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BIOSYNTHESIS OF PSILOTIN FROM [2',3'-$^{13}C_2$,1'-^{14}C,4-^{3}H]PHENYLALANINE STUDIED WITH ^{13}C-NMR

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Summary: The 6-phenyl-dihydro-α-pyrone moiety of psilotin is formed from [2',3'-$^{13}C_2$,1'-^{14}C,-4-^{3}H]phenylalanine in the plant Psilotum nudum with retention of all the isotopes.

Psilotin (3), first isolated from the shoots of *Psilotum nudum*,[2] belongs to a class of natural products which may be considered to be formed from cinnamic acid (1) by the route illustrated in Figure 1,[3] cinnamic acid being formed by the deamination of phenylalanine. The co-

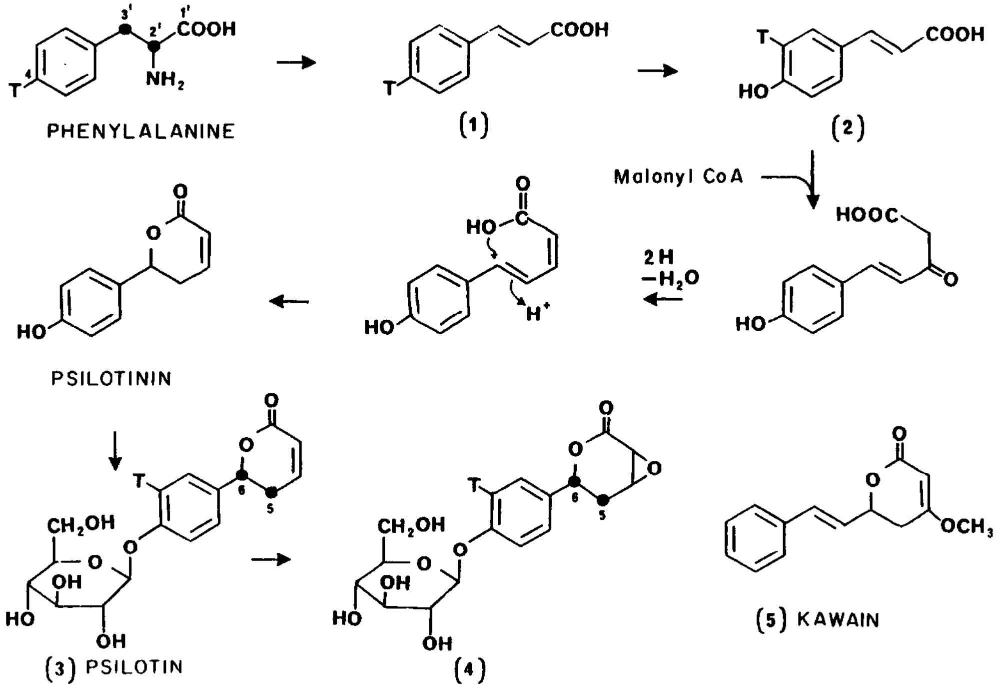

Figure 1. Proposed Biosynthesis of Psilotin from Phenylalanine

enzyme A esters of cinnamic acid and its hydroxylated derivatives serve as starter molecules in a process analogous to polyketide or fatty acid biosynthesis in which acetyl coenzyme A is the starting unit. The most common chain extension is with three acetate units leading to the flavanoids. Kawain (5) is considered to be formed from cinnamate plus two acetate units, and psilotin is thought to arise by the addition of one acetate unit (from malonyl coenzyme A).

This hypothesis has now been tested by feeding isotopically labeled phenylalanine to excised *Psilotum nudum* shoots. Contiguous ^{13}C atoms were introduced into the side chain at C-2' and C-3' so that incorporation of this putative precursor could be established by ^{13}C NMR spectroscopy.[4] This (*RS*)-[2',3'-$^{13}C_2$]phenylalanine was mixed with a small amount of (*S*)-[1'-^{14}C]-phenylalanine[9] so that the specific incorporation could be measured by radioactive assay. (*RS*)-[4-^{3}H]Phenylalanine[10] was also added so that the expected NIH-shift which occurs in the conversion of cinnamic acid to *p*-coumaric acid (2)[11] could be monitored. Table 1 records details of the feeding experiment and the incorporation of all the isotopes of the labeled phenylalanine into psilotin and psilotin epoxide (4).[12]

Table 1. Activities of Psilotin and its Epoxide isolated from *Psilotum nudum* fed (*RS*)-[2',3'-$^{13}C_2$,4-^{3}H]-(*S*)-[1'-^{14}C]Phenylalanine (30 mg, ^{14}C activity: 8.67×10^7 dpm/mmole, $^{3}H/^{14}C = 11.5$, 75% $^{13}C_2$) for 3 days.

	Psilotin	Psilotin Epoxide
Weight (isolated from apical parts)[a]	36.8 mg	4.2 mg
Specific Activity (^{14}C, dpm/mmole)	6.33×10^5	1.03×10^6
$^{3}H/^{14}C$	9.0	8.6
Retention of ^{3}H	78%	75%
% Specific Incorporation (^{14}C)	0.73	1.19
% Specific Incorporation (^{13}C)[b]	0.61	0.90

[a] Psilotin isolated from the basal part of the shoots (66.4 mg) had a lower activity (2.69×10^5 dpm/mmole). The psilotin epoxide (6.5 mg) from the basal parts had an activity of 8.2×10^5 dpm/mmole. [b] Determined by measurement of the intensities of the combined satellite peaks (I_s) and the central peaks (I_c). Then % specific inc. = 1.11 r/A, where $r = (I_s/I_c) - 0.0111$ and A = fraction of $^{13}C_2$ in the phenylalanine (0.75).[13]

Table 2. ^{13}C NMR Spectrum of Psilotin (in D_2O)

Carbon No.	Chemical Shift	Carbon No.	Chemical Shift (ppm from TMS)
2	170.3 (s)	4'	159.6 (s)
3	122.0 (d)	1"	102.9 (d)
4	151.8 (d)	2"	75.6 (d)
5	33.0 (t)	3"	78.8 (d)
6	82.5 (d)	4"	72.1 (d)
1'	135.4 (s)	5"	78.2 (d)
2',6'	130.9 (d)	6"	63.3 (t)
3',5'	119.4 (d)		

The ^{13}C NMR spectrum of psilotin is recorded in Table 2. The chemical shift assignments were made by off-resonance decoupling (the multiplicity of the signals is given in parenthesis) and by comparison with previously assigned compounds: methyl-β-D-glucoside,[14] benzyl acetate,[15] anisole,[15] and dioscorine.[16] Figure 2 illustrates the aliphatic region of the enriched psilotin in which satellites are clearly seen at the signals for C-5 and C-6, indicative of the direct incorporation of the phenylalanine side chain. Similar satellites (J = 36.5 Hz) were observed in the ^{13}C NMR spectrum of the labeled psilotin epoxide at 30.9 and 80.4 ppm which were assigned to C-5 and C-6, respectively. Somewhat to our surprise, the specific incorporation of the racemic [$^{13}C_2$]phenylalanine was almost as high as that of the natural (*S*)-[1'-^{14}C]phenylalanine. Also, the high retention of ^3H relative to ^{14}C indicates that the unnatural (*R*)-phenylalanine (i.e., *D*) is being utilized for the production of psilotin and its epoxide, as efficiently as the L-isomer.[17] Since the enzyme phenylalanine ammonia lyase (E. C. 4.3.1.5)[18&19] only yields cinnamic acid from L-phenylalanine we consider that the present results indicate that the administered D-phenylalanine undergoes a rapid transformation to the L-isomer, possibly via phenyl pyruvic acid.

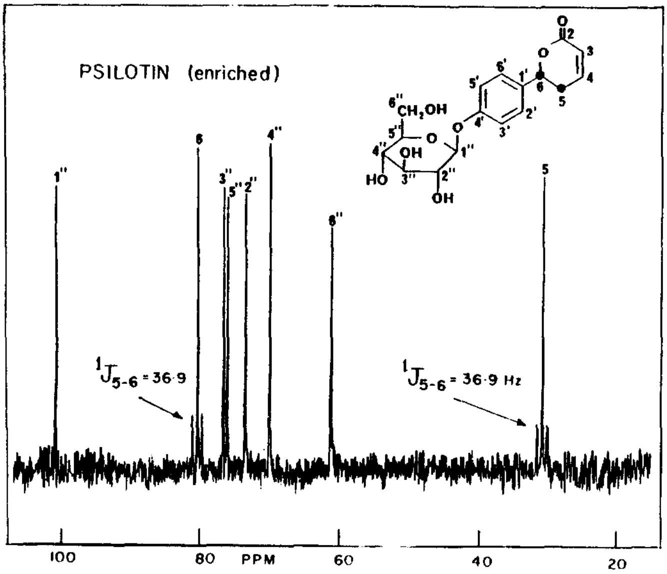

Figure 2. Proton-noise decoupled ^{13}C NMR spectrum of Psilotin (in D_2O) (aliphatic region) derived from [2',3'-$^{13}C_2$]-Phenylalanine.

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1. Contribution No. 182 from this laboratory.
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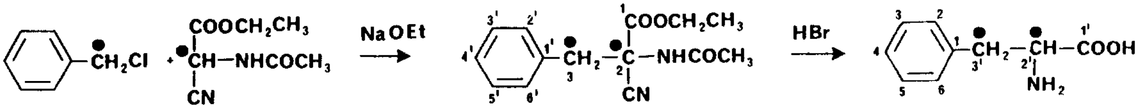
4. The (*RS*)-[2',3'-$^{13}C_2$]phenylalanine (IV) was obtained by hydrolysis of (*RS*)-ethyl-[2,3-$^{13}C_2$]-2-acetamido-2-cyano-3-phenylpropanoate (III) formed by the reaction between [1'-^{13}C]benzyl chloride[5] (I) and ethyl [2-^{13}C]-2-acetamido-2-cyanoacetate (II)[6] in the presence of sodium ethoxide, a reaction sequence previously used for the synthesis of the unlabeled amino acid.[7]



I II III IV

The ^{13}C NMR ($CDCl_3$) of III, δ(ppm from TMS): 170.5 (amide C=O), 165.7, $^1J_{1-2}$ = 57 Hz (ester C=O), 131.5, $^1J_{1'-3}$ = 44 Hz (1'), 130.0 (2',6'), 128.6 (3',5'), 128.3 (4'), 116.6, $^1J_{2-CN}$ = 61 Hz (CN), 63.3 (CH_2 of Et), 58.4, $^1J_{2-3}$ = 34 Hz (2), 41.7, $^1J_{2-3}$ = 34 Hz (3), 22.3 (CH_3 of Ac), 13.7 (CH_3 of Et). Analysis of the peaks at 175, 203, and 262, in the mass spectrum of this compound, which are 2 mass units greater than the corresponding peaks in unenriched material, indicated the presence of 82% $^{13}C_2$. The (*RS*)-[2',3'-$^{13}C_2$]phenylalanine, obtained as colorless plates from aqueous ethanol, mp 272° C, had ^{13}C NMR (D_2O + NaOD), δ(ppm from TMS): 183.4, $^1J_{1'-2'}$ = 50 Hz (1'), 139.5, $^1J_{1-3'}$ = 44 Hz (1), 131.4 (2,6), 130.6, $^3J_{3'-3}$ = 2.7 Hz (3,5), 128.8 (4), 59.3, $^1J_{2'-3'}$ = 33.3 Hz (2'), 42.2, $^1J_{2'-3'}$ = 33.3 Hz (3'). For the highly enriched carbons (C-2' and C-3') the average distance of the inner satellites to the central peaks was 15.8 Hz (Calc.[8] 16.0 Hz). The ratio of the intensities of the satellite peaks to the central peaks of C-2' and C-3' were consistent with the presence of 82% of the $^{13}C_2$ species.

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8. This distance (X) was calculated from the formula: $X = \frac{1}{2}(J_{AB} + V_{AB} - \sqrt{J_{AB}^2 + V_{AB}^2})$, where J_{AB} = coupling constant for the contiguous ^{13}C atoms, and V_{AB} = separation (in Hz) of the coupled carbons.

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