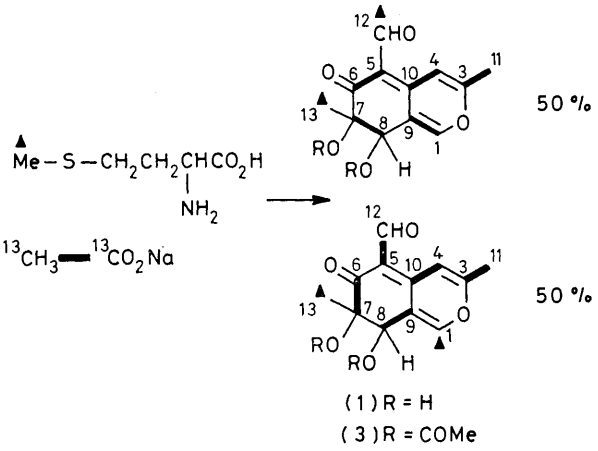
Detection of One Symmetrical Precursor during the Biosynthesis of the Fungal Metabolite Austdiol using [1,2-$^{13}C_2$]Acetate and [*Me*-^{13}C]Methionine

By LINO COLOMBO, CESARE GENNARI, GIULIANA SEVERINI RICCA, and CARLO SCOLASTICO*
(*Istituto di Chimica Organica dell'Università, Centro C.N.R. per le sostanze organiche naturali, via C. Saldini, 50, Milano, Italy*)

and FABRIZIO ARAGOZZINI
(*Istituto di Microbiologia Agraria e Tecnica dell'Università, via Celoria, 2, Milano, Italy*)

Summary A ^{13}C n.m.r. analysis of [1,2-$^{13}C_2$]acetate- and [*Me*-^{13}C]methionine-derived austdiol (**1**) is consistent with the symmetrical aldehyde (**2**) being a biosynthetic precursor of (**1**).

AUSTDIOL (**1**), the main toxic metabolite from *Aspergillus ustus* (Bainier) Thom and Church, was first isolated by Vleggaar *et al.*[1] Its structure was determined by chemical, spectroscopic, and crystallographic evidence.[1,2] The polyketide origin of austdiol (**1**) can be assumed on the basis of the analogy with the experimentally established biosynthesis of similar compounds, *e.g.* citrinin[3] and ascochitine.[4] Our experiments with sodium [1,2-$^{13}C_2$]acetate and [*Me*-^{13}C]methionine now confirm this hypothesis and show that the skeleton of austdiol is derived from a single pentaketide chain, composed of head-to-tail acetate units, and possesses two C_1 units introduced by *S*-adenosylmethionine (Scheme 1). An intriguing labelling pattern was observed (Table) on feeding with [$^{13}C_2$]acetate: quintets were seen for atoms C-5, -6, -7, -8, and -9, while characteristic triplets normally observed in spectra obtained using doubly labelled acetate were only seen for atoms C-11, -3, -4, -10, -12, and -1. Furthermore, by feeding with [*Me*-^{13}C]methionine, an approximately two-fold enrichment at C-13 was observed in comparison with the enrichments at C-12 and C-1.



SCHEME 1

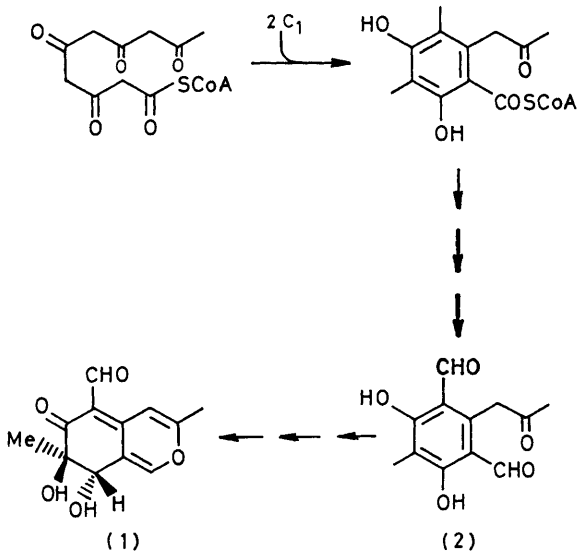
The most likely explanation of these apparent discrepancies is that labelled austdiol (**1**) is a 50:50 mixture of two different labelled molecules as shown in Scheme 1. This labelling pattern is consistent with the biosynthetic pathway shown in Scheme 2, where one of the methyl groups arising from methionine is oxidised and the carboxy-

group of the polyketide is reduced to give the symmetrical dialdehyde (**2**).

TABLE. ^{13}C Chemical shifts (p.p.m.) and ^{13}C–^{13}C coupling constants (Hz) of austdiol diacetate (**3**).[a]

Carbon	Chemical shift	Percentage enrichment[e]	$J(^{13}C–^{13}C)$[b]
1	151·7[c]	5·6	75·5
3	148·2	3·5	50·9
4	106·1	7·4	50·9
5	108·0	2·5	58·7; 58·7
6	190·1	2·0; 1·8	58·6; 41·9
7	80·3	0·2; 2·7	87·9; 41·5
8	67·2	0·7; 2·6	87·1; 48·1
9	116·9	1·2; 1·6	76·0; 48·9
10	165·8	0·5	51·0
11	19·8	15·7	51·0
12	189·0[c]	8·0	59·1
13	17·4[d]	—	—

[a] Austdiol was transformed into austdiol diacetate (**3**) (see ref. 1) in order to obtain a more stable and more soluble product. All spectra were recorded for solutions of (**3**) in CD_3SOCD_3. [b] ^{13}C–^{13}C coupling constants in the proton-noise decoupled ^{13}C n.m.r. spectrum of austdiol derived from [1,2-$^{13}C_2$]acetate. [c] Enhanced in intensity after incorporation of [*Me*-^{13}C]methionine (enrichment 4·8%). [d] Enhanced in intensity after incorporation of [*Me*-^{13}C]methionine (enrichment 12·1%). [e] Percentage enrichments, due to incorporation of [1,2-$^{13}C_2$]acetate, were calculated by means of the formula reported by Vining *et al.* (L. C. Vining, J. L. C. Wright, A. G. McInnes, D. G. Smith, and J. A. Walter, *J. Chem. Soc., Chem. Commun.*, 1974, 282.)



SCHEME 2

The complete spectral assignment (Table) was deduced from literature values,[5] the off-resonance natural abundance spectrum, and the values of individual ^{13}C–^{13}C coupling constants.

(*Received*, 30*th March* 1981; *Com.* 352.)

[1] R. Vleggaar, P. S. Steyn, and D. W. Nagel, *J. Chem. Soc., Perkin Trans.* 1, 1974, 45.
[2] P. S. Steyn and R. Vleggaar, *J. Chem. Soc., Perkin Trans.* 1, 1976, 204, and references therein.
[3] R. H. Carter, M. J. Garson, and J. Staunton, *J. Chem. Soc., Chem. Commun.*, 1979, 1097; J. Barber and J. Staunton, *ibid.*, 1979, 1098; 1980, 552; L. Colombo, C. Gennari, C. Scolastico, F. Aragozzini, and C. Merendi, *ibid.*, 1980, 1133.
[4] L. Colombo, C. Gennari, C. Scolastico, F. Aragozzini, and C. Merendi, *J. Chem. Soc., Perkin Trans.* 1, 1980, 2549, and references therein.
[5] L. Colombo, C. Gennari, G. S. Ricca, C. Scolastico, and F. Aragozzini, *J. Chem. Soc., Perkin Trans.* 1, 1980, 675.

Printed by Heffers Printers Ltd Cambridge England