Biosynthesis of Mavioquinone

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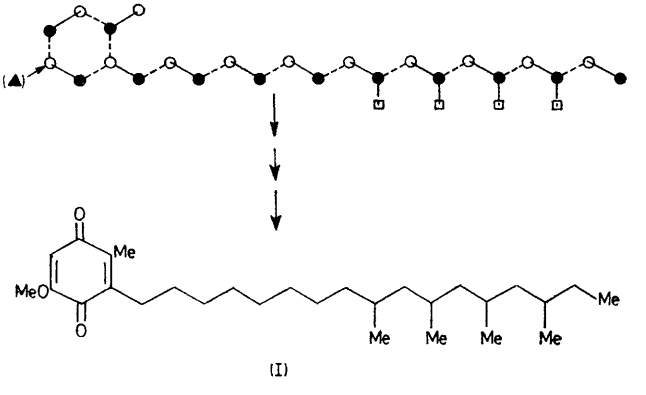
Summary The biosynthesis of mavioquinone in growing cells of *Mycobacterium avium* has been studied using ^{14}C-labelled methionine, acetate, and propionate.

Mycobacterium avium (CIP 802), grown in surface or submerged cultures, produces dihydromenaquinone-9[1,2] and mavioquinone (I).[1,3] It was of interest to compare the biosynthetic origin of mavioquinone to that previously demonstrated for dihydromenaquinones[4] and ubiquinones.[5]

Among the known radioactive precursors of menaquinones, only [$^{14}CH_3$]-L-methionine and, to a lesser extent, [1-^{14}C]- or [2-^{14}C]-acetate were significantly incorporated into mavioquinone (Table 1). Shikimic acid, a common precursor of the quinone ring of mena- and ubi-quinones,[6] and *p*-hydroxybenzaldehyde, a specific precursor of ubiquinones,[5] were not incorporated.

The localization of the label from [$^{14}CH_3$]-L-methionine in mavioquinone was shown by three methods: Zeisel degradation, followed by isolation of MeI (in the form of Ph_3MePI), exchange of the *O*-methyl group by treatment of



(I)

●—O : $MeCO_2H$
D-●—O : $MeCH_2CO_2H$
(▲) : Me from L-methionine

SCHEME

TABLE 1. Incorporation of radioactive precursors into mavioquinone and dihydromenaquinone-9 during growth of *M. avium*[a]

	Mavioquinone			Dihydromenaquinone-9		
	μmol	d.p.m.	d.p.m./μmol	μmol	d.p.m.	d.p.m./μmol
[$^{14}CH_3$]-L-Methionine[b]	8	12840	1605	25	35500	1420
	23	17000	716	61	38000	613
	10	12160	1216	32	44000	1382
(±)-[1,2-^{14}C]Shikimic acid[c] ..	15·3	1200	78	23	25200	1018
[*U*-^{14}C]-*p*-Hydroxybenzaldehyde[d]	12	600	50	30	—	—
Sodium [1-^{14}C]acetate[e]	10	8180	818	27	7530	290
	20	8960	448	50	—	—
Sodium [2-^{14}C]acetate[f]	16	4344	724	33	17960	543
	15	6000	400	19	4630	244
[2-^{14}C]-DL-mevalonic acid[g]	8	624	78	28	840	30

[a] Surface culture, 10—15 days at 37 °C on Sauton's medium containing the indicated precursor in the following amounts; [b] 20 μCi/l; [c] 5 μCi/l; [d] 11 μCi/l; [e] 25 μCi/l; [f] 12·5 μCi/l; [g] 25 μCi/l.

the quinone with BF_3–Et_2O in anhydrous MeOH, followed by recovery of unlabelled quinone as its dihydrodiacetate[3] or dibenzylphosphite adduct,[7] and oxidation with $KMnO_4$ in pyridine and recovery of side chain-derived acids.[3] Consistent results were obtained by the three methods indicating that > 75% of the radioactivity from [$^{14}CH_3$]-L-methionine was incorporated into the *O*-methyl group of mavioquinone, the 25% remaining being randomized in several carbon atoms of the side chain and the ring. The specific incorporation of the methyl group of methionine into the *O*-methyl group of mavioquinone could also be demonstrated by analysis of the mass spectrum of dihydromavioquinone diacetate obtained by reductive acetylation of deuteriated mavioquinone isolated from a growth medium containing [C^2H_3]-L-methionine; the parent peak (M^+ 532) and the main fragmentation peaks at *m/e* 490 ($-CH_2$=CO), 448 ($-2 \times CH_2$=CO), 209, and 167 (OMe-containing fragments) were accompanied by *m*+3 peaks (15—19%) in contrast to the demethoxy fragment at *m/e* 153.

The absence of incorporation of the methyl group of methionine in the ring C-methyl group, and the failure to incorporate common aromatic precursors into the ring carbon atoms suggest an acetate–polymalonate pathway for the biosynthesis of mavioquinone, as proposed for mould toluquinones[8] and probably operating for benzoquinones containing long alkyl side chains (embelin, rapanone[9] *etc.*). The very low yields of incorporation of labelled acetates into mavioquinone have precluded any degradation experiment.

TABLE 2. Incorporation of [3-^{14}C]propionic acid[a] into mavioquinone and dihydromenaquinone-9 of *M. avium*.

	Mavioquinone			Dihydromenaquinone-9		
μCi	μmol	d.p.m.	d.p.m./μmol	μmol	d.p.m.	d.p.m./μmol
25	36	1,070,000	29,700	56	38,700	692

[a] Specific activity: 4·7 μCi/μmol; total culture volume: 2 l.

In contrast, [^{14}C]propionic acid was actively incorporated (Table 2). The specific activities of the branched acids derived from the side chain of mavioquinone (Table 3) indicate that at least 75% of the radioactivity incorporated from [3-^{14}C]propionate was found in the branched part of the side chain; the higher specific activity of longer acids indicates some randomization which seems to exclude any specific incorporation of the remaining radioactivity in the ring part of mavioquinone. When the mixture of branched

TABLE 3. Incorporation of sodium [3-^{14}C]propionate into the main tetramethyl branched acids derived from mavioquinone by $KMnO_4$ oxidation.[a]

Carbon atoms number	μmol	^{14}C Specific activity d.p.m./μmol	% of mavioquinone specific activity
C_{22}	I, 1·20	2332	87
	II, 1·15	2994	96
C_{21}	I 1·13	2358	88
	II, 0·84	3172	102
C_{20}	I, 0·60	2227	83
	II, 0·35	3158	101
C_{19}	I, 0·49	2210	82
	II, 0·26	3038	97
C_{18}	I, 0·30	2191	82
	II, 0·09	2729	87
C_{17}	I, 0·19	2105	78
	II, 0·05	3016	97
C_{16}	I, 0·11	2009	75
	II, 0·07	2309	74

[a] Specific activity of mavioquinone 2685 (expt. I) or 3122 d.p.m./μmol (expt. II). Total methyl esters were reduced in ether with $LiAlH_4$ then acetylated with [$^3H_6$]acetic anhydride (18,895 d.p.m./μmol). Individual acetates were separated by g.l.c. on a SE30 column at 180 °C, collected, and counted for ^{3}H and ^{14}C. ^{3}H labelling was used for quantization of each acid.

acids was degraded by the Kuhn–Roth method, nearly all the radioactivity was recovered in acetic acid.

These results, together with the structural features of mavioquinone, strongly suggest a biogenetic sequence which involves an acetate–methylmalonate–malonate condensation utilizing acetate as a starter unit (Scheme). It is to be noted that other methylmalonate-derived compounds, such as mycocerosic or phthienoic acids and phthiocerol,[10] which are commonly found in most mycobacteria, seem to be absent in *M. avium*.[11] Most of these compounds contain propionate unit(s) in the terminal position; however at least one of them, phthiocerol B,[12] has been shown to contain a terminal group indicating the final incorporation of acetate rather than propionate. Another mycobacterial product, β-leprosol, found in the lipid extract of *M.leprae* and identified as a mixture of monomethyl ethers of 5-n-heptadecyl- and 5-n-pentadecyl-4,6-dimethylresorcinol,[13] probably constitutes a prototype for the biogenetic precursor of mavioquinone. More conclusive proof for the suggested biogenetic pathway to mavioquinone is expected from results for the incorporation of [^{13}C]acetate and [^{13}C]-propionate.

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