Biosynthesis of the Meroterpenoid Metabolites, Austin and Terretonin: Incorporation of 3,5-Dimethylorsellinate

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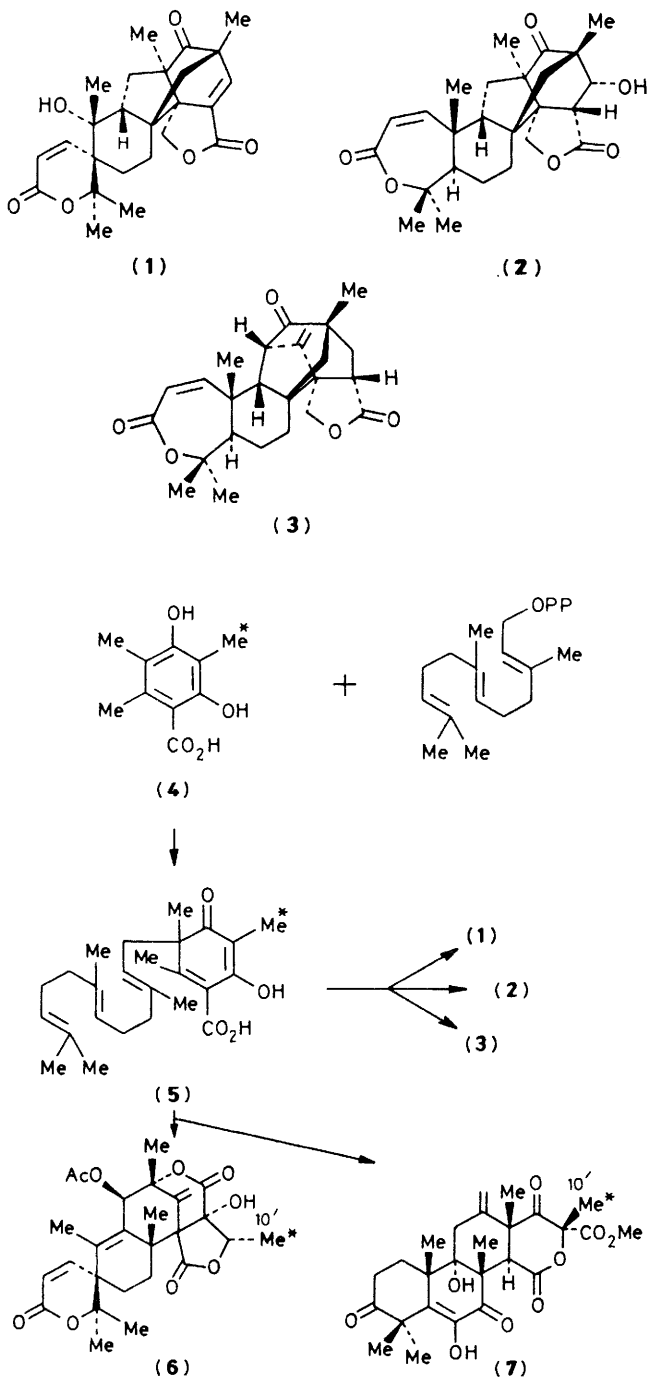
^{14}C and ^{2}H Labelling experiments, together with ^{2}H n.m.r. spectroscopy show that 3,5-dimethylorsellinic acid is a specific precursor of austin and terretonin in *Aspergillus ustus* and *Aspergillus terreus*, respectively, and so substantiate the mixed polyketide–terpenoid origin proposed for these metabolites from the incorporation of ^{13}C-labelled simple precursors.

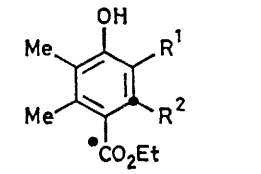
^{13}C and ^{2}H Labelling studies[1–3] have shown that andibenin B (**1**), andilesin A (**2**), and anditomin (**3**), C_{25} metabolites of *Aspergillus variecolor* are formed by a mixed polyketide–terpenoid biosynthetic pathway in which the key step is alkylation of 3,5-dimethylorsellinic acid (**4**) by farnesyl pyrophosphate to give (**5**). We suggested that the mycotoxins austin (**6**) and terretonin (**7**), metabolites of *Aspergillus ustus* and *Aspergillus terreus*, respectively, could also be formed *via* intermediate (**5**), and incorporations of singly and doubly labelled [^{13}C]acetates and [^{13}C]methionine into austin and terretonin result in labelling patterns consistent with these proposals.[4,5] However, as the suggested pathways require unprecedented degrees of modification[6] of the tetraketide-derived phenolic precursor, we have carried out and now report studies to show that 3,5-dimethylorsellinate is indeed a specific precursor of both austin and terretonin (Scheme 1).

Ethyl [*carboxy*,2-$^{14}C_2$]-3,5-dimethylorsellinate (**8**) (39.7 μCi mmol^{-1})[2] was fed to static cultures of *A. ustus* (24 mg to

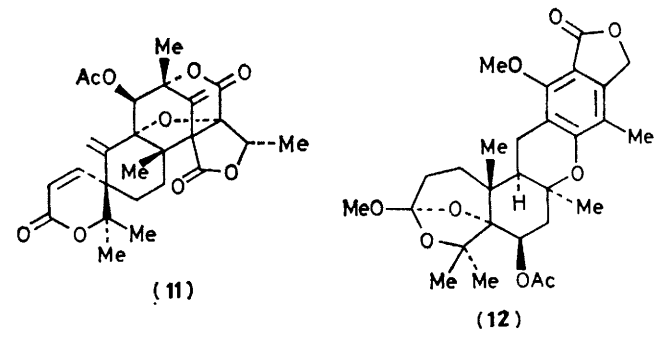


Scheme 1

0.4 l) and *A. terreus* (33 mg to 1 l), respectively, to give austin (36 mg, 1.60 μCi mmol^{-1}) and terretonin (34 mg, 1.01 μCi mmol^{-1}), specific incorporations of 4.0 and 2.5%, respectively. Interestingly, the 2-deoxyorsellinate (**9**) which was incorporated[2] into andibenin B with comparable efficiency to (**8**) was not incorporated into austin to any significant extent (specific incorporation, 0.06%). As with andibenin B, the complexity of the metabolites precluded the degradative studies essential to establish specificity of labelling, so the trideuteriomethyl analogue (**10**)[2] was fed, and the resultant enriched metabolites analysed by 55 MHz ^{2}H n.m.r. spectroscopy. Austin showed only one signal at δ 1.22 p.p.m.; terretonin similarly showed only one signal at δ 1.68 p.p.m.,



(**8**) R^1 = CH_3, R^2 = OH, • = ^{14}C
(**9**) R^1 = CH_3, R^2 = H, • = ^{14}C
(**10**) R^1 = CD_3, R^2 = OH, • = ^{12}C



chemical shifts corresponding in each metabolite to the 10′-methyl hydrogens,† in agreement with our proposed pathways.[4,5] Thus it is clear that 3,5-dimethylorsellinate is a specific precursor to both austin and terretonin and so their meroterpenoid[7] origins are established beyond doubt.

Further evidence for the common biogenetic origins of these metabolites comes from the isolation of austin and dehydroaustin (**11**), a co-metabolite of austin in *A. ustus*,[8] from a chance mutant of the andibenin producing culture of *A. variecolor* which no longer produced andibenin. Another observation to note is the recent isolation of the austalides [*e.g.* (**12**)] from a toxigenic strain of *A. ustus*.[9] Structural analysis suggests they are biosynthesised *via* alkylation of 5-methylorsellinic acid by farnesyl pyrophosphate, *cf.* mycophenolic acid,[10] followed by cyclisation of the farnesyl moiety and oxidative modifications analogous to those occurring in the andibenins and andilesins.

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References

1 J. S. E. Holker and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1978, 626.
2 A. J. Bartlett, J. S. E. Holker, E. O'Brien, and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1981, 1198.
3 T. J. Simpson, *Tetrahedron Lett.*, 1981, 3785.
4 T. J. Simpson and D. J. Stenzel, *J. Chem. Soc., Chem. Commun.*, 1981, 1042.
5 C. R. McIntyre and T. J. Simpson, *J. Chem. Soc., Chem. Commun.*, 1981, 1043.
6 For typical examples, see 'Fungal Metabolites,' W. B. Turner, Academic Press, London, 1971.
7 J. W. Cornforth, *Chem. Br.*, 1968, **4**, 102.
8 T. J. Simpson, D. J. Stenzel, A. J. Bartlett, J. S. E. Holker, and E. O'Brien, *J. Chem. Soc., Perkin Trans.* 1, in the press.
9 R. M. Horak, P. S. Steyn, P. H. van Rooyen, R. Vleggaar, and C. J. Rabie, *J. Chem. Soc., Chem. Commun.*, 1981, 1265.
10 L. Bowen, K. H. Clifford, and G. T. Phillips, *J. Chem. Soc., Chem. Commun.*, 1977, 949.

† Control experiments show that the 10′-methyl signals in the ^{2}H n.m.r. spectra of universally deuteriated austin and terretonin are sufficiently well resolved from other signals to ensure specificity of labelling.