Use of ^{13}C in Biosynthetic Studies. Location of Isotope from Labelled Acetate and Formate in the Fungal Tropolone, Sepedonin, by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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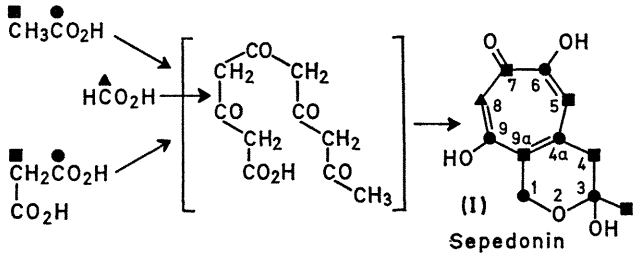
Summary The ^{13}C labelling patterns in sepedonin (3,6,9-trihydroxy-3-methyl-1,3,4,7-tetrahydrocyclohepta[*c*]-pyran-7-one) isolated from cultures of *Sepedonium chrysospermum* fed [1-^{13}C]acetate, [2-^{13}C]acetate, and [^{13}C]formate have been elucidated by ^{13}C nuclear magnetic resonance (^{13}C n.m.r.) spectroscopy.

IN biosynthetic ^{13}C tracer studies labelling patterns can be obtained *directly* by ^{13}C n.m.r. spectroscopy or *indirectly* by detection and integration of ^{13}C–H satellite signals. The latter method has been applied in this laboratory[1a,b] and by other workers[2] to a number of biosynthetic problems, and its advantages and limitations have been enumerated.[1a] However, it was only during the preparation of this manuscript that the first application of ^{13}C n.m.r. in elucidating a biosynthetic pathway was reported.[3]

We have previously shown that sepedonin, produced by cultures of *Sepedonium chrysospermum*, has the structure 3,6,9-trihydroxy-3-methyl-1,3,4,7-tetrahydrocyclohepta[*c*]-pyran-7-one,[4] and have established,[1b] by the ^{13}C–H satellite method, that the methyl group, C-4, and C-5 were labelled by $^{13}CH_3CO_2H$, whereas only C-1 was labelled by $CH_3{}^{13}CO_2H$ and C-8 by $H^{13}CO_2H$ (see Scheme). We postulated that biosynthesis of sepedonin occurred as outlined in the



SCHEME

Scheme. Although this pathway is consistent with results of ^{14}C isotopic tracer studies on simpler fungal tropolones[5] it is not fully proved, since the ^{13}C–H satellite method provided no information on the amounts of ^{13}C tracer at six

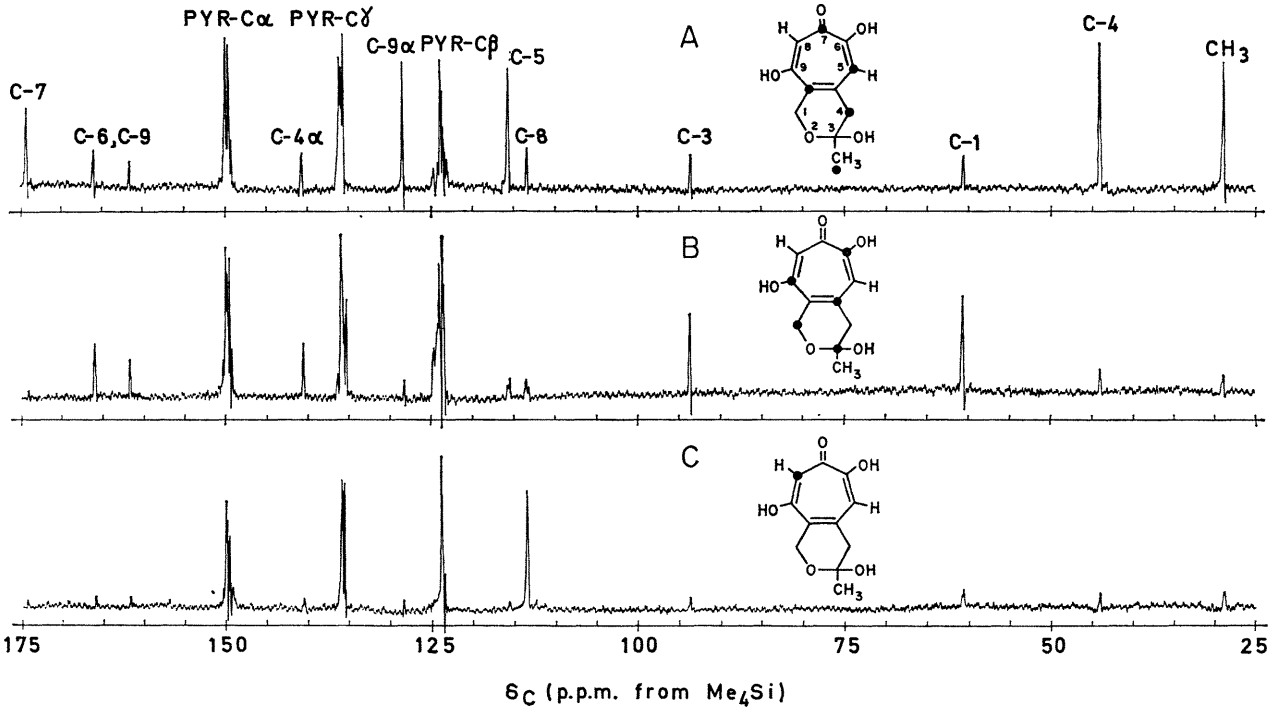


FIGURE. ^{13}C *n.m.r. spectra of sepedonin*: *sample* (A) *from* $^{13}CH_3CO_2Na$, 142 *mg*; (B) *from* $CH_3{}^{13}CO_2Na$, 80 *mg*; *and* (C) *from* $H^{13}CO_3Na$, 85 *mg*. ^{13}C *enrichments were approximately* 4, 5, *and* 20 *times natural abundance in* A, B, *and* C, *respectively*.[1b] *Solvent*, 1·0 *ml pyridine*; 8 *mm tube*. *Spectra recorded on* HA-100 *spectrometer* (25—15 *MHz*) *equipped with* V-3530 RF/AF *sweep unit, a Spectro-System* 100 *for multiscan averaging and* V-3512-1 *heteronuclear noise decoupler*. *Each spectrum is a composite of three equal sections of* 50 *p.p.m., proton noise decoupled with* 50 *scans of* 50 *p.p.m. at* 50 s/*scan, lock signal β-carbons of pyridine*. *Chemical shifts measured relative to β-carbons of pyridine and converted into p.p.m. from* Me_4Si (δ_C) *using* δ_C (*β-carbons of pyridine*) 123·9 *p.p.m.* PYR-C_α,-C_β, *and* -C_γ *refer to carbon resonances of pyridine*.

of the eleven carbon positions in sepedonin. We now show that more conclusive biosynthetic information can be obtained by analysing the same labelled samples of sepedonin by ^{13}C n.m.r.

The proton noise decoupled ^{13}C n.m.r. spectra of sepedonin labelled by $^{13}CH_3CO_2H$ (A), $CH_3{}^{13}CO_2H$ (B), and $H^{13}CO_2H$ (C) are shown in the Figure. As expected sepedonin gave eleven carbon resonances (singlets), and the following could be assigned unambiguously;[6] δ_C 29·0 (CH_3), 44·0 (>CH_2, C-4), 60·6 (–CH_2O–, C-1), 93·6 (>C(OH)O–, C-3), 161·6 and 166·0 (=CO*H*, C-6 and C-9), and 174·3 (>C=O, C-7). The signal at δ_C 113·5 could also be assigned to C-8 because only it has an intensity greater than expected for ^{13}C natural abundance in spectrum C, and our ^{13}C–H satellite results had previously established that position 8 in sepedonin was labelled by $H^{13}CO_2H$. Assignment of C-5 (only carbon other than C-8 bearing one hydrogen) to the signal at δ_C 115·6 was based on CW (single frequency) decoupling experiments. Finally, the signal at δ_C 128·4 was assigned to C-9a, and the one at δ_C 140·6 to C-4a.

The relative intensities of the ^{13}C resonances establish that position 8 in sepedonin is not labelled by either of the two acetate precursors (spectra A and B) whereas this position is specifically labelled by [^{13}C]formate (spectrum C). Moreover, of the ten remaining carbon positions in sepedonin only five were enriched by $^{13}CH_3CO_2H$ (spectrum A) and the other five by $CH_3{}^{13}CO_2H$ (spectrum B). The absence of vicinal ^{13}C–^{13}C coupling together with the latter observations proves that each acetate precursor has labelled alternate carbon atoms of a ten-carbon polyketide intermediate. The five carbons enriched by $^{13}CH_3CO_2H$ were CH_3, C-4, C-5, C-9a (δ_C 128·4), and C-7, whereas those labelled by $CH_3{}^{13}CO_2H$ were C-1, C-3, C-4a (δ_C 140·6), C-6, and C-9. These results provide convincing evidence that sepedonin is formed by insertion of the formate carbon atom between the third and fourth carbon atoms of a ten-carbon polyketide chain derived from acetate (see Scheme), and thus confirm the conclusions based on our more limited ^{13}C–H satellite study.[1b]

Nuclear Overhauser effects, producing enhancements of up to 2·98,[7] preclude quantitative estimation of ^{13}C enrichments by peak area integration of spectra A, B, and C. To obtain quantitative data it would have been necessary first to obtain the enhancements at individual carbon positions by comparing the signal intensities in decoupled and undecoupled ^{13}C natural abundance spectra. This was not done in the present study because there was no ambiguity as to which positions were enriched by the three precursors. However, the Nuclear Overhauser enhancement factors for the C-5 and C-8 resonances might be expected to be very similar; when we integrated these signals in spectrum A we obtained a C-5 : C-8 intensity ratio of 3·9 : 1 which was identical to the ratio obtained by the ^{13}C–H satellite method.

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[1] (a) D. Desaty, A. G. McInnes, D. G. Smith, and L. C. Vining, *Canad. J. Biochem.*, 1968, **46**, 1293; (b) J. Wright, D. G. Smith, A. G. McInnes, L. C. Vining, and D. W. S. Westlake, *ibid.*, 1969, **47**, 945.
[2] M. Tanabe and G. Detre, *J. Amer. Chem. Soc.*, 1966, **88**, 4515; M. Tanabe and H. Seto, *Biochim. Biophys. Acta*, 1970, **208**, 151; A. K. Bose, K. S. Khanchandani, P. T. Funke, and M. Anchel, *Chem. Comm.*, 1969, 1347.
[3] M. Tanabe, H. Seto, and L. Johnson, *J. Amer. Chem. Soc.*, 1970, **92**, 2157.
[4] J. Wright, A. G. McInnes, D. G. Smith, and L. C. Vining, *Canad. J. Chem.*, 1970, **48**, 2702.
[5] R. Bentley, *J. Biol. Chem.*, 1963, **238**, 1895; I. G. Andrew and W. Segal, *J. Chem. Soc.*, 1964, 607; J. H. Richards and L. D, Ferretti, *Biochem. Biophys. Res. Comm.*, 1960, **2**, 107.
[6] J. B. Stothers, *Quart. Rev.*, 1965, **19**, 144; J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, Oxford, 1965, vol. 2, p. 988; E. F. Mooney and P. H. Winson, *Ann. Rev. N. M. R. Spectroscopy*, 1969, **2**, 156.
[7] A. J. Jones, D. M. Grant, and K. F. Kuhlmann, *J. Amer. Chem. Soc.*, 1969, **91**, 5013.