygen skeleton.

The diol (IV) was split to give acetaldehyde and, after oxidation, 5-carboxybenzofuran, which could be decarboxylated; thus data for C(1′)—C(3′) of the side-chain were accessible. After incorporation of [U-^{14}C]phenylalanine [$0{\cdot}3\%$ in (IV)], C(2′) and C(3′) of the side-chain were unlabelled and C(1′) contained $14{\cdot}7\%$ ($= 1{\cdot}03/7$) of the total activity. Hence phenylalanine contributed a C_7 unit—such as benzoic acid, benzaldehyde, or a *p*-hydroxy-analogue, to the biosynthesis of (IV).

At present we have no data for the incorporation of [^{14}C]acetate into (IV), but it seems most probable that C(3′)—C(2′) of the side-chain represent a second acetate-derived unit, with C(2) and C(3) of (IV) also being acetate-derived as in (I).

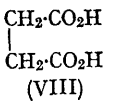
Conceivably it is the 5-formyl-derivative (II) which is the immediate precursor of the other benzofurans, since from it (V) can be derived by a mixed acetoin condensation with acetaldehyde or pyruvate (for which there are numerous parallels), and thence (IV) and (VI); equally, an acceptable oxidation of (V) followed by methylation would yield (I). For the derivation of (II) itself from phenylalanine we postulate [combining the data for (I) and for (IV)], deamination to





(I) R=OMe
(II) R=CHO
(III) R=CH_2·OH
(IV) R=CH(OH)·CH(OH)·CH_3 (1′, 2′, 3′)
(V) R=CO·CH(OH)·CH_3
(VI) R=CH—CH·CH_3 (O)

(VII) R=CH_2·CH_2·OH
(IX) R=H



(VIII)

Ph·CO_2H (X)

PhCH : CH·CO_2H (XI)

p-MeO·C_6H_4·CH : CH·CO_2H (XII)

cinnamic acid (XI), followed by hydration, oxidation, and hydrolysis (as in β-oxidation). This, with *p*-hydroxylation at some stage (or with tyrosine as an alternative starting-point) offers a route to (X) and (XII), and also to *p*-hydroxybenzaldehyde or some similar compound, from which (II) could be obtained by isoprenylation and degradation, or by some other route introducing the two acetate-derived carbons of the furan ring. The isoprenylation route, which we prefer for this step, is that which was recently confirmed for a furanocoumarin (pimpinellin) in a higher plant.[2] The breakdown of aromatic amino-acids by a form of β-oxidation is also well-recognized in higher plants but relatively novel in fungi, and this appears to be the first evidence for the generation of an actual acetate unit by this route. Secondary metabolites of the "shikimic acid series" are relatively less common in fungi than in green plants, so that any comparative data are of value, particularly since, in the natural state, most fungi employ plant material as their nutrient source. For example, it is quite probable that wild growths of *S. subpileatum*[1] produce these benzofurans from the lignin breakdown products which other white-rot fungi utilize in different ways.[3]

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[3] J. D. Bu'Lock and D. C. Walker, *J. Chem. Soc.*, 1967, 336.