161. Differential Hydrogen Exchange During Biosynthesis of Cytochalasins B and D

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Summary

Sodium [2-^{13}C,2-$^{2}H_3$]acetate was incorporated into cytochalasin B (**1**) by *Phoma exigua* and into cytochalasin D (**2**) by *Zygosporium masonii*. The ^{13}C-NMR. and ^{2}H-NMR. of the metabolites showed that most of the deuterium was lost except at carbon atoms which are in polyketide chain-initiating units.

Cytochalasins B (**1**) and D (**2**) are members of an expanding[2]) class of microbial metabolites which display a large variety of useful biological effects, inclusing the ability to extrude viable cell nuclei [3]. Their importance as tools in cell biology has stirred interest in chemical synthesis [4] and microbiological production [5] of these compounds, with special emphasis on generation of functionally modified or isotopically labelled derivatives. Our previous biosynthetic studies determined that both **1** and **2** are formed from L-phenylalanine, several one C-atom units originating from methionine, and nine intact acetate units [6]. The present work deals with incorporation of doubly-labelled sodium [2-^{13}C,2-$^{2}H_3$]acetate into cytochalasins B (**1**) and D (**2**), and with detection of differential exchange of the deuterium by ^{2}H-NMR. and ^{13}C-NMR.

The realization that the fate of H-atoms during biosynthesis of secondary metabolites offers valuable mechanistic insights has led to expanded use of ^{2}H-NMR. and ^{3}H-NMR. spectroscopy in recent studies [7–9]. However, the lack of sensitivity due to the quadrupolar nature of deuterium and safety considerations for the radioactivity of tritium frequently require relatively high incorporation of precursor into product. These problems can be partly circumvented by the use of precursors labelled with both ^{2}H and ^{13}C [10]. C-atoms bearing deuterium are easily detected in ^{1}H-decoupled ^{13}C-NMR. spectra because of isotope shift, spin-spin coupling, quadrupole broadening, and reduced nuclear *Overhauser* effect

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[2]) Recent additions to this class of compounds include the aspochalasins [1] and chaetoglobosin K [2].

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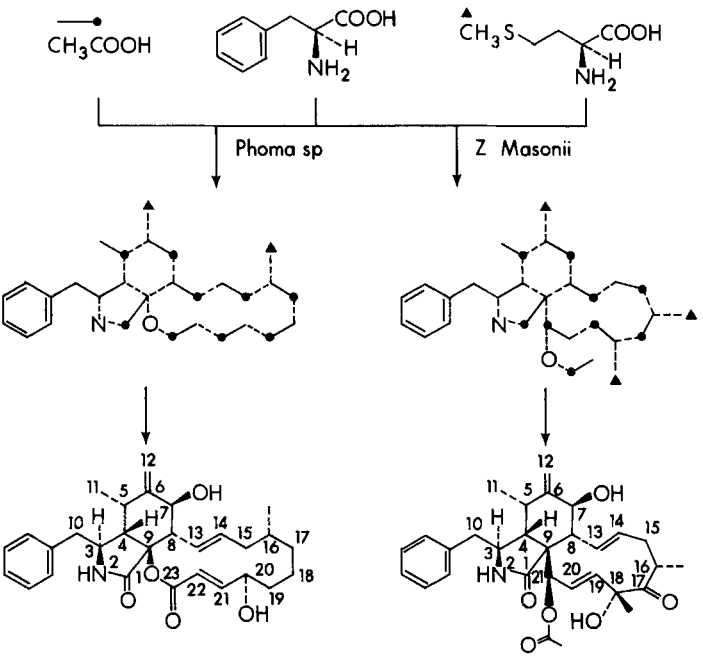

1 Cytochalasin B **2** Cytochalasin D

[11]. Application of this methodology to polyketide biogenesis by incorporation of sodium [2-^{13}C,2-^{2}H$_3$]acetate supported an earlier report [9] that considerable hydrogen exchange can occur during formation of C-chains by the acetate/malonate pathway [12–15]. Although protium can also be used as a tracer, as demonstrated by incorporation of sodium [1,2-^{13}C$_2$,2-^{1}H$_3$]acetate into citrinin in a D_2O medium [16], the prohibitive cost of large scale fermentations and reduced yield due to biochemical isotope effects pose severe problems[3]).

Sodium [2-^{13}C,2-^{2}H$_3$]acetate (isotopic purity 93% ^{13}C, 98% ^{2}H) was prepared by exchange [18] of commercially available [^{13}C]acetate and incorporated into cytochalasin D (**2**) by a growing culture of *Zygosporium masonii*. The ^{13}C-NMR. spectrum of **2** showed that the signals for C(6), C(8), C(14), C(16), C(18), C(20) and C(9) were strongly enhanced singlets [19], but that the peaks for C(11) and the methyl of the *O*-acetyl group appeared as natural abundance singlets superimposed on small multiplets. Examination of the ^{1}H-decoupled 30.7-MHz-^{2}H-NMR. spectrum confirmed that deuterium was primarily localized at these two C-atoms. Both the *O*-acetyl methyl and C(11) appeared as ^{13}C-coupled doublets of $J = 20.3$, 19.0 Hz, respectively. Since no other deuterium signals were observed, the other hydrogen-bearing carbon atoms originating from C(2) of acetate had exchanged most if not all of their deuterium during biosynthesis. Incorporation of sodium [1-^{13}C,2-^{2}H$_3$]acetate into cytochalasin D (**2**) by *Z. masonii* gave the

[3]) *Cf.* Recent review by *Garson & Staunton* on new NMR. methods for tracing the fate of hydrogen in biosynthesis [17].

expected enrichments [19] of ^{13}C at C(1), C(5), C(7), C(13), C(15), C(17), C(19), C(21) and the carbonyl C-atom of the *O*-acetyl group, but no deuterium label could be detected at any of these C-atoms. This suggests that either intramolecular 1,2-shifts of hydrogen do not occur on the polyketide part of the molecule during biosynthesis, or that exchange with medium must precede such shifts.

Commercially available sodium [2-^{13}C,2-$^{2}H_3$]acetate (isotopic purity 90.1% ^{13}C, 98% ^{2}H) was incorporated into cytochalasin B (**1**) in the presence of unlabelled malonate using *Phoma exigua*. Isolation of the metabolite and measurement of the ^{1}H-decoupled ^{13}C-NMR. spectrum showed that C(6), C(8), C(9), C(14), C(16), C(18), C(20) and C(22) were strongly enhanced singlets[4]), but that C(11) was a natural abundance singlet superimposed on a deuterium-coupled multiplet. Although it was not possible to obtain good ^{2}H-NMR. spectra, the ^{13}C-NMR. clearly demonstrated that most of the deuterium label had been exchanged during biosynthesis except for that at C(11). Thus in both **1** and **2** only the starter units of the acetate/malonate polyketide retain large amounts of hydrogen label.

Our results support the proposal of *Staunton et al.* [12] that [2-^{13}C,2-$^{2}H_3$]acetate can be used to identify these starter units. One deuterium can of course be lost from acetate units which are incoporated as malonate during the dehydration step of normal fatty acid biosynthesis [21]. Apparently a non-stereospecific hydrogen exchange which occurs at a post-malonate stage [22] may account for most of the remaining deuterium loss. Recent work on palmitate biosynthesis showed that the smallest amount of hydrogen exchange occurred at the methyl C-atom (starter unit), the next acetate unit suffered the maximum amount of label loss, and succeeding units retained progressively more label [15]. This type of hydrogen exchange may also be a complicating factor in studies on enoyl reductase in which the yeast enzyme [23] and the *E. coli* enzyme [24] appear to operate with different stereochemistry.

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Experimental Part

The general experimental procedures for incorporation of labelled precursors into cytochalasins B (**1**) and D (**2**) by *Phoma exigua* and *Zygosporium masonii* have been described [6] [20]. In the feeding experiment of cytochalasin B (**1**) the culture medium (3 l) was treated with 770 mg [2-^{13}C,2-$^{2}H_3$]acetate and 315 mg malonic acid per liter, the pH was adjusted with 1N NaOH.

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The ^{13}C-NMR. spectra were measured on *Bruker* WH90 and WH400 spectrometers, ^{2}H-NMR. spectra were obtained on *Bruker* WH90 and WH200 spectrometers. We are grateful to Mr. *K. Aegerter*

[4]) The mode of incorporation of sodium [2-^{13}C]acetate into cytochalasin B (**1**) by *Phoma* has been reported [20].

(University of Basel), Mr. *G. Bigam* and Dr. *T. Nakashima* (University of Alberta) for making these measurements.

Sodium [2-^{13}C,2-^{2}H$_3$]acetate was purchased from *Prochem-British Oxygen Company Ltd.* for the incorporation into cytochalasin B (**1**) or was prepared by repeated exchange (7 days, 150°) of commercially available *(Prochem-British Oxygen Company Ltd.)* sodium [2-^{13}C]acetate (93% isotopic purity) with D_2O [18] for the incorporation of cytochalasin D (**2**). Sodium [1-^{13}C,2-^{2}H$_3$]acetate was synthesized in an analogous manner.

REFERENCES

[1] *W. Keller-Schierlein & E. Kupfer,* Helv. *62*, 1501 (1979).
[2] *J.P. Springer, R.H. Cox, H.G. Cutler & F.G. Crumley,* Tetrahedron Lett. *1980*, 1905.
[3] 'Cytochalasins, Biochemical and Cell Biological Aspects', S.W. Tanenbaum (Ed.), Elsevier/North Holland Press, Amsterdam 1978.
[4] *G. Stork, Y. Nakahara, Y. Nakahara & W.J. Greenle,* J. Am. Chem. Soc. *100*, 7775 (1978); *P.A. Clark & P.L. Fuchs,* ibid. *101*, 3567 (1979); *T. Schmidlin & Ch. Tamm,* Helv. *63*, 121 (1980).
[5] *R.A. Zabel, C.A. Miller & S.W. Tanenbaum,* Appl. Environ. Microbiol. *37*, 208 (1979); *S. Udagawa, T. Muroi, H. Kurata, S. Sekita, K. Yoshihira, S. Natori & M. Umeda,* Can. J. Microbiol. *25*, 170 (1979).
[6] *J.C. Vederas & Ch. Tamm,* Helv. *59*, 558 (1976); cf. *Ch. Tamm,* in [3], pp. 15–51.
[7] *J.A. Elvidge, D.K. Jaiswal, J.R. Jones & R. Thomas,* J. Chem. Soc. Perkin I, *1977*, 1080; *L.J. Altman, C.Y. Han, A. Bertolino, G. Handy, D. Laungani, W. Muller, S. Schwartz, D. Shanker, W.H. de Wolf & F. Yang,* J. Am. Chem. Soc. *100*, 3235 (1978).
[8] *B.W. Bycroft, C.M. Wels, K. Corbett & D.A. Lowe,* J. Chem. Soc. Chem. Commun. *1975*, 123; *P.M. Dewick & D. Ward,* ibid. *1977*, 338; *D.E. Cane & S.L. Buchwald,* J. Am. Chem. Soc. *99*, 6132 (1977); *D.E. Cane & P.P.N. Murthy,* ibid. *99*, 8327 (1977); *C.R. Hutchinson, A.H. Heckendorf, J.L. Straughn, P.E. Dadona & D.E. Cane,* ibid. *101*, 3358 (1979); *D.E. Cane & R.B. Nachbar,* Tetrahedron Lett. *1980*, 437; *S. Damtoft, S.R. Jensen & R.B. Nielsen,* J. Chem. Soc. Chem. Commun. *1980*, 42.
[9] *Y. Sato, T. Oda & H. Saito,* Tetrahedron Lett. *1976*, 2695.
[10] *M. Imfeld, C.A. Townsend & D. Arigoni,* J. Chem. Soc. Chem. Commun. *1976*, 541; *A. Battersby, R. Hollenstein, E. McDonald & D.C. Williams,* ibid. *1976*, 543; *A.I. Scott, M. Kajiwara, T. Takahashi, I.M. Armitage, P. Demou & D. Petrocine,* ibid. *1976*, 544.
[11] *J.B. Stothers,* in 'Topics in Carbon-13 NMR Spectroscopy', Vol. 1, *G.C. Levy* (Ed.), John Wiley & Sons, New York 1974, pp. 234–238.
[12] *M.J. Garson, R.A. Hill & J. Staunton,* J. Chem. Soc. Chem. Commun. *1977*, 624, 921.
[13] *U. Sankawa, H. Shimada, T. Sato, T. Kinoshita & K. Yamasaki,* Tetrahedron Lett. *1977*, 483.
[14] *U. Sankawa, H. Shimada & K. Yamasaki,* Tetrahedron Lett. *1978*, 3375; *A. Banerji, R. Hunter, G. Mellows & K. Sim,* J. Chem. Soc. Chem. Commun. *1978*, 843; *R. Hunter & G. Mellows,* Tetrahedron Lett. *1978*, 5051.
[15] *A.G. McInnes, J.A. Walter & J.L.C. Wright,* Tetrahedron Lett. *1979*, 3245.
[16] *J. Barber & J. Staunton,* J. Chem. Soc. Chem. Commun. *1979*, 1098.
[17] *J.M. Garson & J. Staunton,* Chem. Soc. Rev. *8*, 539 (1979).
[18] *L.H. Jones & E. McLaren,* J. Chem. Phys. *22*, 2796 (1954).
[19] *J.C. Vederas, W. Graf, L. David & Ch. Tamm,* Helv. *58*, 1886 (1975).
[20] *W. Graf, J.L. Robert, J.C. Vederas, Ch. Tamm, P.H. Solomon, I. Miura & K. Nakanishi,* Helv. *57*, 1801 (1974).
[21] *K. Bloch & D. Vance,* Ann. Rev. Biochem. *46*, 263 (1977).
[22] *B. Sedgwick & J.W. Cornforth,* Eur. J. Biochem. *75*, 465 (1977); *B. Sedgwick, J.W. Cornforth, S.J. French, R.T. Gray, E. Kelstrup & P. Willadsen,* ibid. *75*, 481 (1977).
[23] *B. Sedgwick & C. Morris,* J. Chem. Soc. Chem. Commun. *1980*, 96.
[24] *R.H. White,* Biochemistry *19*, 9 (1980).