Biosynthetic Study of Betaenone B: Origin of the Oxygen Atoms and Accumulation of a Deoxygenated Intermediate using P-450 Inhibitor

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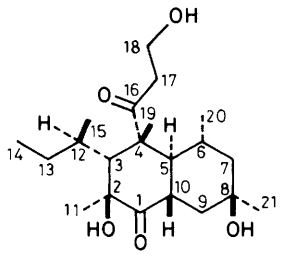
The biosynthetic origin of oxygen atoms in betaenone B (**1**) was established by feeding experiment using [1-^{13}C, $^{18}O_2$] acetate and by cytochrome P-450 inhibitor treatment of *Phoma betae*; incorporation patterns and accumulation of the plausible intermediate enabled us to propose a biosynthetic pathway for (**1**) involving an intramolecular Diels–Alder reaction at a late stage.

Hydroxylation is an important step at later stages of the biosynthesis of natural products, *i.e.* erythromycin,[1] rifamycin,[2] and trichothecene.[3] In this type of reaction the most common enzyme is cytochrome P-450, inhibitors for which have recently been reported.[4] These were used successfully to study the biosynthetic or metabolic pathways rather than their antifungal activity.[5] We now report the first application of cytochrome P-450 inhibitors for polyketide compounds.

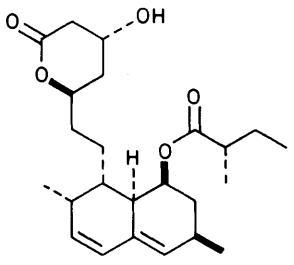
Betaenone B (**1**) was isolated as the phytotoxin produced by *Phoma betae* Frank, and is a major metabolite in the betaenone family. In previous work, we showed that (**1**) was biosynthesized from eight acetate/malonate and five C_1-units.[7] If we hypothesize that (**1**) is biosynthesized *via* an intramolecular Diels–Alder reaction, as for mevinolin (**2**) and other decalin polyketides,[8] the α-ketol moiety in (**1**) must be derived from an alkene intermediate. In addition, the lack of a C-3 hydroxy group in diplodiatoxin (**3**)[9] implies that the C-8 hydroxylation in (**1**) occurs after ring formation. Therefore, we assume that (**1**) may be biosynthesized *via* an intermediate (**4**) (Scheme 1).

Incorporation of [1-^{13}C, $^{18}O_2$]acetate was first carried out to examine the origin of the oxygen atoms. An isotopic shifted signal at C-16 (Δ+0.05 p.p.m.) was observed in the ^{13}C n.m.r. spectrum of the [1-^{13}C, $^{18}O_2$]acetate-enriched (**1**), but not at C-1 and C-18. This result suggests that the oxygen atom at C-1 did not originate from acetate. The absence of an upfield shifted signal at C-18 is possibly due to washing-out of labelled oxygen at the carboxy group stage after hydrolysis of enzyme-bound polyketide.

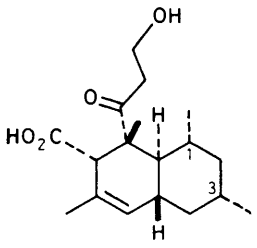
We then studied inhibition of biosynthesis. Thus, a solution



(1)



(2)



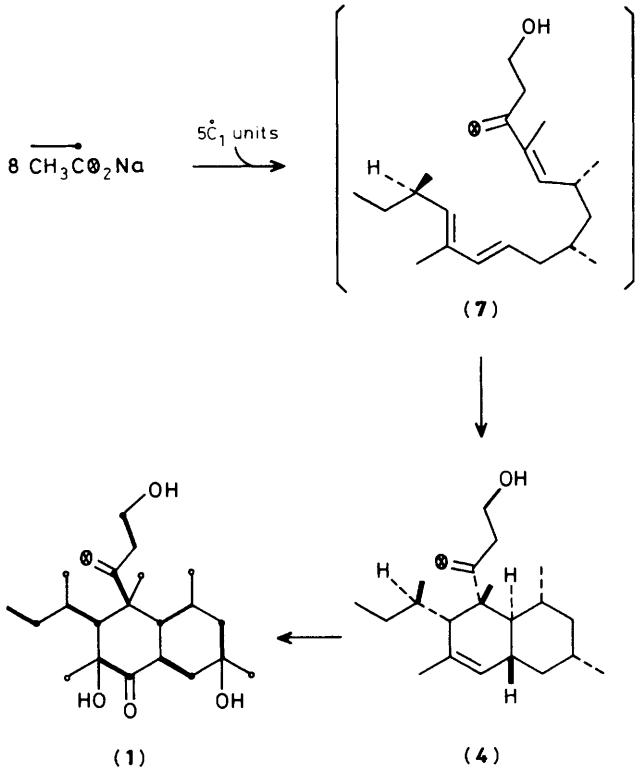
(3)

in dimethyl sulphoxide (DMSO) of ancymidol (**5**)[10](5, 10, 20, and 40 mg, respectively) was added to four culture flasks containing 100 ml of 2% potato–sucrose medium after fermentation for 4 days. The production of (**1**) was suppressed in proportion to the concentration of (**5**) and a new metabolite (**4**) was detected in mycelial extracts. Treatment with (**5**) (40 mg) resulted in almost complete inhibition of formation of (**1**) to give 8.7 mg of (**4**) after SiO_2 chromatography. A similar result was obtained with the plant growth retardant, S-3307D (**6**).[11]

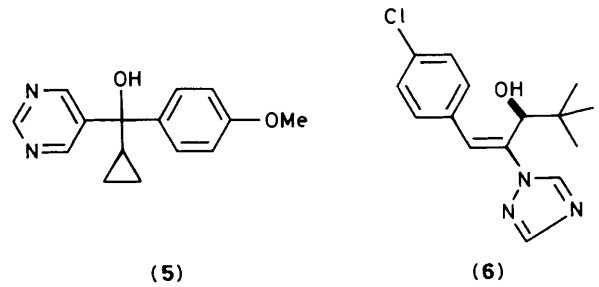
The intermediate (**4**), which we named probetaenone I, was isolated as a colourless oil: $C_{21}H_{36}O_2$; $[\alpha]_D^{24}$ −9.4° (*c* 2.5, MeOH). The ^{13}C n.m.r. spectrum of (**4**) showed 21 signals (CH_3×6, CH_2×5, CH×7, C×3). N.m.r. analysis (^1H, ^{13}C, and ^1H–^1H, ^1H–^{13}C-COSY in C_6D_6) showed the presence of the partial structure (A), together with HO–CH_2CH_2C=O [δ_c 58.04 (t), 41.40 (t), 215.03 (s); δ_H 3.794 (ddd, *J* 4.4, 6.6, 11.4 Hz), 3.713 (ddd, *J* 4.4, 5.9, 11.4 Hz), 2.430 (ddd, *J* 4.4, 6.6, 18.3 Hz), 2.284 (ddd, *J* 4.4, 5.9, 18.3 Hz); $\nu_{max.}$ (neat) 3400 and 1695 cm^{-1}], CH_3–CH_2 [δ_c 13.25 (q), 26.95 (t); δ_H 0.890 (t, *J* 8.1 Hz)], CH_3–C (δ_c 17.78 (q), 53.85 (s); δ_H 0.932 (s)], and 2 × CH_3–CH [δ_c 21.70 (q), 22.33 (q), 33.94 (d), 34.76 (d); δ_H 0.855 (d, *J* 6.2 Hz), 0.918 (d, *J* 7.3 Hz)].† In addition to these data, the marked similarity of the ^1H n.m.r. spectrum of (**1**) to the spectra of (**2**) and (**3**), and the appearance of unusually upfield shifted methyl group (δ_H 0.683, C-20), which is characteristic of betaenones, confirmed the structure of (**4**) as that shown in Scheme 1.

In conclusion, the inhibitor experiment suggests that (**4**) is an intermediate in the biosynthesis of (**1**) and the incorporation results are consistent with this. On the basis of these results, we propose a biosynthetic pathway for (**1**) (Scheme 1) in which an intramolecular Diels–Alder reaction of the triene (**7**) is probably a key step. Furthermore, cytochrome P-450 inhibitor might be useful for studies of biosynthetic pathways since it inhibits the oxidation, giving a less oxidized carbon skeleton. The synthesis of radioactive (**7**) is in progress.

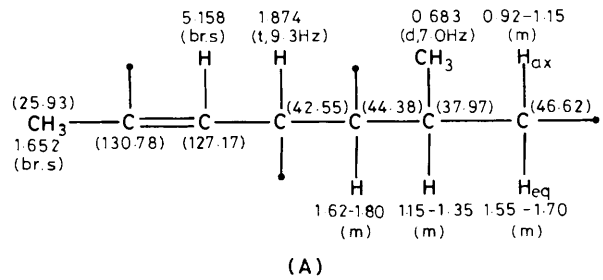
† The ^{13}C n.m.r. signals of (**4**) were assigned from COSY (^1H–^1H and ^1H–^{13}C) and chemical shift data, and by comparison with data for (**1**).



Scheme 1



(5) (6)



(A)

We thank Dr. K. Kamoshita, Sumitomo Chemical Co., Ltd., for a generous gift of S-3307D.

Received, 15th October 1987; Com. 1521

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