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NEW BENZOFURANS FROM *STEREUM SUBPILEATUM*, THEIR BIOSYNTHESIS, AND RELATED PROCESSES OF AROMATIC AMINOACID METABOLISM IN A BASIDIOMYCETE

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Abstract—From *Stereum subpileatum* cultures are obtained 5-methoxy-, 5-hydroxy-, 5-formyl-, 5-hydroxymethyl-, 5-carboxy-, 5-(1,2-dihydroxypropyl)-, 5-(1,2-epoxypropyl)-, and 5-(1-oxo-2-hydroxypropyl)-benzofuran, 2-(2-hydroxyethyl)-4-methoxyphenol, succinic acid, and a C_{12} benzofuran acid of uncertain structure. Precursor-incorporation studies with product degradations show that in these compounds (*a*) the two carbons of the furan ring derive from acetate, probably *via* mevalonate; (*b*) the benzene ring and one carbon at $C_{(5)}$ derive from a C_6C_1 intermediate formed alternatively from C_6C_3 aminoacids by side-chain degradation or from sugars *via* shikimic acid; (*c*) the rest of the side-chain in the 5-propyl-derivatives arises from a metabolic intermediate close to enolpyruvate and is attached by an acetoin-condensation type of process. Analytical data suggest that the central route to these benzofurans is *via* the 5-formyl-compound, which is consistent with the biogenetic pathways proposed.

INTRODUCTION

Reports of flavour contamination in beer-casks, infected with the Basidiomycete *Stereum subpileatum* Berk and Curt, led Birkinshaw and co-workers to study the steam-volatile metabolites of this fungus. They were able to isolate cinnamic acid, cinnamaldehyde, and 5-methoxybenzofuran (I).[1] Though (I) was the simplest of natural benzofurans, its biosynthesis was not obvious *a priori* and seemed to merit investigation. Here we describe the simultaneous formation of several new co-metabolites of (I) and the results of some precursor-incorporation studies, which clarify the question of the origin of these benzofurans and also throw light on the general pathways of aromatic acid metabolism in this species.[2]

RESULTS

Characterization of Metabolites

Details of the extraction, separation, and characterization of the compounds (I)–(XI) are given in the Experimental. The characterizations were largely based upon the NMR and mass-spectroscopic data. Chemical characterization steps included: cleavage of the diol (VI) to acetaldehyde and the aldehyde (III); conversion of (III) into the carbinol (IV) and the acid (V); conversion of (I) into (II) and into 2-ethyl-4-methoxyphenol; comparisons of (I) and (VI) with authentic synthetic samples. With the exceptions of (I) and (obviously) (X), these compounds are all new as natural products; structure (XI) is not established with complete certainty.

[1] J. H. Birkinshaw, P. Chaplen and W. P. K. Findlay, *Biochem. J.* **66**, 188 (1957).

[2] A preliminary account of part of this work is: J. D. Bu'Lock, A. T. Hudson and B. Kaye, *Chem. Commun.* 814 (1967).

Production

In our earlier work,[2] following Birkinshaw *et al.*,[1] *S. subpileatum* was grown (as surface mats) on 5% malt extract, and then afforded mainly (I) and the new diol (VI) in roughly equal amounts.[3] For some subsequent work we used (initially by accident) 10% malt extract, on which (VI) is the major metabolite, with relatively smaller yields of (I) and the aldehyde (III). Quantitative extractions from such cultures showed that the first benzofuran to appear was the aldehyde (III), as shown in Fig. 1; in this same experiment, the maximum yields were: of the diol (VI), 530 mg/l. at 28 days; of the aldehyde (III), 56 mg/l. at 21 days; of the ether (I), 180 mg/l. at 35 days.

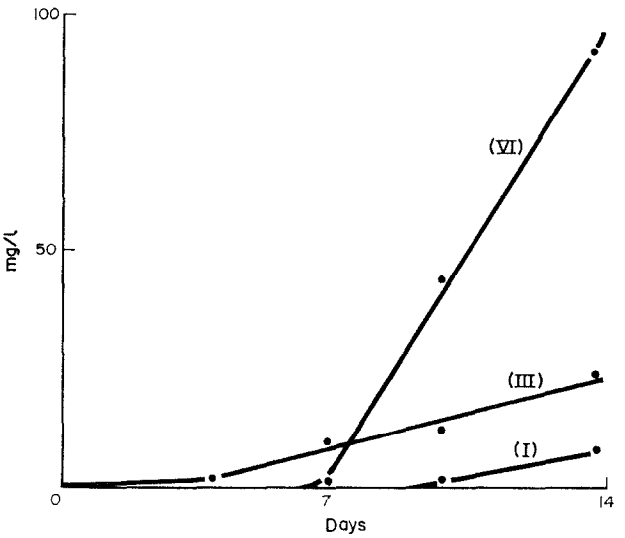


Fig. 1. Yields of the major metabolites (I), (III) and (VI) in surface cultures of *S. subpileatum*.

Tracer Incorporation

The results of several series of experiment, in which various tracers were fed to full-grown mycelial mats with 3–7 days' further incubation, are summarized in Table 1 in terms of per cent incorporation into the major metabolites. The purified products from these experiments were degraded by the routes summarized in Fig. 2 and fully described in the experimental section, and the results of these degradations in terms of the location of labelling in the metabolites are presented in Table 2. Detailed protocols for the individual tracer experiments are available on request.[4]

DISCUSSION

Origin of the Benzofuran Ring

The origin of $C_{(2)}$ and $C_{(3)}$ of, e.g. (I) was our first concern. In benzofurans from higher plants, which sometimes carry an isopropyl residue at $C_{(2)}$ and sometimes do not, these

[3] The earlier workers (cf. Ref. 1) isolated the steam-volatile products and hence compound (VI) would not have been detected.

[4] A. T. Hudson, Ph.D. Thesis, University of Manchester (1968).

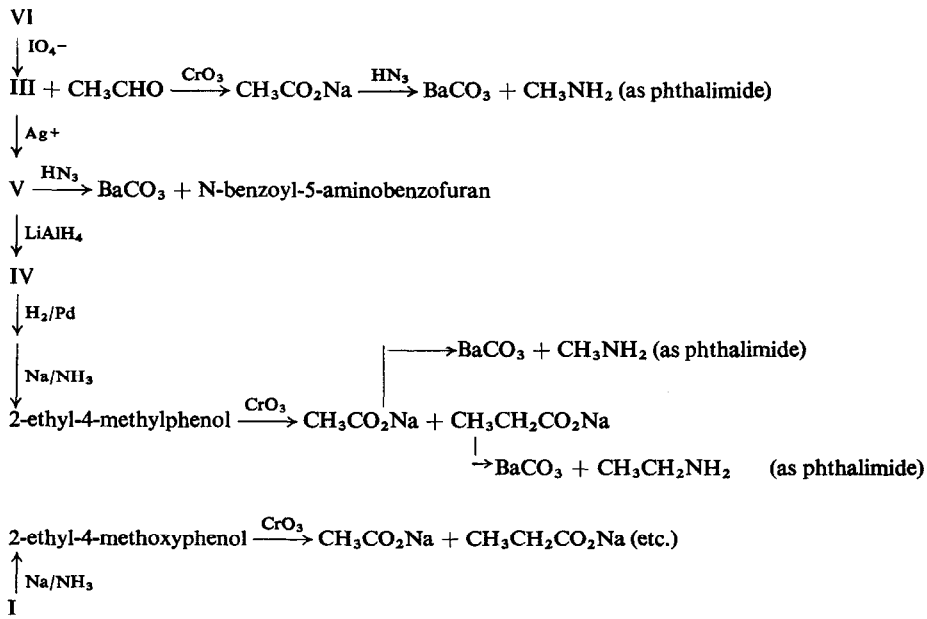


FIG. 2. DEGRADATION SCHEME FOR LABELLED METABOLITES.

TABLE 1. PERCENTAGE INCORPORATION OF ^{14}C INTO *S. subpileatum* BENZOFURANS

Series A	Isolated (% total ^{14}C fed)
Fed (at 5 weeks on 5% malt)	[as (I) only]
2′-^{14}C-DL-Phenylalanine	0·09
2′-^{14}C-DL-Tyrosine	0·03
1′-^{14}C-DL-Phenylalanine	0·18
1-^{14}C-Acetate (Na)	0·38
2-^{14}C-Acetate (Na)	0·58
6-^{14}C-D-Glucose	0·16

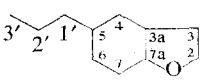
Series B	Isolated (% total ^{14}C fed)			
Fed (at 2 weeks on 10% malt)	(I)	(III)	(VI)	(I)+(III)+(VI)
U-^{14}C-L-Phenylalanine	0·06	0·11	0·35	0·52
1-^{14}C-Acetate (Na)	0·01	0·01	0·03	0·05
2-^{14}C-DL-Lactate (Na)	0·003	0·02	0·08	0·10
6-^{14}C-D-Glucose	0·01	0·01	0·03	0·05

carbons derive from mevolonate; a phenol is alkylated in the *ortho*-position by a dimethylallyl group and cyclization, sometimes with loss of a C_3 unit, follows.[5]

In our case, the specific incorporation of 1- and 2-^{14}C-acetate into $C_{(3)}$ and $C_{(2)}$ respectively (Table 2) is entirely consistent with such a mechanism. An alternative, involving direct attachment of a C_2 unit as such, is not excluded but cannot be very plausibly formulated. The data do exclude the possibility that the C_8 benzofuran skeleton is any kind of polyketide. An initially attractive hypothesis was that, since the carbon-oxygen skeleton of (II) is the same as that of homogentisic acid, it might arise as a unit by the same pathway as that leading to homogentisate in animals, i.e. from tyrosine via *p*-hydroxyphenylpyruvate with 1,2-shift of the side-chain. Taken alone, the observation that 2′-^{14}C-phenylalanine and

TABLE 2. DISTRIBUTION OF ^{14}C IN *S. subpileatum* BENZOFURANS*

Series A† Fed	% Total ^{14}C in C atom No.							
	2	3	3a	4–7a	5	1′	2′	3′
2′-^{14}C-DL-Phenylalanine	30	5	2	65	—	—	—	—
2′-^{14}C-DL-Tyrosine	30	7	2	63	—	—	—	—
1′-^{14}C-DL-Phenylalanine	0	80	0	22	—	—	—	—
1-^{14}C-Acetate (Na)	0	67	0	33	—	—	—	—
2-^{14}C-Acetate (Na)	33	0	0	70	—	—	—	—
6-^{14}C-D-Glucose	0	0	0	100	—	—	—	—
Series B‡								
U-^{14}C-Phenylalanine	0	0	15·0	71·5	—	14·7	0	0
1-^{14}C-Acetate (Na)	0	98	0	1	—	1	0	0
2-^{14}C-DL-Lactate (Na)	0	75	0	13	3	2	10	0
6-^{14}-D-Glucose	12·5	0	0	49·5	0	0	0	39

* Numbering 

† Only 5-methoxybenzofuran, (I), was isolated.

‡ (I), (III) and (VI), degraded separately, gave identical distributions within the common structure; tabulated results are for diol (VI) only.

-tyrosine label $C_{(2)}$ of (I) fairly selectively would seem to support this, but since in the parallel experiment $C_{(1)}$ of phenylalanine (which would be absent in homogentisate) specifically labels $C_{(3)}$ of (I) with similar (low) efficiency shows that a different explanation is required. It is in fact very doubtful whether the homogentisate pathway as such occurs in these fungi at all. Instead, comparison of the ^{14}C-phenylalanine data with those for ^{14}C-acetate strongly suggests that both 1-^{14}C- and 2-^{14}C-phenylalanine only label (I) indirectly, *viz* by way of 1-^{14}C- and 2-^{14}C-acetate, and this observation is fully consistent with the view, developed further below, that the C_6C_3 aminoacids in *Stereum* can be broken down by a type of β-oxidation to a C_6C_1 unit and acetyl-CoA.

[5] H. G. FLOSS and U. MOTHES, *Phytochem.* **5**, 169 (1966).

Presence of a C_6C_2 Unit and its Origin

When only the 5-methoxy-compound (I) was available (Table 2, series A) the only clue to the origin of the benzene ring, to which $C_{(3)}$ and $C_{(2)}$ are attached as above, came from the incorporation of 6-^{14}C-glucose, specifically into certain (unlocated) atoms of the benzene ring (Table 2) and with fair efficiency (Table 1). In this series of experiments, the benzene ring was also labelled by ^{14}C from acetate, somewhat specifically but obviously indirectly. Though this latter effect was not observed in series B, both sets of data are consistent with formation of the benzene ring by way of the shikimic acid pathway, given that in series B the different nutritional conditions (younger cultures, richer medium) suppressed the 'back-incorporation' of acetate into shikimate *via* sugars.

With the realization that (VI) is also a major metabolite, it became apparent that the benzene ring could be part of either a C_6C_1 or a C_6C_3 unit, neither of which was contributing to the extra carbon atoms in the furan ring and either of which could appear as a bare C_6 unit in (I). The incorporation data for U-^{14}C-phenylalanine into (VI) show that, even with this potential C_6C_3 donor, it is actually a C_6C_1 unit which is involved (Table 2); this raises immediately the question of the origin of the remaining carbon atoms of the side-chain in (VI), which is considered subsequently.

Two alternative routes from the shikimic pathway to C_6C_1 precursors need to be considered. In one, hydroxybenzoic acids, etc. are formed directly from C_6C_1 intermediates at or near the shikimic acid level; in the other, synthesis continues *via* prephenate to the C_6C_3 aminoacids and thence degradatively by way of cinnamic acids and β-cleavage to *p*-hydroxy-benzoic acid, -benzaldehyde, or -benzoyl-CoA.[6] In higher plants the *simultaneous* operation of both pathways is conceivable[7] but has not been directly demonstrated. In higher fungi, the degradative route is widespread[8] and should *a priori* be particularly considered in white-rot lignin-utilizing species such as *S. subpileatum*.

In our data, the incorporation of added phenylalanine and tyrosine into the benzofurans is clearly by the 'degradative' pathway with generation of both a C_6C_1 and a C_2 ('acetate') unit. However, if this were the only pathway to the C_6C_1 unit, labelling from 6-^{14}C-glucose should be found in $C_{(1)}$ of the side-chain as well as in two carbon atoms of the benzene ring,* whereas by the 'direct' pathway labelling should reach the benzene ring only. The experimental data accord only with the latter alternative. It therefore appears that in this species the C_6C_1 unit can be formed by either pathway, according to the carbon source available.

Origin of the Side-chain

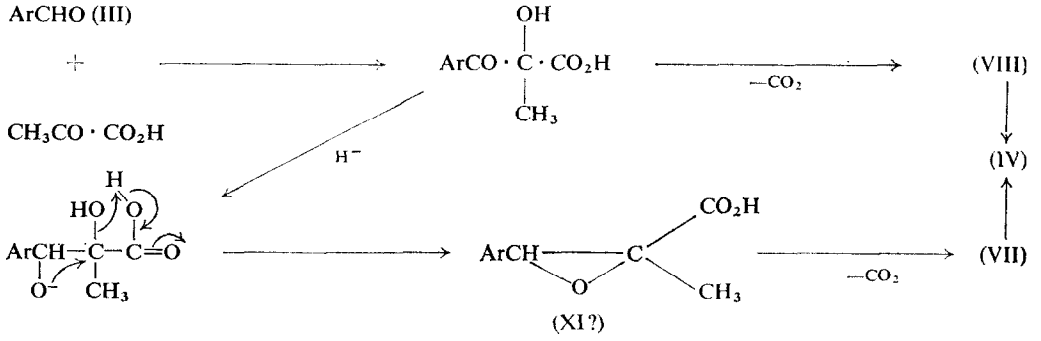
On the origin of the CH_3CHOH unit which is attached to the C_6C_1 group in (VI), the co-occurrence of the hydroxyketone (VIII) emphasizes that some type of mixed acetoin condensation, e.g. between the aldehyde (III) and an 'acetaldehyde donor', is most likely, given the well-known ability of many fungi to effect such reactions with exogenous aldehydes. However, the relationship of the epoxide (VII) to such a reaction scheme, and the precise identity of the 'acetaldehyde donor', are less clear.

* In shikimic acid, two labelled ring atoms via 4-^{14}C-tetrose and 3-^{14}C-phosphoenolpyruvate; in prephenic acid, an additional label from 3-^{14}C-phosphoenolpyruvate in the side-chain.

[6] M. H. Zenk and E. Leistnev, *Lloydia* **31**, 275 (1968).
[7] P. M. Dewick and E. Haslam, *Chem. Commun.* 673 (1968).
[8] G. H. N. Towers, *Biochem. J.* **106**, 507 (1968) and earlier papers.

The 'acetaldehyde unit' is not formed by the reduction of acetyl-CoA, since it is negligibly labelled by ^{14}C-acetate. When ^{14}C-lactate is fed, as a source of labelled pyruvate, it is clear from Table 2 that most of the observed incorporation into (VI) is into $C_{(2)}$ and $C_{(3)}$ and hence via acetate. However, the presence of some labelling in the C_6C_1 unit shows that some incorporation is also occurring through sugars, and the acetaldehyde unit simultaneously acquires comparable labelling. Conversely, when in feeding 6-^{14}C-glucose most of the labelling is passing via sugars into the C_6C_1 unit, but some is reaching the acetyl-derived atoms of the furan ring (presumably via triose and pyruvate), the acetaldehyde unit again acquires labelling. It follows from the tracer data that the 'acetaldehyde donor' is a metabolic intermediate lying somewhere between triose phosphate and pyruvic acid. The situation is thus very similar to that which has been described for a C_3 unit in canescin.[9]

In this connection the structure assigned on purely chemical grounds to the C_{12} benzofuran, (XI), has obvious relevance. Even if the suggested structure is not precisely correct, this acid clearly contains the structural elements of the aldehyde (III) and of pyruvate, and it could well be a precursor, not only of the diol (VI) but also of the otherwise inexplicable epoxide (VII).* A conventional thiamine-dependent condensation of (III) with pryuvate would give the aroyl-lactic acid; as shown in the scheme, this could decarboxylate directly, to give the acyloin (VIII), or alternatively, by hydride reduction generate (XI), from which the epoxide (VII) could arise. The diol (VI) could then be formed either from (VII) or from (VIII).



Other Products

The phenol (II) and its ether (I) could plausibly be formed from the aldehyde (III) by direct (Dakin-type) oxidation; the phenol (IX) is clearly related to these two compounds but there is nothing to indicate whether a benzofuran is an intermediate in its formation. The accumulation of succinic acid, although quite large, is not very unusual. Exhaustive studies of the acidic fraction, in particular by GLC of trimethylsilylated material, failed to confirm the presence of benzoic, cinnamic, or *p*-hydroxycinnamic acids, which were reported by us in a preliminary communication[2] on the basis of GLC studies of methylated material.

* It is *a priori* less likely that the epoxide could be formed from the diol, and equally there is no evidence for the presence of the alternative precursor 5-propenylbenzofuran (nor any very consistent pathway by which this might be produced).

[9] A. J. Birch, F. Gager, L. Mo, A. Pelter and J. J. Wright, *Australian J. Chem.* **22**, 2429 (1969).

Conclusions

The initially simple objective of accounting for the biosynthesis of a single product (I), led to surprising complexities. The range of metabolites was considerably extended [(I)–(XI)] and direct or circumstantial evidence was obtained for a variety of processes:

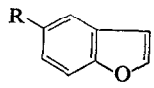
(a), Alkylation of an aromatic ring, leading to formation of the benzofuran system [and presumably of the hydroxyethyl side-chain of (IX)];

(b), β-cleavage of aromatic C_6C_3 acids to reactive acetate and a C_6C_1 compound, which furnishes the aromatic ring of compounds (I)–(IX) and (XI);

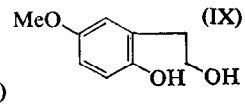
(c), independent formation of the same C_6C_1 intermediate from shikimic acid by the 'direct' route;

(d), involvement of a pyruvic acid derivative in an acetoin-type condensation, with subsequent transformations of the product giving rise to compounds (VI)–(VIII);

(e), formation of (I) and (II) by oxidation of the aldehyde (III).



(I) R = OMe
(II) R = OH
(III) R = CHO
(IV) R = CH_2OH
(V) R = CO_2H
(VI) R = $CHOH \cdot CHOH \cdot CH_3$
VII) R = $CH\text{-}CH \cdot CH_3$ (O)
(VIII) R = $CO \cdot CHOH \cdot CH_3$



(IX)

$HO_2C \cdot CH_2CH_2 \cdot CO_2H$ (X)

EXPERIMENTAL

Growth and Extraction Methods

Inocula taken from 2% malt–agar plates of *S. subpileatum* (Forest Products Research Laboratory Strain 271A) were supported on glass wool in 'penicillin flasks' containing 750 ml (*ca.* 4 cm) of 5% or 10% malt extract medium, using 5 or more inocula per flask. Mats were normally grown for 3 weeks at 20° in nominal darkness; the mycelium contained negligible quantities of the compounds under investigation and was removed.

The medium was acidified (pH 3), filtered, and continuously extracted with ether. Evaporation of the ether gave an oil which was separated into 'neutral' and 'acidic' fractions by partition between ether and 10% aq. NaOH. The netural fraction was resolved by chromatography on silica gel with slowly increasing proportions (20%–75%) of diethyl ether in hexane. Thus in a radiochemical run, 0·5 l. of medium gave 0·25 g 'neutral' fraction from which were isolated (in order of elution): (I), 20 mg; (III) 30 mg; (IV), 6 mg; (VIII), 5 mg; (VI), 170 mg. In other runs, very small amounts of (VII) (1 mg from 6 l. medium) were isolated similarly. Products were purified by repeated column or TLC according to the amount available.

The 'acidic' fraction (2 g accumulated material) was dissolved in 10% aq. NaOH (50 ml) with 50 g of NaCl. Exhaustive extraction with ether and evaporation of the extract gave an oil (0·3 g), which on chromatography on silica gel afforded (VI) and about 25% of (IV). The alkaline solution was acidified (4N aq. HCl at 0°) and continuously extracted with ether; the extract was evaporated on to washed Celite (20 g) which was then extracted in a Soxhlet thimble successively (*a*) for 1 hr with hexane (fraction A, 0·4 g), (*b*) overnight with hexane (fraction B, 0·3 g), and (*c*) exhaustively with ether (fraction C, 0·9 g). Fraction A was taken up in ether and extracted with saturated aq. $NaHCO_3$, acidification of which gave (V); fraction C was triturated with a little ether and gave (X). Fraction B (200 mg) was resolved by partition chromatography on dichlorodimethylsilanetreated Embacel kieselguhr (100–120 mesh) in amyl acetate, eluting with aqueous buffer (0·2 M NaOH–KH_2PO_4, pH 7; 0·1 M HCl–Na borate, pH 9; 0·15 M NaOH–Na_2HPO_4, pH 11) giving, in order of (overlapping) elution (V), (IX), the C_{12} benzofuran, and (II), which were further purified individually by TLC on silica gel F in EtOAc.

Characterization of Products

5-Methoxybenzofuran (I). M.p. 31·5–32°, λ_{max} 247, 293, 300 nm, was identical with synthetic[10] material. In the NMR spectrum (CCl_4) (OMe, 3Hs, 6·23τ), following published observations[11] and our own data on related compounds, the aromatic protons could be assigned as follows (J values in c/s); H(2) d, 2·46τ; H(3) dd, 3·36τ; H(4) dd, 3·05τ; H(6) dd, 3·15τ; H(7) dt, 2·68τ ($J_{2,3}$ 2·2; $J_{3,7}$ 0·9; $J_{4,6}$ 2·9; $J_{4,7}$ 1·1; $J_{6,7}$ 9·0). With such data as a guide it was confirmed that the other benzofurans of *S. subpileatum* were all 5-substituted. In the mass spectrum of (I) the main fragmentations are:

$$M^+ = 148(100\%) \xrightarrow{-CH_3} 133\ (70\%) \xrightarrow{-CO} 105\ (45\%) \xrightarrow{-CO} 77\ (82\%)$$

$$\downarrow -CH_2O$$

$$118\ (12\%) \xrightarrow{-CO} 90\ (10\%) \xrightarrow{-H} 89\ (16\%) \xrightarrow{-C_2H_2} 63\ (35\%)$$

in which the sequence 118 → 63 is typical of simple benzofurans.

5-Hydroxybenzofuran (II). $C_8H_6O_2$ (high-resolution *m/e*), m.p. 60–61°, λ_{max} 247, 254, 295 nm (248, 256, 325 nm in alkali) was identical with synthetic[12] material and gave authentic (I) with CH_2N_2.

5-Formylbenzofuran (III). $C_9H_6O_2$ (high-resolution *m/e*), m.p. 36–37°, λ_{max} 238, 253, 262, 300 nm, ν_{max} 1700 cm^{-1}, formed a 2,4-dinitrophenylhydrazone $C_{15}H_{10}N_4O_5$ (high-resolution *m/e*) and was identical with material prepared from (VI). In the NMR spectrum the formyl proton is at 0·25τ and $H_{(4)}$ and $H_{(6)}$ are shifted downfield (1·91 and 2·18τ). The mass spectrum shows M^+ at *m/e* 146 (95%) with prominent peaks at 145 (100%), 118 (15%), 117 (84%), 90 (15%), 89 (70%), 63 (37%).

5-Hydroxymethylbenzofuran (IV). $C_9H_8O_2$ (high-resolution *m/e*), m.p. 30–31°, λ_{max} 245, 251, 270, 275, 279, 286 nm, was identical to material prepared from (III) by reduction with $LiAlH_4$. In the NMR spectrum the $—CH_2OH$ group gives signals at 7·46τ (1H, br.) and 5·29τ (2H, s.).

5-Carboxybenzofuran (V). M.p. 190–191°, λ_{max} 245, 250, 275, 285, 293 nm, was identical to synthetic[13] material and to material produced from (III) by oxidation with alkaline $AgNO_3$.

5-(1,2-Dihydroxypropyl)-benzofuran (VI), $C_{11}H_{12}O_3$ (high resolution *m/e*), m.p. 63–65°, λ_{max} 247, 253, 270, 276, 280, 286 nm, $[\alpha]_D$-16·4° in EtOH, was cleaved to (III) and MeCHO with periodate (see below) and with Me_2CO–$CuSO_4$ gave an acetonide, $C_{14}H_{16}O_3$ (high resolution *m/e*), an oil. In the NMR spectrum the side-chain gives rise to signals at 9·0τ (3H, d), 6·96τ (2H, br, lost in D_2O), 6·05τ (H, complex), and 5·31τ (H, d: J = 4 c/s). In the NMR spectrum of the acetonide the Ar—CH<C/O> proton is at 4·71τ (d, J = 6·5 c/s) but its splitting does not establish the relative configurations of the two asymmetric centres. In the mass spectrum the principal fragmentations are

$$M^+ = 192\ (11\%) \xrightarrow{-CH_3CHOH} 147\ (100\%) \xrightarrow{-H} 146\ (22\%)$$

followed by breakdown as for (III).

5-(1,2-Epoxypropyl)-benzofuran (VII), λ_{max} 238, 253, 264, 272, 276, 278 nm, was an oil. Fragmentation in the mass spectrometer partly involved rearrangement following electron impact,[14] the principal processes being:

$$M^+ = 174\ (20\%) \xrightarrow{-CH_3CHO} 130\ (100\%) \xrightarrow{-CO} 102\ (60\%)$$

$$\downarrow \qquad \searrow -CH_3 \rightarrow 159\ (49\%)$$

$$\text{(rearrangements)} \xrightarrow{-CHO} 145\ (65\%) \xrightarrow{-CO} 117\ (34\%)\ \text{etc.}$$

$$-C_2H_5 \qquad -C_2H_5CO$$

[10] S. TANAKA, *J. Am. Chem. Soc.* **73**, 872 (1959).
[11] J. A. ELVIDGE and R. G. FOSTER, *J. Chem. Soc.* 590 (1963).
[12] P. KARRER, A. GLATTFELDER and F. WIDMER, *Helv. Chim. Acta* **3**, 541 (1920).
[13] F. DURO and P. CONDORELLI, *Ann. Chim. Roma* **53**, 1582 (1963).
[14] H. E. AUDIER, J. F. DUPIN, M. FETIZON and Y. HOPPILLIARD, *Tetrahedron Letters* 2077 (1966).

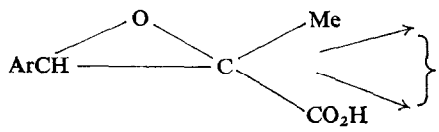
5-(1-*Oxo-2-hydroxypropyl*)-*benzofuran* (*VIII*). $C_{11}H_{10}O_3$ (high-resolution *m/e*), λ_{max} 238, 252, 264 295 (br) nm, ν_{max} 3450, 1685 cm^{-1}, was an oil. The principal fragmentation process in the mass spectrometer was

$M^+ = 190\,(6\%) \xrightarrow{-CH_3CHOH} 145\,(100\%)$, followed by breakdown as for (III).

2-(2-*Hydroxyethyl*)-4-*methoxyphenol* (*IX*). $C_9H_{12}O_3$ (high-resolution *m/e*), m.p. 87–89° (lit.[15] 88–89°), λ_{max} 227, 291 nm (242, 310 nm in alkali), showed the expected NMR peaks [3·1–3·4τ, 3H complex (3 Ar-H); 6·09τ 2H tr. (carbinol CH_2); 6·29τ, 3H s (Ar-OMe); 7·19τ, 2H tr (benzyl CH_2)]. In the mass spectrometer the principal fragmentations are:

$$M^+ = 168\,(90\%) \xrightarrow{-H_2O} 150\,(70\%) \xrightarrow{-CH_3} 135\,(95\%)$$
$$\Big\downarrow -CH_2OH$$
$$137\ (100\%)$$

C_{12} benzofuran. Was obtained from the bicarbonate-soluble fraction in rather small amount (< 3 mg) and underwent decomposition during manipulation. The best material had λ_{max} 245, 251, 269, 274, 278, 285 nm [close to data for (VI) and its acetonide] and ν_{max} 1735 cm^{-1} (confirming the acid function). The sample used for NMR measurements had partly decomposed but showed tertiary C-Me and five benzofuran protons, the chemical shifts of the protons at $C_{(4)}$ and $C_{(6)}$ being similar to those of the acetonide of (VI). In the mass spectrum the molecular ion (base peak) $C_{12}H_{10}O_4$ (high-resolution *m/e*) showed a direct loss of CO_2 (*m/e* 174, 16%; metastable at 139) with further fragmentation similar but not identical to that of the epoxide (VII). The most plausible interpretation is in structure (XI), with fragmentations thus:



(XI)

$ArCOCH_2CH_3^+ \longrightarrow ArCHO^+,\ ArCO^+$

174 (16%) 146 (60%) 145 (100%)

$ArCH_2COCH_3^+ \longrightarrow ArCH_3^+,\ ArCH_2^+$

132 (80%) 131 (90%)

^{14}C-*Feeding—General*

Tracer-substrates (50–100 μc) in filter-sterilized aqueous solution were added to 2 or 4 mycelial mats 2 or 5 weeks from inoculation (see Table 1) and the culture media were collected 3–7 days later. All substances (except $BaCO_3$) were recrystallized to constant specific activity, which was determined using a calibrated end-window-counter and samples of 'infinite thickness' (*ca.* 10 mg in 0·3 cm^2), counting not less than 10^5 counts and for not less than 9×10^3 sec. Dilutions, where necessary, were carried out with recrystallized materials and factors checked both gravimetrically and by counting.

Degradation Procedures

Cleavage of (VI). A mixture of (VI) (0·2 g) and sodium metaperiodate (2·0 g) in 0·07 M phosphate buffer, pH 7, was shaken overnight in a stoppered flask. The acetaldehyde produced was flushed out with N_2 into saturated 2,4-dinitrophenylhydrazine in 2N-HCl, giving acetaldehyde 2,4-dinitrophenylhydrazone (72%). The aqueous mixture left in the flask afforded, by ether extraction, the aldehyde (III) (100%), which for counting purposes was dissolved in ethanol (0·17 g in 4 ml) to which aq. $AgNO_3$ (0·2 g in 2 ml) was added followed by aq. NaOH to alkalinity. After 30 min the mixture was filtered and the filtrate and aqueous washings were washed with ether, acidified, and extracted with ether to give the acid (V) (88%).

Oxidation of acetaldehyde-DNP. The sample (0·13 g) was refluxed for 1 hr with 5N chromic acid (8 ml) and conc. H_2SO_4 (2 ml). Excess oxidant was removed with aq. hydrazine (60%), and the solution was neutralized with aq. NaOH (25%), then acidified with phosphoric acid and distilled until the distillate was no longer acidic (*ca.* 50 ml). The distillate was titrated with 0·01 N NaOH and evaporated to dryness; the solid residue was taken up in the minimum of aq. acetone–propanone (9:2:1) and chromatographed on "Amberlite CG-50" resin in the same solvents, collected 3 ml fractions and titrating each. This gave the Na salt from 29 mg of acetic acid (85%).

Degradation of acetic acid. The above sodium acetate was taken up in 1 ml of conc. H_2SO_4 and NaN_3 (30 mg) added at 0° in a stream of N_2 passing into baryta traps. The temperature was taken to 70° over 30′ until no more CO_2 was evolved. The precipitated $BaCO_3$ was dried for counting (92%). The acidic residue was made alkaline at 0° with 40% sq. NaOH, diluted with water (20 ml) and distilled (10 ml) into 20% aq. HCl. The acidic solution was evaporated to dryness and to the crude CH_3NH_2, HCl, taken up in 0·8 ml.

[15] E. ZBIRAL, E. L. MENARD and J. M. MULLER, *Helv. Chim. Acta* **48**, 404 (1965).

AcOH, were added anhydrous NaOAc (0·04 g) and phthalic anhydride (0·06 g). The mixture was refluxed for 30 min, diluted with water (5 ml) and extracted with hexane (3 × 5 ml), evaporation of which afforded *N*-methylphthalimide which after chromatography on alumina was crystallized for counting (70%).

Decarboxylation of (V). The acid (V) (0·04 g) was refluxed with redistilled $SOCl_2$ to give the acid chloride, m.p. 63–65° (98%), to which, dissolved in acetone (4 ml), NaN_3 (0·03 g) in water (1 ml) was added at 0°. After 30′ at 0°, 25 ml of water was added and after stirring for a further 10′ at 0° the acid azide, m.p. 98·5–99° (98%) was filtered off. This was refluxed for 2 hr in dry benzene to give the isocyanate as an oil (96%), which was taken up in 20% aq. HCl at 0° and then warmed on a steam bath in a stream of N_2 passing into baryta traps until all the CO_2 was collected as $BaCO_3$ for counting (70%). Basification of the residue gave 5-aminobenzofuran as a colourless oil (79% yield) (full spectroscopic characterization) which was converted into the benzoyl derivative $C_{15}H_{11}O_2N$ (by microanalysis) (recrystallized for counting, 98%), m.p. 141–142°. No satisfactory further degradation of this amine was developed.

Degradation of (V) through 2-ethyl-4-methylphenol. The acid (V) (0·3 g) in tetrahydrofuran (25 ml) with $LiAlH_4$ (0·06 g) was refluxed overnight; excess reagent was destroyed with wet ether, and after filtration evaporation of the filtrate gave crude (IV), purified by chromatography in benzene–ether on silica gel to material identical with the natural product (see above) (90%). This product (0·22 g), treated overnight with hydrogen while shaking in 5 ml EtOH with 0·05 mg 5% PD-C and 0·1 ml concentrated aq. HCl, gave, after chromatography of the product on alumina in hexane–ether, 5-methyl-2,3-dihydrobenzofuran (oil) (87%). This product (0·1 g) was dissolved in ether (10 ml) and ammonia was distilled from sodium (20 ml). The solution was stirred under reflux and small pieces of clean Na added until the blue colour developed and was maintained for 5·5 hr, after which excess NH_4Cl was added and the NH_3 allowed to evaporate. The residue, in water (10 ml), was extracted with ether, from which chromatography in benzene–ether afforded 2-ethyl-4-methoxyphenol (oil) (94%). This was subjected to Kuhn–Roth oxidation as described for acetaldehyde 2,4-dinitrophenylhydrazone; in this case fractionation of the Na salts afforded both propionic (15 mg) and acetic (21 mg) acids, degraded as for acetic acid (above) to samples of $BaCO_3$ and *N*-methyl- (70%) and *N*-ethylphthalimide (78%) for counting. Another part of the 5-methyl-2,3-dihydrobenzofuran was subjected to similar Kuhn–Roth oxidation to acetic acid (72%).

Degradation of (I). Ammonia (10 ml) was distilled from Na on to (I) (94 mg) and to the solution small pieces of clean Na were added until the characteristic blue colour developed (20 min). The NH_3 was allowed to evaporate and saturated aq. NH_4Cl (10 ml) was added; ether extraction gave 2-ethyl-4-methoxyphenol, b.p. 65–67°/0·5 mm (76%), which, treated overnight with fresh phenyl isocyanate, gave the phenylurethane, m.p. 112–114°, for counting. Kuhn–Roth oxidation of this, as above, gave acetic and propionic acids which were separated and each degraded to samples of $BaCO_3$ and *N*-methyl- and *N*-ethylphthalimide respectively.