# Biosynthesis of Betaenone B, Phytotoxin of *Phoma betae* Fr.

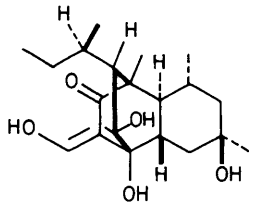
**Hideaki Oikawa, Akitami Ichihara,* and Sadao Sakamura***

*Department of Agricultural Chemistry, Hokkaido University, Sapporo 060, Japan*

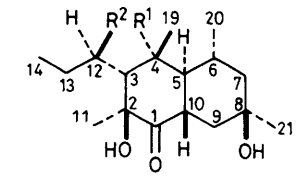
The incorporation of [1-$^{13}$C]-, [2-$^{13}$C]-, [1,2-$^{13}$C]-acetate, and [Me-$^{13}$C]methionine, indicated that betaenone B was biosynthesized from eight acetate units *via* a polyketide pathway, and all *C*-methyl groups except the C-14 methyl were derived from methionine.

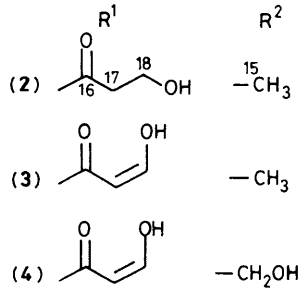
In previous papers, we have reported the isolation of betaenones, A (**1**), B (**2**), and C, (**3**), the phytotoxins of *Phoma betae* Fr.,[1,2] the fungus that causes leaf spot disease on sugar beet. Their structures were established by spectroscopic methods, chemical correlations, and *X*-ray crystallographic analysis. Betaenones A, (**1**), and C, (**3**), which have a unique enolic β-ketoaldehyde moiety, possess stronger phytotoxic activity than the others and (**3**) especially exhibited a high inhibitory effect on the RNA and protein syntheses of starfish gastrula,[2] and on the root elongation of rice seedlings.[2]

From a structural point of view, we assumed that betaenones are biosynthesised through a polyketide pathway. When the microbial metabolites of the polyketide have branching *C*-methyl groups, there are two possibilities, (a) the alkylation of the polyketide by a $C_1$ pool, or (b) the condensation of propionate and acetate to give the polyketide (Scheme 1).[3] However no fungal metabolite produced by route (b) has been reported, although homo-orsellinc acid[4] and aurovertin[5] have been biosynthesised using propionate as a starter unit instead of acetate. In this communication, we describe the biosynthetic pathway to the structurally distinct betaenone B, (**2**), and the origin of the branching methyl groups. We believe that this report is the first biosynthetic study on (**2**) or structurally related phytotoxins, *e.g.*, stemphyloxin I, (**4**),[6] and diplodiatoxin.[7]



(**1**)



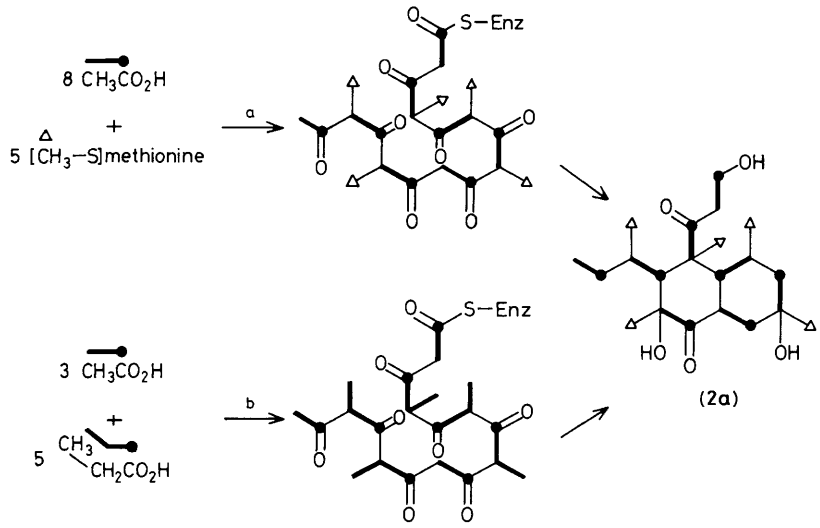


(**2**) $R^1$ = $-CH_3$ (15)

(**3**) $R^1$ = $-CH_3$

(**4**) $R^1$ = $-CH_2OH$

In the $^1$H n.m.r. spectrum of betaenone B, (**2**), all proton signals were clearly assigned by proton decoupling experiments with the exception of two kinds of methyl groups (3 Me-C-, 2 Me-CH), which were assigned using the nuclear




Scheme 1

**Table 1.** $^{13}C$ N.m.r. data for betaenone B, (**2**).

| C- | $\delta_c$[a]/p.p.m. | $J_{cc}$/Hz | Enrichment[b] |
|---|---|---|---|
| 1 | 216.9 | 36.8 | ● |
| 2 | 77.3 | 36.8 | ★ |
| 3 | 57.3 | 35.3 | ● |
| 4 | 52.9 | 38.2 | ★ |
| 5 | 46.6 | 33.8 | ● |
| 6 | 29.1 | 33.8 | ★ |
| 7 | 47.7 | 38.2 | ● |
| 8 | 68.7 | 37.5 | ★ |
| 9 | 41.5 | 42.7 | ● |
| 10 | 40.3 | 42.7 | ★ |
| 11 | 23.9 | | △ |
| 12 | 35.8 | 35.5 | ★ |
| 13 | 25.1 | 34.6 | ● |
| 14 | 13.6 | 34.6 | ★ |
| 15 | 23.4 | | △ |
| 16 | 217.6 | 38.2 | ● |
| 17 | 43.9 | 38.2 | ★ |
| 18 | 54.8 | 38.2 | ● |
| 19 | 20.4 | | △ |
| 20 | 21.5 | | △ |
| 21 | 31.1 | | △ |

[a] Relative to $Me_4Si$. [b] Enrichment from [1-$^{13}C$]acetate (●), [2-$^{13}C$]acetate (★), and [Me-$^{13}C$]methionine (△).

Overhauser enhancement method and comparison with the spectrum of betaenone D.[2] On the basis of these observations, 17 signals in the $^{13}C$ n.m.r. spectrum of (**2**) were unambiguously assigned by chemical shift consideration, the INEPT method, and selective proton decoupling. The remaining 4 signals (2 C=O, 2 Me–CH) were assigned by consideration of long-range selective proton decoupling. Thus, irradiation of 17-H changed the signal pattern of C-16, and a similar relationship was observed between 15-H and C-12, and further confirmed by a labelling experiment using [1,2-$^{13}C$]acetate. Prior to this, preliminary experiments with [1-$^{14}C$]acetate as the precursor established conditions which give good incorporation (7.90%).

The $^{13}C$-labelled phytotoxins were prepared in feeding experiments with 90% enriched sodium [1-$^{13}C$], [2-$^{13}C$]-, [1,2-$^{13}C$]-acetate and 98.57% enriched [Me-$^{13}C$]methionine. The labelled precursors were administered to the potato medium containing 2% sucrose (4 days after innoculation). After a further 12 day fermentation, the crude phytotoxins were extracted with EtOAc and chromatographed on a silica gel column using $CHCl_3$–MeOH (98:2) and benzene–MeOH (92:8) as eluents.

The $^{13}C$ n.m.r. spectrum of [1-$^{13}C$]acetate-derived betaenone B showed eight enhanced signals, attributed to C-1, C-3, C-5, C-7, C-9, C-13, C-16 and C-18, whereas the spectrum of betaenone B derived from [2-$^{13}C$]acetate showed enhanced signals representative of C-2, C-4, C-6, C-8, C-10, C-12, C-14, and C-17. Incorporation of eight acetate units with [1,2-$^{13}C$]acetate was revealed by detecting eight pairs of carbon–carbon coupling signals as indicated in (**2a**).

Use of [Me-$^{13}C$]methionine gave strong signal enhancement for five methyl carbons; C-11, C-15, C-19, C-20, and C-21. These results confirmed that the methyl group branches on the polyketide chain are derived from methionine (Table 1).

We conclude that betaenone B was biosynthesized through pathway (a) rather than (b). This conclusion is closely related to the other polymethylated fungal polyketides, *e.g.*, citreoviridin[8] and radiclonic acid.[9]

Though the biosynthetic pathway linking the betaenones (**1**), (**2**), and (**3**) is not clear at present, betaenone C, (**3**), would be a precursor of betaenone A, (**1**), since the latter is formed by intramolecular aldol condensation of the former.[1]

We are grateful to Dr. S. Naito, Hokkaido National Agricultural Experimental Station, Sapporo, for the generous gift of the strain, *Phoma betae* Fr.

*Received, 20th February 1984; Com. 225*

## References

1 A. Ichihara, H. Oikawa, K. Hayashi, S. Sakamura, A. Furusaki, and T. Matsumoto, *J. Am. Chem. Soc.*, 1983, **105**, 2907.
2 A. Ichihara, H. Oikawa, M. Hashimoto, S. Sakamura, T. Haraguchi, and H. Nagano, *Agric. Biol. Chem.*, 1983, **47**, 2965.
3 W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, pp. 76—82; W. B. Turner and D. C. Aldridge, 'Fungal Metabolites II,' Academic Press, London, New York, 1983.
4 K. Mosbach, *Acta Chem. Scand.*, 1964, **18**, 1591.
5 P. S. Steyn, R. Vleggaar, and P. L. Wessels, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1298.
6 I. Barash, S. Manulis, Y. Kashman, J. P. Springer, M. H. M. Chen, J. Clardy, and G. A. Strobel, *Science*, 1983, **220**, 1065.
7 P. S. Steyn, P. L. Wessels, C. W. Holzapfel, D. J. Potgieta, and W. K. A. Louw, *Tetrahedron*, 1972, **28**, 4775.
8 D. W. Nagel, P. S. Steyn, and N. P. Ferreia, *Phytochemistry*, 1972, **11**, 3215.
9 H. Seto, T. Sasaki, and H. Yonehara, *Tetrahedron Lett.*, 1977, **47**, 4083.