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# Evidence for an Octanoate Synthase Operating During the Biosynthesis of Piliformic Acid in *Poronia Piliformis*

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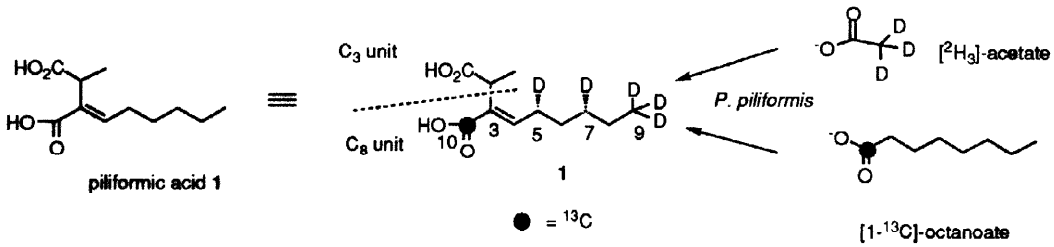
***Abstract***: Sodium [1-$^{13}$C]-octanoate is incorporated intact into the $C_8$ unit of piliformic acid **1** in *Poronia piliformis* whereas sodium [3-$^{13}$C]-decanoate **2** is incorporated into this unit only after prior β-oxidation to [1-$^{13}$C]-acetyl-CoA. Also 8-fluorooctanaote **3** is utilised by *P. piliformis* as an intact unit to generate 9-fluoropiliformic acid **5** whereas 10-fluorodecanoate **4** is not. These results suggest that octanoate is biosynthesised *de novo* for secondary metabolism and is not assimilated from higher fatty acids by β-oxidation. 




**Scheme 1**

Piliformic acid **1** has been isolated[1] as a secondary metabolite in several closely related fungi of the xylariaceous genera. These include *Hypoxylon deustum*, the slow growing dung fungus *Poronia piliformis* and four *Xylaria* strains, *X. polymorpha*, *X. longipes*, *X. mali* and *X. hypoxylon*. A recent biosynthetic investigation[2] in both *P. piliformis* and *X. mali* has shown that piliformic acid **1** is constructed from a $C_3$ unit, derived from the citric acid cycle intermediate oxaloacetate, and from a $C_8$ octanoic acid moiety as shown in Scheme 1. Sodium [1-$^{13}$C]-octanoate was incorporated predominantly as an intact unit with isotope enrichment at C-10. Additionally the stereochemical configuration of the resultant deuterium atoms at C-5 and C-7 after incorporation of sodium [$^2H_3$]-acetate, was consistent with the operation of an enoyl reductase from a fungal fatty acid synthase (FAS), rather than that from a polyketide synthase (PKS)[2]. This study therefore established the operation of a fungal FAS and not a PKS operating during piliformic acid biosynthesis. This FAS may have a sole function in delivering octanoate for piliformic acid biosynthesis. Alternatively octanoate may derive from the β-oxidation of the higher fatty acids synthesised by a FAS of primary metabolism. In an effort to delineate these two possibilities we now report an investigation into the incorporation of sodium [3-$^{13}$C]-decanoate **2** into piliformic acid. Decanoate is an intermediate in the β-oxidation of saturated fatty acids and oleic acid[3] and should be vulnerable to further β-oxidation and generate [1-$^{13}$C]-octanoyl-CoA. Incorporation of octanoate derived in this manner will label C-10 only and would

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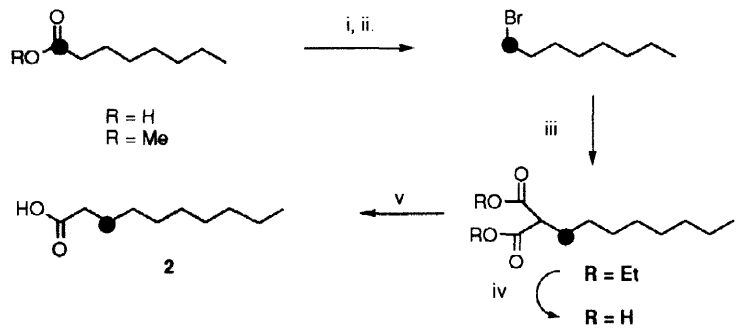
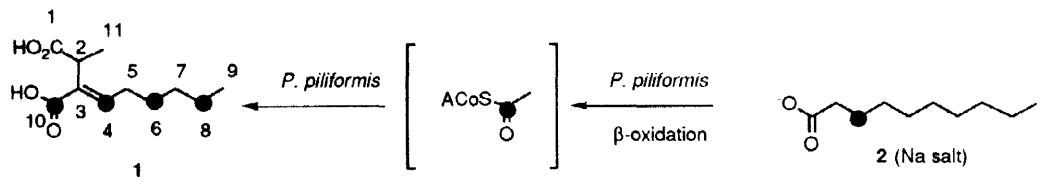
support the latter hypothesis where the catabolism of primary metabolites is routed to secondary metabolism. On the other hand if there is a dedicated octanoyl-FAS linked to piliformic acid biosynthesis, then isotope from sodium [3-$^{13}$C]-decanoate will be located in an alternating pattern (at C4, C6, C8 & C10), after degradation of the labelled substrate by β-oxidation and then incorporation *de novo* from [1-$^{13}$C]-acetyl-CoA.




**Scheme 2** i, $LiAlH_4$, $Et_2O$, reflux, 1h, 92%; ii, aq HBr, 47%, $H_2SO_4$, reflux, 6h, 83%; iii, NaOEt, EtOH, diethyl malonate, reflux, 4h, 62%; iv, $H_2O$, KOH, EtOH, reflux, 4h, then HCl, 85%; v, 160°C, 1h, 56%.

A synthetic route to [3-$^{13}$C]-decanoic acid **2**[4] was developed and is illustrated in Scheme 2. The isotopically labelled material was administered as the sodium salt of **2**, to the medium of a freshly inoculated culture of *P. piliformis* and after 6 weeks piliformic acid **1** was isolated as previously described[1]. The resultant $^{13}$C-NMR indicated that the incorporation was low (~1%) and the labelling pattern, which is shown in Scheme 3, is consistent with the β-oxidation of [3-$^{13}$C]-decanoate to [1-$^{13}$C]-acetyl-CoA, and then incorporation into piliformic acid **1**. There was no evidence for a unique enrichment at C-10, the carboxylate group which would have become labelled if [1-$^{13}$C]-octanoate had been assimilated after one β-oxidation cycle.



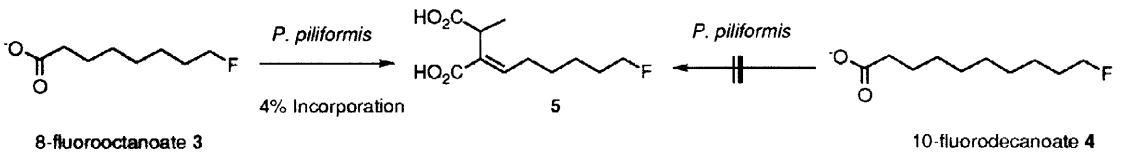

**Scheme 3**

| Carbon No | δC | % Incorp. | Carbon-No | δC | % Incorp. | Carbon-No | δC | % Incorp. |
|---|---|---|---|---|---|---|---|---|
| 1 | 179.6 | 0.24 | 5 | 28.8 | n.d. | 9 | 13.9 | -0.02 |
| 2 | 37.5 | -0.02 | 6 | 28.0 | 2.25 | 10 | 171.5 | 1.02 |
| 3 | 131.1 | -0.22 | 7 | 31.5 | 0.49 | 11 | 15.42 | 0 |
| 4 | 147.3 | 1.53 | 8 | 22.4 | 1.44 | - | - | - |

**Table 1** Incorporation data[5] from **2** determined after $^{13}$C NMR analysis of the resultant piliformic acid **1**.

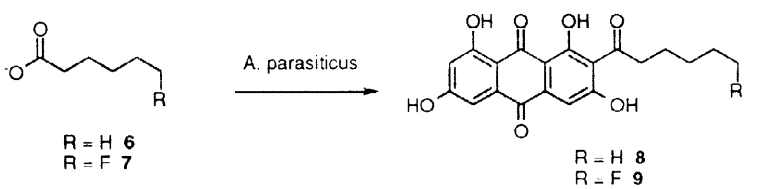
In two further experiments we have studied the incorporation of sodium 8-fluorooctanoate **3**[6] and 10-fluorodecanoate **4**[6] into piliformic acid **1** as shown in Scheme 4. Fluorine at the terminus of the aliphatic chain is not expected to perturb the size or lipophilicity of the molecule and can be used in this case as an alternative to isotopic labelling. In each case the piliformic acid produced was isolated, treated with diazomethane and the resultant dimethyl ester purified by chromatography. The resultant $^{1}$H- and $^{19}$F- NMR spectra from the 8-fluorooctanoate **3** experiment indicated the presence of dimethyl 9-fluoropiliformate, ($\delta_H$ =

4.5, d t, J = 47, J = 6Hz; $\delta_F$ = -218, t, 47), at a similar level (4%) to that found for the incorporation of sodium [1-$^{13}$C]-octanoate (5.5%) in the previous study[2]. GC-MS analysis provided further support for the presence of the dimethyl ester of **5**. The sample was applied *via* splitless injection and a small peak (~4%) eluted, overlapping the front edge of the dominant dimethyl piliformate peak, with the expected ion at 260 (1.35%, $M^+$) and diagnostic fragment ions at 229 (100%, - OMe) and 201 (2.3%, - $CO_2Me$).




**Scheme 4**

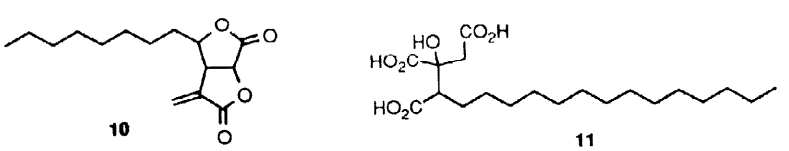
The result indicates that *P. piliformis* can utilise octanoate and 8-fluorooctanoate **3** to a similar extent. On the other hand, there was no evidence for the incorporation of fluorine from 10-fluorodecanoate **4**. This observation is consistent with that for the sodium [3-$^{13}$C]-decanoate experiment and reinforces the conclusion that octanoate generated during β-oxidation, is not released for piliformic acid **1** biosynthesis, but is channelled towards further β-oxidation to acetyl-CoA.



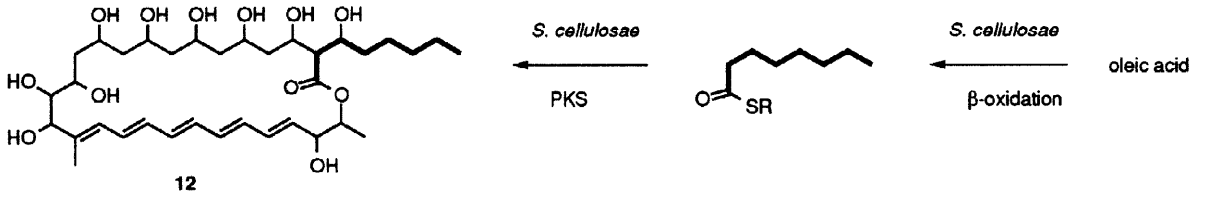

**Scheme 5**

Short chain fatty acids are involved in the biosynthesis of other fungal metabolites. For example hexanoate **6** is the starter unit[7] for the assembly of norsolorinic acid **8**, an early intermediate in aflatoxin biosynthesis in *Aspergillus parasiticus* as illustrated in Scheme 5. The biochemical and genetic evidence[8,9] in that system suggest that there is a FAS which assembles the hexanoate unit from acetate/malonate and a separate PKS which then utilises the hexanoate as a starter unit for anthraquinone biosynthesis. The FAS must physically associate with the PKS before each is functional, therefore this FAS has a unique function in secondary metabolism. The stereochemical incorporation of deuterium from [$^2H_3$]-acetate into the hexanoate starter of **6** is the same[10] as that found[2] into the octanoate unit of piliformic acid **1** (Scheme 1), and more generally into fungal FAS's, but not PKS's[2,11]. 6-Fluorohexanoate **7** is also efficiently utilised by the PKS[12] to generate the fluorinated analogue **9** of norsolorinic acid **8**, in a process similar to that found here for 8-fluorooctanoate **3** to generate 9-fluoropiliformic acid **5**. Thus these two systems display some common characteristics.

Piliformic acid **1** is a member of a much larger class of fungal and lichen metabolites[2] which combine both short and long chain fatty acids with a $C_3/C_4$ unit from the citric acid cycle. Avenaciolide **10**[13] from



*Aspergillus avenaceus* and caperatic acid **11**[14] serve as illustrative examples. These observations on piliformic acid, following from those on norsolorinic acid **8** reviewed above, suggest that more generally fungi do not rely on the delivery of *short chain* fatty acids from primary metabolism but instead posess short FAS's of secondary metabolism which have evolved to construct these units to order.




**Scheme 6**

In a contrasting study[15] in the bacterium *Streptomyces cellulosae* the polyene antibiotic fungichromin **12** incorporates an octanoate unit which was shown to derive from the β-oxidation of oleic acid as shown in Scheme 6. Therefore, from the limited evidence to date, eucaryotic and procaryotic short chain fatty acids present in secondary metabolites seem to derive from dedicated FAS's and β-oxidation respectively. This may originate in the higher degree of compartmentalisation in fungi. Whether the constituent long chain fatty acids of other fungal metabolites eg. **11** and those which incorporate oleate[2,16], are derived from primary metabolism or from a dedicated FAS remains an intriguing question.

**Acknowledgements**

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**References and notes**

1. Anderson, R.; Edwards, R. L.; Whalley, A.J.S. *J. Chem. Soc., Perkin Trans., 1*, **1985**, 1481.
2. Chesters, N. C. J. E.; O'Hagan, D. *J. Chem. Soc., Perkin Trans., 1*, **1997**, 8227.
3. Oleic acid is degraded to *cis*-dodec-3-enoyl-CoA, isomerised to *trans*-dodec-2-enoyl-CoA and then hydrated to β-hydroxydodecanoyl-CoA, before scission to decanoyl-CoA. Zubay, G. *Biochenistry*, Maxwell-MacMillan, 2nd ed, 1988.
4. The starting material [1-$^{13}$C]-octanoic acid was purchased from the Aldrich Chemical Co. Selected analytical data for **2**; M.p 29.5°C (30-32°C); IR (KBr) υ = 2860, 1715, 1470, 1450 and 1300 cm$^{-1}$, $^{1}$H-NMR ($CDCl_3$); 2.34 (2H, m, 2-$CH_2$), 1.50 (2H, dm, $^{1}J_{CH}$ = 124Hz, 3-$CH_2$), 1.26 (12H, m, $CH_2$'s), 0.88 (3H, t, $^{3}J_{HH}$ = 6.7 Hz, $CH_3$), $^{13}$C-NMR; 24.6 (C-3).
5. Scott, A.I.; Townsend, C.A.; Ohada, K. *J. Am. Chem. Soc.*, **1974**, *96*, 8069.
6. Compounds **3** and **4** were prepared by treatment of the ω-hydroxy methyl esters of octanoate and decanoate respectively with diethylamino sulfur trifluoride (DAST). Hydrolysis and neutralisation then generated **3** and **4** as sodium salts. Selected spectroscopic data for **3** $^{1}$H-NMR ($D_2O$); 4.39 (2H, dt, $^{2}J_{HF}$=47.6Hz, $^{3}J_{HH}$ = 5.9Hz, $CH_2F$), 2.01 (2H, t, 7.4Hz, $COCH_2$), 1.3-1.6 (4H, m, $CH_2$'s), 1.17 (6H, m, $CH_2$'s). $^{19}$F-NMR; -216.0 (t.t, $^{2}J_{HF}$ = 47.0Hz, $^{3}J_{HF}$= 24.8Hz), FAB-MS (glycerol); 93 (100%, glycerol), 115, 133, 185, 207, 277 (M + glycerol$^+$, 2.6%).
Selected spectroscopic data for **4** $^{1}$H-NMR ($D_2O$); 4.38 (2H, dt, $^{2}J_{HF}$ =4 7.6Hz, $^{3}J_{HH}$ = 6.2Hz, $CH_2F$), 2.10 (2H, t, $COCH_2$), 1.3-1.7 (4H, m, $CH_2$'s), 1.1 (10H, m, $CH_2$'s). $^{19}$F-NMR; -215.9 (t.t, $^{2}J_{HF}$ = 47.4Hz, $^{3}J_{HF}$ = 26.7Hz), FAB-MS (glycerol); 93 (100%, glycerol), 115, 185, 207, 242, 285, 305 (M + glycerol$^+$, 10%).
7. Townsend, C.A.; Christensen, S.B. *Tetrahedron*, **1983**, *39*, 3575.
8. Watanabe, C.M.H.; Wilson, D.; Linz, J.E.; Townsend, C.A. *Chem. Biol.*, **1996**, *3*, 463.
9. Yu, J.; Chang, P-k.; Carey, J.W.; Wright, M.; Bhatnager, D.; Cleveland, T.E.; Payne, G.A.; Linz, J.E. *Appl. Environ. Microbiol.*, **1995**, *61*, 2365.
10. Townsend, C.A.; Brobst, S.W.; Ramer, S.E.; Vederas, J.C. *J. Am. Chem. Soc.*, **1988**, *110*, 318.
11. (a) Arai, K.; Rawlings, B.J.; Yoshizawa, Y.; Vederas, J.C. *J. Am. Chem. Soc.*, **1989**, *111*, 3391.
12. McKeown, D.S.J.; McNicholas, C.; Simpson, T.J.; Willett, N.J. *J. Chem. Soc., Chem. Commun.*, **1996**, 301.
13. Tanabe, M.; Hamasaki, T.; Suzuki, Y. *J. Chem. Soc., Chem. Commun.*, **1973**, 212.
14. Asahina, Y.; Shibata, S. *Chemistry of Lichen Substances*, Jap. Soc. for the Promotion of Science, Ueno, Tokyo, **1954**.
15. Noguchi, H.; Harrison, P.H.; Arai, K.; Nakashima, T.T.; Trimble, L.A.; Vederas, J.C. *J. Am. Chem. Soc.*, **1988**, *110*, 2938.
16. O'Hagan, D. *The Polyketide Metabolites*, Ellis Horwood Ltd., Chichester, **1991**.