Biosynthesis of the Fungal Tropolones. Puberulonic and Puberulic Acids

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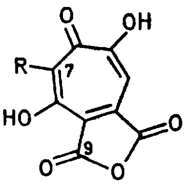
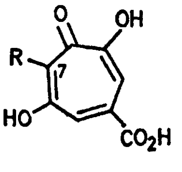
Summary In contrast to earlier studies, C-9 of puberulonic acid (**3**) was found to be derived from acetate.

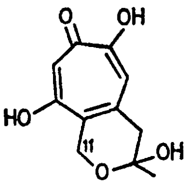
It has been recognised for several years that acetate, malonate, and methionine, serve as specific precursors in the biosynthesis of the fungal tropolones stipitatonic (**1**) and stipitatic (**2**) acids[1] in *Penicillium stipitatum*, puberulonic (**3**) and puberulic (**4**) acids[2] in *P. aurantio-virens* and sepedonin (**5**)[3] in *Sepedonium chrysospermum*.



(1) R=H
(3) R=OH

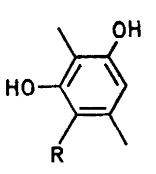


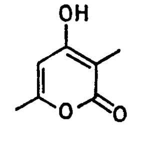
(2) R=H
R=OH



(5)

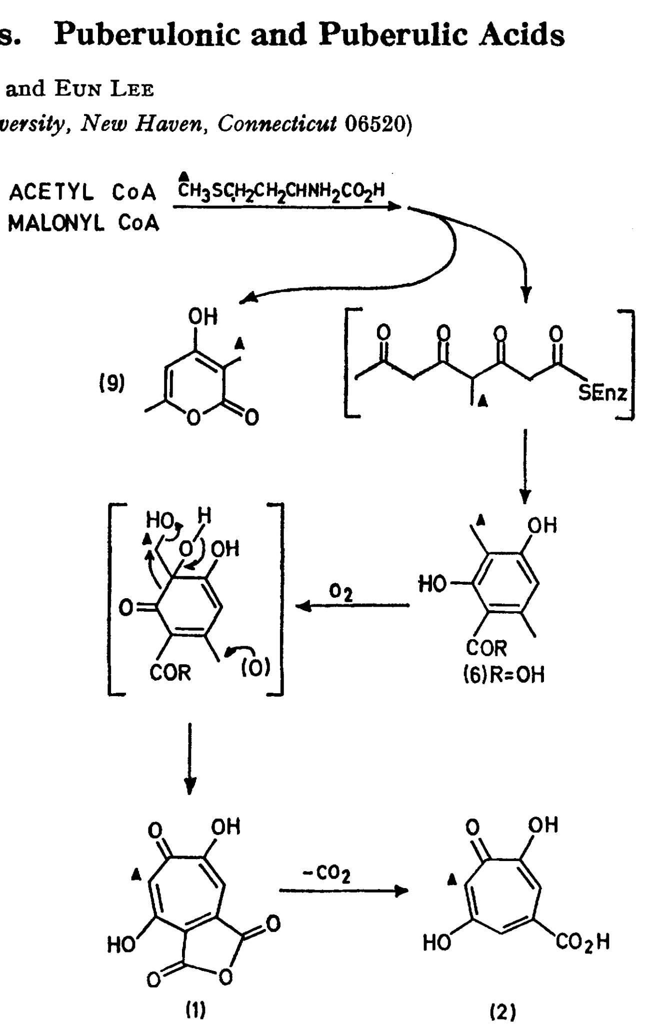
(6) R=CO$_2$H
(7) R=CHO
(8) R=CO$_2$CH$_2$Me





(9)

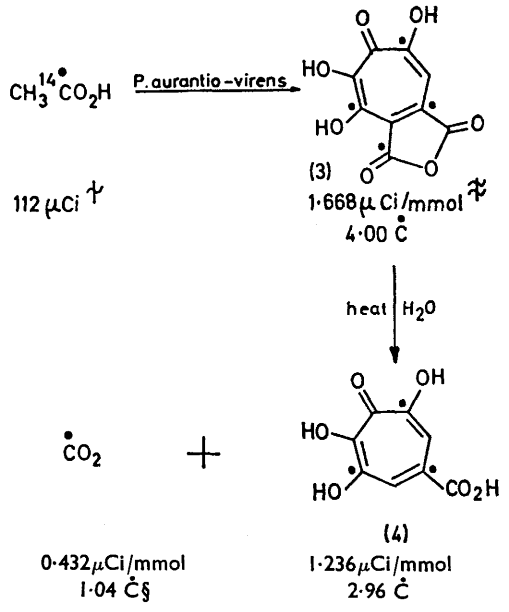
Recently it was shown[4] that in *P. stipitatum* a linear condensation of acetate-malonate units undergoes C-methylation by methionine, *then* aromatisation to (**6**) (or its biologically active derivative), followed by oxidative ring expansion[5] to (**1**) (Scheme 1).



Scheme 1

In light of these results, the closely related 7-hydroxy acids (**3**) and (**4**) which occur in several species of *Penicillia* exhibit an anomalous pattern of labelling, for it is reported that C-9 of (**3**) is derived, not from C-1 of acetate as in (**1**), but from an unknown "C$_1$" pool.[2,6] Furthermore, the corresponding position C-11 in (**5**) is labelled in the normal polyketide sense by C-1 of acetate.[3] Since a different origin of such similar structures seemed unlikely, the problem was re-examined.

[1-^{14}C]-Sodium acetate was fed to growing cultures of *P. aurantio-virens* NRRL 2138 and radioactive (**3**) isolated by



SCHEME 2

a known procedure.[7] After sublimation and recrystallisation to constant activity, hydrolytic decarboxylation of (**3**) yielded radioactive carbon dioxide (trapped as barium carbonate) and (**4**) (Scheme 2).

The ratio of specific activities clearly indicates that C-9 of (**3**) indeed originates from C-1 of acetate. The corrected labelling pattern of (**3**) and the structural analogy suggest that (**1**) and (**3**) are biosynthesised from the same set of precursors, *i.e.*, one acetate, three malonates, and one methionine. Further evidence of a common pathway in the biosynthesis of both compounds was provided by the discovery of small quantities of (**1**) and (**9**)[8] in the *P. aurantio-virens* fermentation medium which produces (**3**) and (**4**), although addition of (**1**) (labelled from [1-^{14}C]-acetate) to *P. aurantio-virens* cultures resulted in non-radioactive (**3**). Further feeding experiments with possible intermediates and analogues have also proved unsuccessful.¶ Thus [$^{14}CO_2H$]-3-methylorsellinic acid (**6**) and [^{14}CHO]-β-orcaldehyde (**7**) were rapidly destroyed by the organism without incorporation into (**3**). [3-$^{14}CH_3$]-Ethyl 3-methylorsellinate (**8**) was neither degraded nor utilized to a noticeable extent.

Finally and most remarkably, [$^{14}CO_2H$]-3-methylorsellinic acid (**6**) was not incorporated to a measurable extent into (**1**) in *P. aurantio-virens*, in sharp contrast to its demonstrated precursor relationship to (**1**) in *P. stipitatum*.[4] In spite of these negative results with large substrates, it seems certain that the biosynthesis of (**3**) and (**4**) follow the pattern of the other fungal tropolones.

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† Fed to one 500 ml Czapek-Dox submerged culture at the ninth day of growth and harvested at the sixteenth day of growth.

‡ Diluted approximately five-fold with authentic (**3**).

§ As $BaCO_3$.

¶ [5-$^{14}CH_3$]-Dimethylorsellinic acid was fed to *P. aurantio-virens* cultures before the results of the [1-^{14}C]-acetate feeding experiment were known. It was degraded rapidly and there was no incorporation to (**3**).

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