Mavioquinone, A New Quinone from *Mycobacterium avium*

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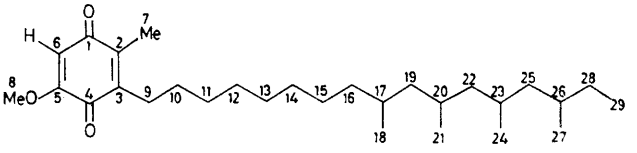
Summary Mavioquinone, a new quinone isolated from lipid extracts of *M. avium*, has been shown to be the 5-methoxy-2-methyl 3-(9,11,13,15-tetramethylheptadecyl)-1,4-benzoquinone (I).

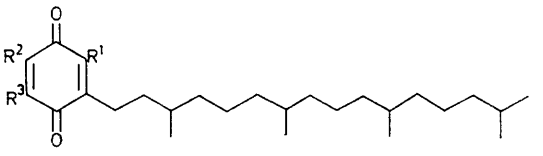
SEVERAL years ago, during investigations of the occurrence of menaquinones in Myco- and Coryne-bacteria, Beau *et al.*[1] reported the isolation of small amounts of a new benzoquinone, exclusively found in *Mycobacterium avium*, and subsequently named mavioquinone. This quinone is easily obtained, together with dihydromenaquinone-9,[2] by acetone extraction of wet cells and fractionation of the lipid material by silica-gel chromatography [yield: 10 mg/100 g wet bacteria, from its characteristic u.v. absorption: λ_{max} (cyclohexane) 269 nm; ϵ 15,150]. Mavioquinone has now been shown to possess structure (I).

Mass spectrometry established a molecular formula of $C_{29}H_{50}O_3$ corresponding to a benzoquinone substituted by a methoxy and a methyl group, plus a saturated hydrocarbon side chain of 21 carbon atoms (M^+ 446, main fragments at *m/e* 167, 153, and 138). Similar results were obtained from the dihydro-diacetate (M^+ 532, main fragments at *m/e* 490, 448, 209, 167 and 151—153). The nature of substituent groups was confirmed by the n.m.r. spectra: singlets at δ 5·89 (1H, quinone) or 6·45 (diacetate), 3·80 (OMe, quinone) or 3·75 (diacetate), and 2·05 (Me, quinone) or 2·0 (diacetate). A doublet of 15 $\pm$ 2 protons in the region δ 0·8—0·9 indicated the presence of 5 $\pm$ 1 methyl groups in the side chain.

The localization of substituents on the quinone ring was determined by comparison of n.m.r. and u.v. spectra of mavioquinone and model dihydrophytyl quinones (II) (synthesised by phytylation of the three different methoxymethyl-1,4-benzohydroquinones[3] followed by catalytic hydrogenation of the side chain, oxidation to quinones with Ag_2O, and chromatographic separation of products).



(I)



(II)

	R^1	R^2	R^3
a;	Me	H	OMe
b;	Me	OMe	H
c;	H	Me	OMe
d;	H	OMe	Me
e;	OMe	H	Me
f;	OMe	Me	H

TABLE 1. Characteristic fragmentation patterns observed in the mass spectra of some individual methyl esters of the homologous acids obtained by $KMnO_4$ oxidation of mavioquinone. G.l.c. on SE-30 3% at 100—250 °C. Mass spectrometry: ion source 230 °C; 70 eV.

Carbon number	$Me[CH_2CHMe]_4[CH_2]_nCO_2Me$ *n*	M^+	Base peak *m/e*	Main fragments *m/e* (% of base peak)
C_{22}	8	354	354	74(42);[a] 87(21), 101(3), 129(8), 143(17), 157(6);[b] 171(8);[b,c] 213(12), 255(9), 297(3);[c] 199(17), 241(20), 283(13), 325(3);[d] 167(12), 209(13), 251(6), 293(1);[e] 311(7), 354(3).[f]
C_{21}	7	340	340	74(73);[a] 87(25), 101(3), 115(4), 129(14), 143(25);[b] 157(10);[b,c] 199(14), 241(14), 283(3);[c] 185(18), 227(20), 269(15), 311(4);[d] 153(17), 195(17), 237(6);[e] 297(7).[f]
C_{18}	4	298(5)	74[a]	87(33), 101(6);[b] 115(16);[b,c] 157(16), 199(8), 241(4);[c] 143(25), 185(13), 227(9), 269(2);[d] 111(35), 153(30), 195(9);[e] 222(78);[g] 266(7).[g]
C_{17}	3	284(10)	57[h]	74(48);[a] 87(17);[b] 101(12);[b,c] 143 (19), 185(5);[c] 129(33), 171(11), 213(9);[d] 97(33), 139(17), 181(7);[e] 208(7);[g] 241(8).[f]
C_{16}	2	270(3)	87[b]	74(24);[a] 129(6), 171(6);[c] 115(11), 157(6);[d] 83(21), 125(24);[e] 213(2).[f]
C_{15}	1	256(4)	101[d]	74(70);[a] 115(10), 157(10);[c] 143(20);[d] 69(24), 111(26);[e] 199(9).[f]
C_{14}	0	242(9)	88[i]	101(60), 143(2);[c] 129(6), 171(4);[d] 97(20), 139(7);[e] 185(2);[f] 152(3).[j]

[a] MacLafferty rearrangement ion $CH_2=C(OH)OMe^+$. [b] Fragments corresponding to $^+[CH_2]_nCO_2Me$ ions. [c] Methyl ester-containing ions $^+[CH_2CH(Me)]_m-[CH_2]_nCO_2Me$ resulting from a prebranching fragmentation (m = 0, 1, 2, or 3). [d] Methyl ester-containing ions $^+[CH(Me)CH_2]_m-[CH_2]_nCO_2Me$ resulting from a postbranching fragmentation (m = 1, 2, 3, or 4). [e] Ions resulting from ions in footnote d by loss of the MeOH unit. [f] Rearrangement ions arising through elimination of a branched part of the chain: $[-CH(Me)-CH_2- + H] \rightarrow M - 43$ or $[-CH(Me)-CH_2-CH_2- + H) \rightarrow M - 57$. [g] $M - 76$ ions resulting from a sequential elimination of MeOH and $-CH_2=CHOH$ units involving rearrangement of an activated C-6 hydrogen.[6] [h] Particularly prominent hydrocarbon ion, together with others not quoted in the table, and corresponding to $MeCH_2[CH(Me)CH_2]_m-[CH_2]_n^+$ ions. [i] MacLafferty rearrangement ion involving an α-methyl group: $MeCH=C(OH)OMe^+$. [j] $M - 90$ rearrangement ion arising as in footnote g through successive elimination of MeOH and MeCH=CHOH units, involving an α-methyl group and characteristic of 2,6-dimethyl methyl esters.[6]

Among the six possible isomers, only 2-dihydrophytyl-6-methoxy-3-methylbenzoquinone (IIa) had similar properties to mavioquinone, as follows. (i) Their chromatographic migration on silica-gel plates was nearly identical in several solvent systems, (ii) the single quinone hydrogen appeared as a singlet in their n.m.r. spectra, corresponding to a position *ortho*- to the OMe group, (iii) the weak u.v. absorption band of the two quinones in the 300—400 nm region was centred at 351 nm [347 nm for (IIb)], and (iv) the respective hydroquinone diacetates absorbed at 274 (shoulder) and 279 nm while the corresponding derivative of (IIb) showed characteristic peaks at 274, 278, and 281 nm.

TABLE 2. ^{13}C chemical shifts of mavioquinone (I).

Assignments	Chemical shifts	
	Measured[a]	Calculated[b]
C-1, C-4	182·04—189·76	—
C-2, C-3	141·16—143·20	—
C-5	158·41	—
C-6	107·04	—
C-7	12·15	—
C-8	56·02	—
C-9, C-10, C-11	26·32—28·60—28·92	—
C-12, C-13	30·02	29·96
C-14[c]	29·83	30·21
C-15	26·84	27·27
C-16	36·59	37·16
C-17[c]	29·63	30·45
C-18	20·54	20·12
C-19	45·23	44·11
C-20, C-23	27·52	28·38
C-21, C-24	21·06	20·61
C-22	45·82	44·36
C-25	44·74	43·86
C-26	31·58	32·52
C-27	20·02	19·63
C-28	29·44	29·85
C-29	11·18	10·87

[a] In p.p.m. downfield from internal Me_4Si (solvent $CDCl_3$). [b] From Lindeman and Adams' equation (ref. 7). [c] Assignments possibly inverted.

The unusual side chain structure was elucidated by degradation and natural abundance ^{13}C-n.m.r. spectroscopy. Oxidation of mavioquinone by $KMnO_4$ in pyridine[4] afforded a series of C_{14}—C_{22} homologous aliphatic branched acids which were separated and identified as their methyl esters by g.l.c. (on several polar and non polar phases) combined with mass spectrometry; the data in Table 1, particularly for the lower members of this series, show all the prominent features expected for tetramethyl branched methyl esters[5,6] substituted in the ω-2, ω-4, ω-6, and ω-8 positions.

Natural abundance ^{13}C-n.m.r. spectra confirmed the proposed structure; the ^{13}C assignments in Table 2 for the different carbon atoms of the distal part of the side chain entirely agree with the chemical shifts predicted on the basis of Lindeman and Adam's equation[7] developed for C_5—C_9 paraffins.

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