The Role of Leucine in Terpenoid Metabolism. Incorporation of [2-^{13}C]- and [3-^{13}C]-Leucines into Sesquiterpenoids by Tissue Cultures of *Andrographis paniculata*

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Incorporation of [2-^{13}C]- and [3-^{13}C]-leucines into paniculides by tissue cultures of *Andrographis paniculata* shows that (a) (3*S*)-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) from leucine breakdown *is not* incorporated intact into paniculide and (b) leucine *is* incorporated by breakdown to acetyl-CoA and its subsequent incorporation *via* HMG-CoA and MVA.

The possible involvement of leucine in the biosynthesis of isoprenoids has long been the subject of conjecture.[1] The literature of the past thirty years contains scattered reports of the incorporation of leucine into a variety of terpenoids in animals, plants, and micro-organisms.[2,3] These reports derive special interest from the fact that (3*S*)-3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) (**1**) stands at the cross-roads of two metabolic pathways: (i) the catabolic pathway from leucine to acetoacetate and acetyl-CoA[4] and (ii) the anabolic pathway from acetoacetyl-CoA and acetyl-CoA to mevalonic acid and thence to terpenoids[5] (Scheme 1). From this stems the intriguing question: 'is HMG-CoA, formed from leucine, incorporated into terpenoids intact or *via* prior breakdown to acetyl-CoA and acetoacetate?'

We have sought to answer this question for the incorporation of leucine into the sesquiterpenoids paniculides A (**2**) and B (**3**) by tissue cultures of *Andrographis paniculata* and here report our findings.

Experiment A demonstrates conclusively that HMG-CoA from leucine breakdown is not incorporated intact into paniculide. Leucine, labelled at C-2, will label C-1 of HMG-CoA and, if this is incorporated intact, C-5 of MVA and hence C-4, C-8, and C-12 of paniculide.

(2*RS*)-[2-^{13}C]Leucine was synthesised by the method of

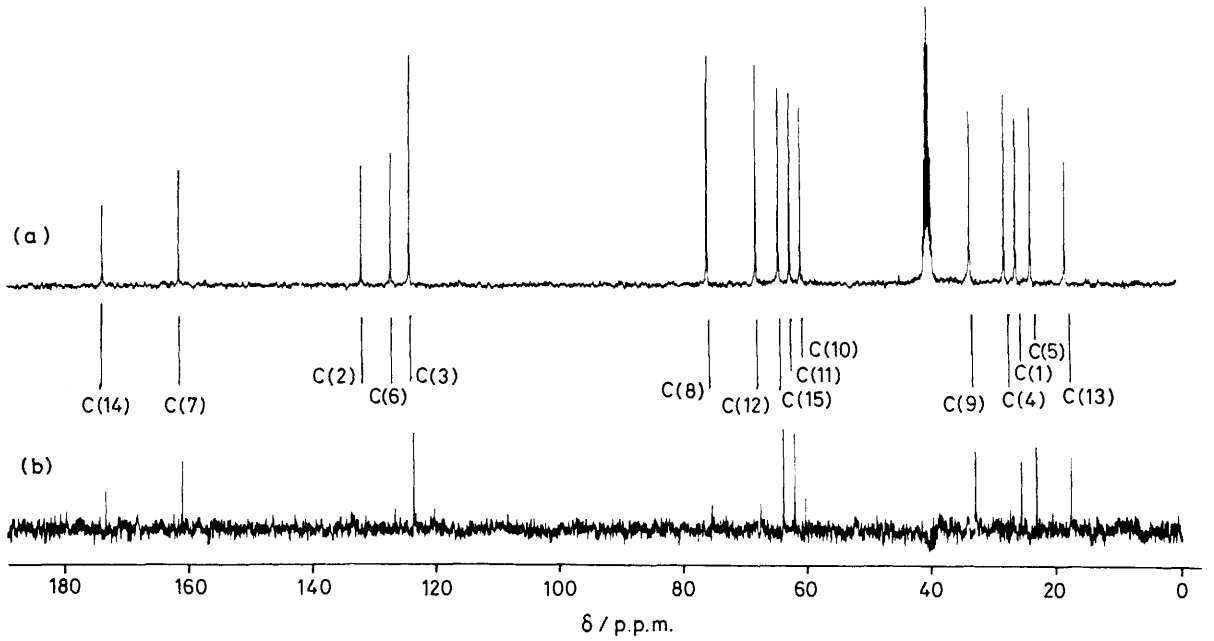
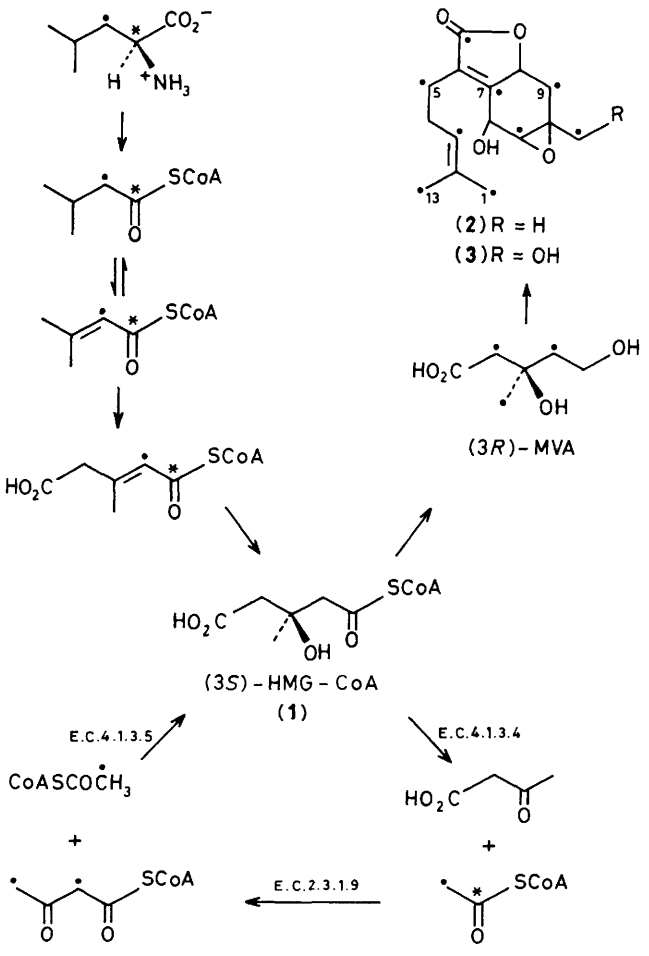


Figure 1. Labelling pattern of paniculide B derived from [3-^{13}C]leucine. (a) Natural abundance ^{13}C n.m.r. spectrum of paniculide B (**3**) [0.25 mmol in $(CD_3)_2SO$; 4000 scans; 90.65 MHz]; (b) ^{13}C n.m.r. difference spectrum of paniculide B labelled from [3-^{13}C]leucine — paniculide B at natural abundance [conditions as in (a)].



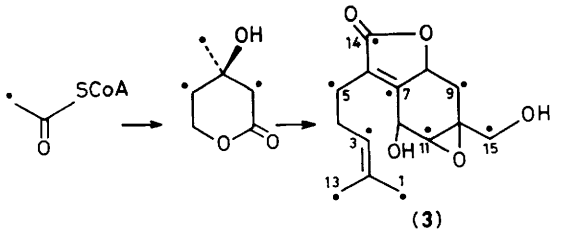
Scheme 1

Pichat[6] for [2-^{14}C]leucine. *Andrographis paniculata* was grown in suspension culture[7] (15 × 100 ml; 2.55 g freeze-dried weight of callus) for 21 days following transfer from solid medium and then the medium was replaced with fresh medium (1.5 l) containing (2*RS*)-[2-^{13}C]leucine (75 mg; 70 atom % ^{13}C) and (2*S*)-[U-^{14}C]leucine (1 μCi). After 7 days paniculide B (**3**) (34 mg; $I_{spec.}$[8] 15.5%) was isolated by preparative t.l.c. No peak enhancement whatever could be detected in the ^{13}C n.m.r. spectrum at 25.2 MHz of paniculide B or the derived diacetate (peak height normalisation with respect to unlabelled acetate carbons; calculated peak enhancement, from [U-^{14}C]leucine incorporation, approximately three-fold for each of three labelled carbons). In a parallel experiment, using modified culture conditions, label was incorporated solely into paniculide A (**2**) (18 mg; I_{spec} 28.3%). Again, no peak enhancement could be detected in the ^{13}C n.m.r. spectrum (calculated enhancement approximately six-fold for each of three peaks).

These results show unequivocally that HMG-CoA from leucine breakdown is not incorporated intact into paniculides. Clearly also in these experiments, label is not incorporated *via* [1-^{13}C]acetyl-CoA.† All the radioactivity from [U-^{14}C]leucine must therefore enter paniculide *via* acetoacetate.

Experiment B shows that leucine is incorporated into paniculide by breakdown to acetyl-CoA and acetoacetate and subsequent incorporation of these fragments via *HMG-CoA and MVA.* [3-^{13}C]- and [3-^{14}C]-Leucines were synthesised from [1-*C]isobutyric acid *via* reduction and displacement of isobutyl *p*-bromobenzenesulphonate with acetamidomal-

† The observed results require incorporation of *labelled* acetyl-CoA into acetoacetyl-CoA in Experiment B but not in Experiment A. This apparent discrepancy could be due to the differing ages of the cultures at the time of precursor administration (based on optimised conditions) in the two experiments. Thus acetyl-CoA acetyltransferase (E.C. 2.3.1.9) might function only in Experiment B at a time when *labelled* acetyl-CoA is available.



(3)

Scheme 2

onic ester, incorporating 51% of label from Ba^*CO_3.[9] *Andrographis* callus was grown in suspension culture[7] (700 ml) for 7 days following transfer from solid medium and then [3-^{13}C, 3-^{14}C]-leucine (87 mg; 90 atom %; 2.8 μCi) was added and growth continued for 16 days. Extraction of the callus (freeze-dried weight 6.5 g) afforded paniculide B [181 mg; I_{spec} (after two recrystallisations) 1.66%]. Comparison of the natural abundance spectrum (a) of paniculide B and the difference spectrum (b) (paniculide B labelled from [3-^{13}C]leucine – paniculide B at natural abundance) (Figure 1) clearly shows that [3-^{13}C]leucine uniformly labels (peak enhancement 18% for each of 9 peaks, calculated from I_{spec}) C-1, C-3, C-5, C-7, C-9, C-11, C-13, C-14, and C-15 of paniculide B, thus supporting breakdown to [2-^{13}C]acetyl-CoA prior to its incorporation† *via* [2,3′,4-$^{13}C_3$]MVA (Scheme 2). Incorporation of [U-^{14}C]leucine under identical conditions into paniculide B (I_{spec} 3.63%) suggests that the acetoacetate formed from C-4, C-5, and C-5′ of leucine must be incorporated about 2.5 times as efficiently into paniculide B as is acetyl-CoA (from C-2 and C-3 of leucine). This point is receiving more detailed attention.

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