Biosynthesis of the 3-Hydroxy-3-methylglutarate Portion of the Pyrrolizidine Alkaloid Dicrotaline

Alastair A. Denholm and David J. Robins*

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK

The necic acid portion of dicrotaline **1**, 3-hydroxy-3-methylglutaric acid **3**, is not formed specifically from acetate, mevalonate or 3-hydroxy-3-methylglutarate, but label from C-5 of isoleucine **4** is incorporated specifically into the methyl group of this necic acid.

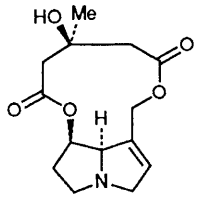
Pyrrolizidine alkaloids are widely distributed and show a range of structural types[1] and biological activities.[2] Many of the alkaloids, such as dicrotaline **1**, are macrocyclic diesters of (+)-retronecine **2**. Most of the esterifying (necic) acids are C_{10} diacids, which were first thought to be terpenoid in origin, but the high degree of oxygenation and unusual substitution patterns of these diacids indicated a different biogenesis. Experiments with ^{3}H- and ^{14}C-labelled precursors on the few necic acids that have been studied have shown that they are derived from common α-amino acids such as isoleucine **4**, its

biological precursor threonine, valine and leucine **6**. Thus senecic acid **5** is formed from two molecules of L-isoleucine with loss of both carboxy groups (Scheme 1).[3] In a similar fashion trichodesmic acid **7** is derived from leucine **6** and isoleucine **4**.[4] Dicrotaline **1** contains the simplest diacid found in these alkaloids, 3-hydroxy-3-methylglutaric acid **3** (HMG). HMG is a well known metabolite and (as its coenzymeA ester) is produced by condensation of three molecules of acetate on the pathway to mevalonate and terpenoid compounds. We now provide evidence that HMG in dicrotaline is not formed from the acetate pathway, and that isoleucine **4** is involved in its construction in a novel manner.

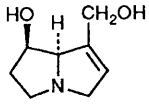
Dicrotaline **1** was first isolated from *Crotalaria dura* and *C. globifera* by Marais,[5] and was the first macrocyclic pyrrolizidine alkaloid to be synthesized.[6] Recently, dicrotaline **1** was found to be the major constituent of *C. lachnosema*.[7] Plants were grown from seeds of *C. lachnosema* supplied by Dr A. R. Mattocks, and one six-month-old plant was used for each feeding experiment. To begin this work, possible precursors of HMG, namely acetate and mevalonate, in ^{14}C-labelled form were fed together with the internal reference [1,4-^{3}H]putrescine (1,4-diaminobutane) dihydrochloride (^{3}H : ^{14}C ratio *ca.* 2) into *C. lachnosema* by the wick method.[8] Dicrotaline **1** was extracted[6] after ten days and purified by preparative TLC. Results (Table 1, expts. 1 and 3) show that acetate and mevalonate were incorporated into dicrotaline **1** much less efficiently than putrescine [a known precursor of (+)-retronecine **2**[9]]. Alkaline hydrolyses of these dicrotaline samples to give (+)-retronecine **2** and 3-hydroxy-3-methylglutaric acid **3** showed that putrescine predominantly labelled the (+)-retronecine portion (100–101% of the alkaloid activity in the base; 2–4% in the acid), whereas the ^{14}C-activity from acetate and mevalonate was spread between the acid and base portions (Table 1). Repetition of these experiments using ^{14}C-labelled precursors alone yielded similar results (Table 1, expts. 2 and 4). Next, feeding [3-^{14}C]-HMG to *C. lachnosema* gave a reasonable incorporation of ^{14}C with only a small increase in the ^{3}H : ^{14}C ratio (Table 1, expt. 5). However, hydrolysis of the labelled dicrotaline showed that the ^{14}C-activity was spread between the acid and base portions with more activity in the base portion. This experiment was repeated using only [3-^{14}C]-labelled material, producing a similar result (Table 1, expt. 6). Furthermore, Kuhn–Roth oxidation of dicrotaline obtained after feeding [3-^{14}C]-HMG afforded a sample of barium acetate containing only 5.2% of the total alkaloid radioactivity. This result shows clearly that HMG is not a specific precursor for the acid portion of dicrotaline, and that it is probably degraded to acetyl coenzymeA and acetoactate before incorporation into dicrotaline **1**.

The next set of precursors tested were the ^{14}C-labelled amino acids threonine, isoleucine, valine and leucine. The last two were poorly incorporated into dicrotaline with a large increase in the ^{3}H : ^{14}C ratio and the radioactivity was split between the acid and base portions (Table 1, expts. 7 and 8). The failure of leucine to label the HMG portion of dicrotaline **1** specifically is perhaps somewhat surprising, since 3-hydroxy-3-methylglutaryl coenzymeA is a known breakdown product of leucine. However, threonine and isoleucine were each efficiently incorporated into dicrotaline **1** with relatively small increases in the ^{3}H : ^{14}C ratios (Table 1, expts. 9 and 10). Moreover, basic hydrolyses of these dicrotaline samples demonstrated that most of the ^{14}C-activity was located in the acid portions. [In all four experiments with amino acids (Table 1, expts. 7–10), the ^{3}H-activity from putrescine was located mainly in the base portion (94–102%) with very little radioactivity in the acid moiety (2–5%).]

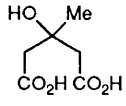
Isoleucine **4** has the same carbon skeleton as HMG and could be converted into HMG by a simple series of reactions involving removal of the amino group, oxidation of the C-5



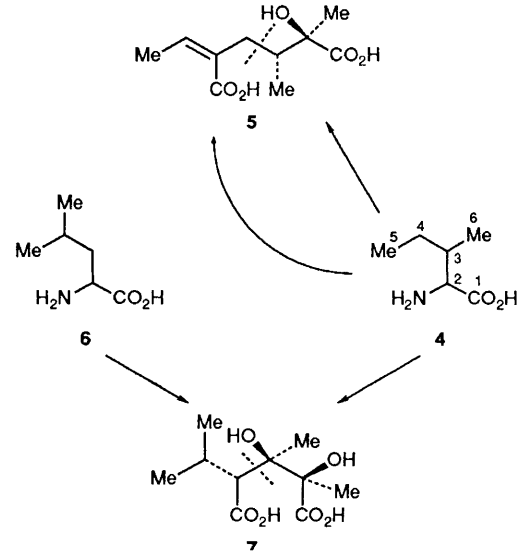
1



2



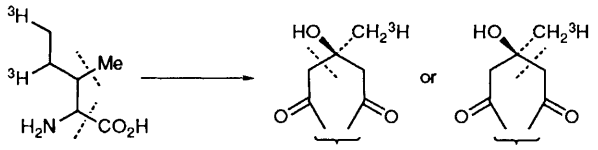
3



Scheme 1

Table 1 Incorporation of ^{14}C-labelled precursors into dicrotaline **1** in *C. lachnosema* plants with [1,4-^{3}H]putrescine as reference

Expt.	Precursor	^{3}H : ^{14}C ratio Start	^{3}H : ^{14}C ratio End	^{14}C Incorpn. (%)	^{14}C in base (%)	^{14}C in acid (%)
1	[1-^{14}C]acetate	1.88	30.5	0.02	60	45
2	[1-^{14}C]acetate	—	—	0.012	55	45
3	DL-[2-^{14}C]mevalonate	1.98	18.6	0.04	43	58
4	DL-[2-^{14}C]mevalonate	—	—	0.034	54	49
5	[3-^{14}C]-3-hydroxy-3-methylglutarate	1.9	3.55	0.40	56	42
6	[3-^{14}C]-3-hydroxy-3-methylglutarate	—	—	0.23	59	42
7	L-[U-^{14}C]valine	1.67	13.6	0.068	65	29
8	L-[U-^{14}C]leucine	1.66	10.9	0.079	49	49
9	L-[U-^{14}C]threonine	0.65	1.44	0.25	14	88
10	L-[U-^{14}C]isoleucine	1.61	3.09	0.26	5	94



Scheme 2

methyl group to a carboxy group and hydroxylation at C-3. However, there is another possibility for the incorporation of isoleucine into HMG. By analogy with the formation of the 'right-hand' halves of senecic acid **5** and trichodesmic acid **7**, isoleucine could provide a $MeC(OH)CH_2CO_2H$ portion of HMG with loss of the C-6 methyl group and carboxy group. In order to distinguish between these two possibilities, L-[4,5-^{3}H]isoleucine was administered to *C. lachnosema*. A reasonable total incorporation of 0.57% was observed (no allowance was made for any loss of ^{3}H). The labelled dicrotaline was subjected to a Kuhn–Roth degradation which yielded a sample of barium acetate containing 90.5% of the total alkaloid radioactivity. This result demonstrates that isoleucine is not converted into HMG by simple reactions not affecting the carbon skeleton. It is extremely unlikely that isoleucine undergoes the unprecedented rearrangement necessary to form HMG with C-4 or C-5 of isoleucine becoming the methyl group of HMG. The most likely explanation for this result is that incorporation of ^{3}H occurs from the C-5 position of isoleucine into the methyl group of HMG and loss of ^{3}H necessarily takes place from C-4 of isoleucine (Scheme 2). The carboxy group and the methyl group corresponding to C-6 of isoleucine must also be removed. Isoleucine would thus contribute four of the six carbon atoms of HMG as shown in Scheme 2. The origin of the remaining two carbon atoms in HMG has still to be determined.

We are grateful to Dr A. R. Mattocks, MRC Toxicology Unit, Surrey, for the gift of seeds of *C. lachnosema*, and we thank the SERC for a Studentship (to A. A. D.).

Received, 21st September 1990; Com. 0/04286B

References

1 D. J. Robins, *Nat. Prod. Rep.*, 1984, **1**, 235; 1985, **2**, 213; 1986, **3**, 297; 1987, **4**, 577; 1989, **6**, 221; 1989, **6**, 577.
2 A. R. Mattocks, *Chemistry and Toxicology of Pyrrolizidine Alkaloids*, Academic Press, London, 1986.
3 R. Cahill, D. H. G. Crout, M. V. M. Gregorio, M. B. Mitchell and U. S. Muller, *J. Chem. Soc., Perkin Trans. 1*, 1983, 173.
4 J. A. Devlin and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1984, 1329.
5 J. S. C. Marais, *Onderstepoort J. Vet. Sci. Anim. Ind.*, 1944, **20**, 61.
6 K. Brown, J. A. Devlin and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1819.
7 A. R. Mattocks and N. Nwude, *Phytochemistry*, 1988, **27**, 3289.
8 D. J. Robins and J. R. Sweeney, *J. Chem. Soc., Perkin Trans. 1*, 1981, 3083.
9 D. J. Robins, *Chem. Soc. Rev.*, 1989, **18**, 375.