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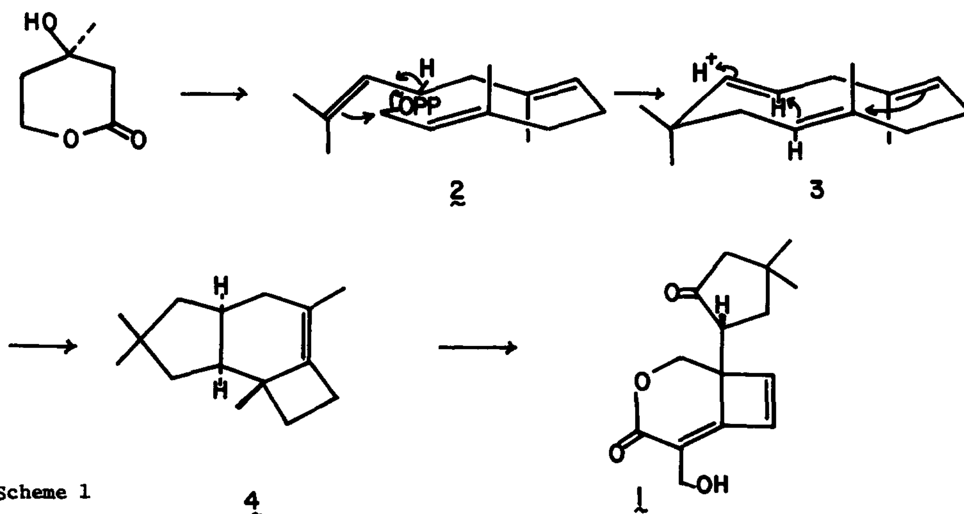
BIOSYNTHESIS OF FOMANNOSIN. ^{2}H NMR EVIDENCE AGAINST POSTULATED HYDRIDE SHIFTS

David E. Cane*[1] and Robert B. Nachbar
Department of Chemistry, Brown University, Providence, Rhode Island 02912

ABSTRACT: ^{2}H NMR analysis of fomannosin (1) derived from [5,5-$^2H_2$]-mevalonate established the presence of deuterium at C-5 and C-10, whereas no isotope was located at C-12.

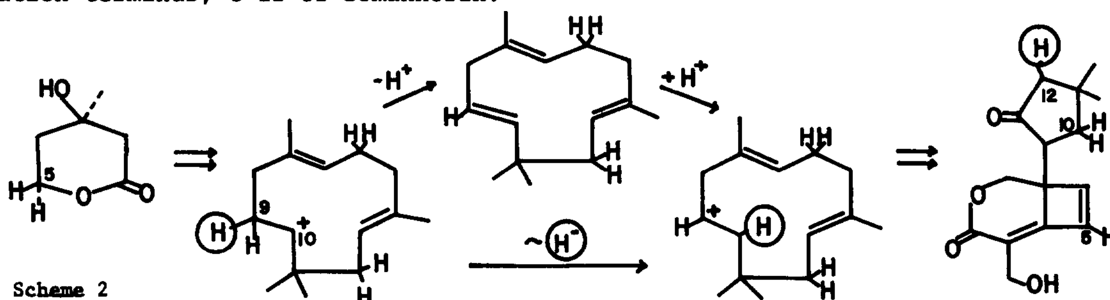
In previous reports on the biosynthesis of the phytotoxic fungal metabolite fomannosin (1) we have described experiments on the incorporation of [1,2-$^{13}C_2$]-acetate which support a biosynthetic pathway in which intramolecular cyclization of trans,trans-farnesyl pyrophosphate (2), derived from mevalonate, generates humulene (3) by attack on the _si_ face of the distal double and loss of a proton from C-9.[2a] Reprotonation at C-10 initiates further cyclization to a protoilludyl cation. Subsequent oxidative metabolism, including cleavage of the six-membered ring, would then yield fomannosin. Independent evidence for such a pathway has come from the isolation of Δ^6-protoilludene (4) from a fomannosin producing strain of _Fomitopsis insularis_.[3] Similar schemes have been proposed for a group of closely related metabolites, including the illudins, marasmic acid, hirsutic acid, and the coriolins and a stereochemical analysis of each pathway has been advanced.[2a,b]



Scheme 1

In considering the proposed intermediacy of humulene, a reasonable alternative

to the above deprotonation - reprotonation sequence would be a simple 1,2-hydride shift which would interconvert the two postulated humulyl cations. Since the protons at C-9 of humulene originate at C-5 of mevalonate, the two mechanistic alternatives can readily be distinguished by incorporating the appropriately labeled mevalonate and establishing the presence or absence of isotope at the presumed migration terminus, C-12 of fomannosin.



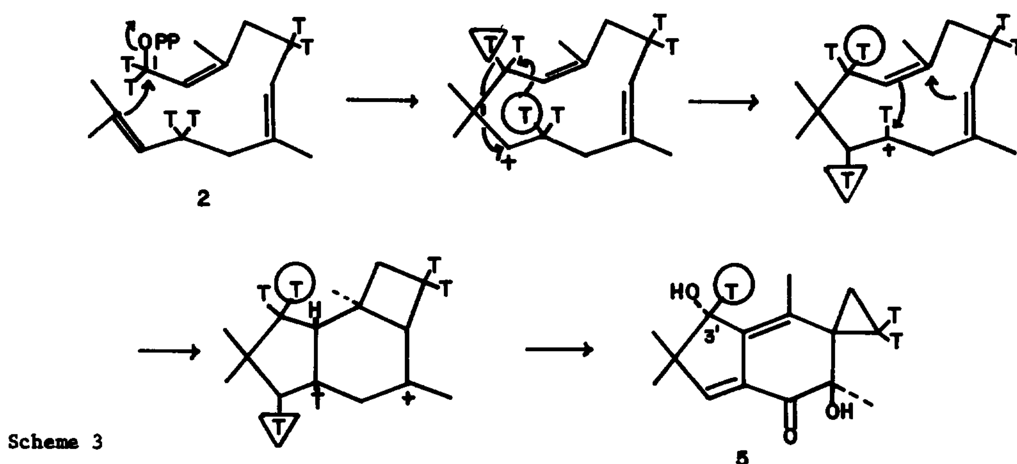
Scheme 2

To this end 2.21 mmol of sodium [5,5-$^{2}H_2$]-mevalonate[5] (99 atom% ^{2}H), containing internal standard [2-^{14}C]-mevalonate (8.30 x 10^{5} dpm/mmol) was administered to 4 250-ml surface cultures of Fomes annosus and the resulting fomannosin (0.35 mmol, 5.45 x 10^{3} dpm/mmol) isolated as previously described[2] and rigorously purified by repeated silica gel chromatography. The observed specific activity of 1 corresponded to a calculated ^{2}H enrichment of 0.2% per labeled site. Analysis of the resultant ^{2}H nmr spectrum[5,6] established the presence of excess deuterium at three positions: D-10β (1.64 ppm, 1 D), D-10α (1.51 ppm, 1 D), and D-5 (6.40 ppm, 0.4 D).[7] No signals could be detected which would correspond to deuterium at C-12, thereby firmly excluding any hydride shift to this position. This conclusion is further corroborated by the results of radioisotopic incorporation experiments. Thus feeding of [2-^{14}C,5RS-^{3}H]-mevalonate ($^{3}H/^{14}C$, 1.96)[9a] gave fomannosin ($^{3}H/^{14}C$, 1.04; 1.14 x 10^{5} dpm ^{14}C/mmol),[9b] corresponding to retention of only three of six tritium equivalents. Such a result is consistent only with the originally proposed deprotonation - reprotonation sequence since the alternative hydride shift would require retention of 4/6 of the original tritium activity. Similarly incorporation of 3R,5S-[5-^{3}H],3RS-[2-^{14}C]-mevalonate ($^{3}H/^{14}C$, 2.98)[9a] gave fomannosin which had retained only one of three tritium atoms ($^{3}H/^{14}C$, 2.30; 2.80 x 10^{4} dpm ^{14}C/mmol;[9b] theoretical $^{3}H/^{14}C$, 1.99, based on 3R-mevalonate). The latter result also established that the hydrogen at C-5 of fomannosin must be derived from H-5re of mevalonate.

The reduction in the relative intensity of the D-5 resonance in the ^{2}H nmr spectrum was unanticipated and is the consequence of an isotope effect on the loss of the mevalonate H-5si-derived hydrogen during formation of the cyclobutene double bond of 1. Interestingly this isotope effect is not evident from the radioisotopic incorporation experiments which indicate retention of near integral amounts of tritium. The apparent discrepancy between the two sets of results arises from the use of intramolecularly double labeled precursor for the ^{2}H nmr experiment. When tritiated substrates are employed, no two isotopes reside in the same molecule.

Any isotope effect on the loss of mevalonate H-5si-derived tritium therefore has no effect on the relative retention of the remaining H-5re-derived atom. On the other hand, when both isotopes are carried on the same molecule, as is the case for [5,5-$^{2}H_{2}$]-mevalonate, isotopic discrimination at one site necessarily results in a depletion of both labels.[10] Since it is statistically unlikely that two labeled mevalonates will be incorporated into the same molecule of metabolite, the isotope effect is observed as a reduction in the relative amount of deuterium which appears in the product at the affected site.

The absence of any deuterium at C-12 of fomannosin is also significant in light of a proposal by Hanson for an unusual series of hydride shifts in the biosynthesis of illudin M, a metabolite biogenetically closely related to fomannosin.[11] Feeding of [5RS-^{3}H] and [5R-^{3}H]-mevalonates and subsequent degradation had established the presence of isotope derived from H-5re of mevalonate at C-3' of illudin M. This isotope would normally be expected to have been derived by way of 1-Hre of the intermediate farnesyl pyrophosphate. When, however, attempted incorporation of [1RS-^{3}H,2-^{14}C]-farnesyl pyrophosphate gave illudin M which was devoid of tritium activity, it was felt necessary to postulate the sequence of hydride shifts illustrated in scheme 3. Unfortunately these conclusions were never substantiated by degradations to establish the positions of labeling, leaving open the possibility that the low incorporation of ^{14}C (0.02%) and the loss of tritium were a consequence of prior degradation and random incorporation of the precursor. A similar sequence of hydride shifts in the biosynthesis of fomannosin, itself also derived from a protoilludyl intermediate, would in fact have resulted in deuterium at C-12. Our present results therefore cast serious doubts on the validity of the claimed hydride shifts during illudin M biosynthesis.[12]



Scheme 3

References and Notes

1. Fellow of the Alfred P. Sloan Foundation, 1978-1980; National Institutes of Health Research Career Development Award, 1978-1983.

2. a) D. E. Cane and R. B. Nachbar, J. Am. Chem. Soc., 100, 3208 (1978); 101, 1908 (1979); b) For references to the original literature, cf. ref. 2a.

3. S. Nozoe, H. Kobayashi, S. Urano, and J. Furukawa, Tetrahedron Lett., 1381 (1977).

4. D. E. Cane, R. B. Nachbar, J. Clardy, and J. Finer, Tetrahedron Lett., 4277 (1977).

5. cf. D. E. Cane and S. L. Buchwald, J. Am. Chem. Soc., 99, 6132 (1977); D. E. Cane and P. P. N. Murthy, ibid., 99, 8327 (1977); and ref. cited therein.

6. Proton decoupled ^{2}H NMR spectra were recorded at 41.44 MHz on a Bruker HX 270. From 3200 transients a S/N 6:1 was obtained for the D-10 resonances.

7. The corresponding proton chemical shifts for the cyclopentanone ring of 1 were assigned by detailed decoupling experiments at 270 MHz. H-9 (δ3.18), H-10α (1.58), and H-10β (1.74) constitute an ABX system in which the observed $J_{9-10\alpha}$ = 12.6 Hz and $J_{9-10\beta}$ = 8.8 Hz correspond to trans and cis vicinal couplings respectively. Irradiation of the upfield methyl resonance H-15 (1.10) caused the H-10α signal to sharpen with no accompanying increase in integrated area, indicating a long range coupling due to a trans-diaxial relationship of C-15 and H-10α.[8] A similar sharpening of the resonance at 1.95 allowed assignment of this signal to H-12α. No effect was observed on the resonances corresponding to H-10β and H-12β (2.22) nor did irradiation of the downfield H-14 methyl singlet (1.16) alter the remainder of the spectrum. Additional long range couplings between H-9 and H-12β (^{4}J = 1.3 Hz) and H-10β and H-12β (^{4}J = 2.1 Hz) further confirm the above assignments. The assignment of the methyl resonances also agrees with that previously arrived at by an independent approach.[2]

8. M. Barfield, A. M. Dean, C. J. Fallick, R. J. Spear, S. Sternhell, and P. W. Westerman, J. Am. Chem. Soc., 97, 1482 (1975) and ref. cited.

9. a) Determined for the benzhydrylamide; b) Determined by recrystallization of the derived dihydrofomannosin camphanate ester.[2,4]

10. For a recent example of use of intramolecular double labeling for the indirect detection of isotope effects see M. H. O'Leary and J. F. Marlier, J. Am. Chem. Soc., 100, 2582 (1978).

11. J. R. Hanson, T. Marten, and R. Nyfeler, J. C. S. Perkin I, 876 (1976). This claim has also been cited uncritically in at least one review: "Terpenoids and Steroids," Vol. 7, Specialist Periodical Reports, Chemical Society, 1977, pp. 192-193;

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