STRUCTURE OF THE DIHYDROMENAQUINONE-9 of *Mycobacterium phlei*[x].

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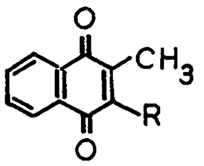
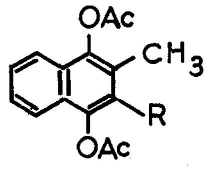
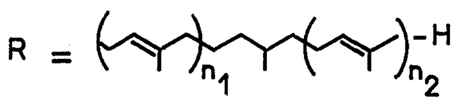
Gale *et al.* (1963) have shown that the vitamin K_2 of *M. phlei* is a dihydromenaquinone-9[xx] (I) (n_1, $n_2 > 0$). The molecular weight of this compound ($M^+ = 786$) and of the corresponding hydroquinone diacetate (IV) ($M^+ = 872$) have been confirmed by mass spectrometry (Lederer, 1964; Beau *et al.*, 1966) and we have shown recently that all of the strains of Mycobacteria examined contain the same MK-9(H_2) (Beau *et al.*, 1966); Corynebacteria contain the lower isoprenolog MK-8(H_2)(II)(Scholes and King, 1965; Beau *et al.*, 1966).







(I) : $n_1 + n_2 = 8$
(II) : $n_1 + n_2 = 7$

(IV) : $n_1 + n_2 = 8$
(V) : $n_1 + n_2 = 7$

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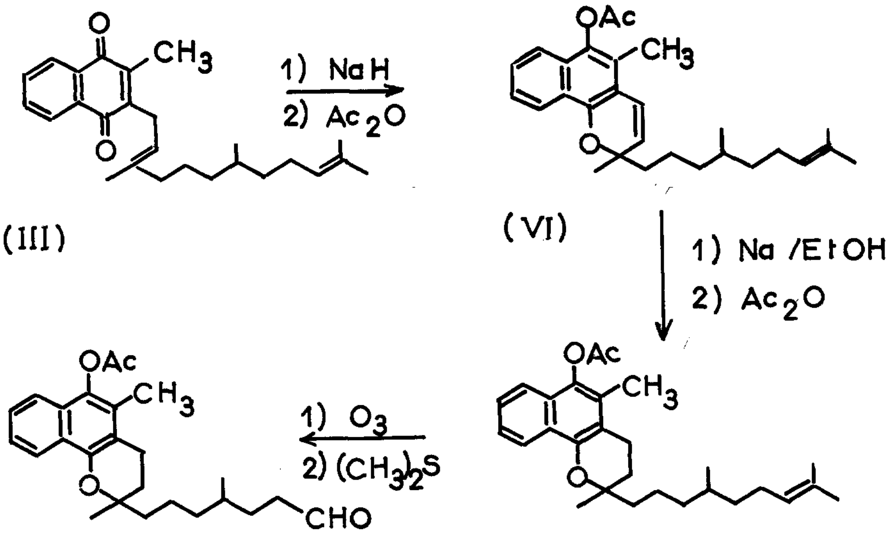
[xx] Nomenclature and abbreviations as recommended by the I. U. P. A. C., I. U. B. Committee (Biochim. Biophys. Acta, 1965, 107, 5).

Mass spectra of all these compounds showed, after loss of the terminal isoprene unit (C_5H_9= 69 mass units), six (I, IV) or five (II, V) successive losses of 68 mass units (C_5H_8): we thus proposed that the saturation in the side chain was in the second isoprene unit starting from the quinone ring (I : n_1=1 , n_2=7) (Lederer, 1964).

We now report confirmation of this structure, obtained by degradation and synthesis.

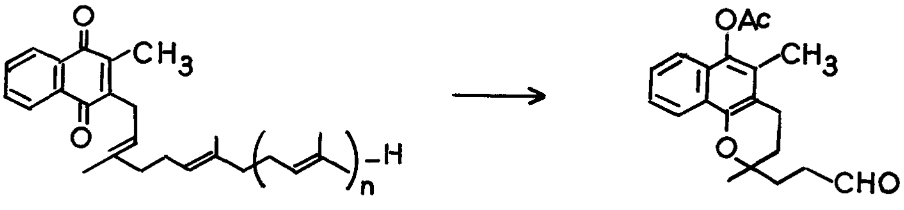
Synthetic II-dihydromenaquinone-3[xxx](III)(Azerad and Cyrot, 1965) was cyclized by sodium hydride treatment and the chromenol acetylated to the I-dihydromenachromenyl-2 acetate (VI) (Wagner et al., 1963); then the chromenyl acetate (VI) was reduced by sodium in ethanol (Schudel et al., 1963) to the I-dihydromenachromanyl-2 acetate (VII). This was



[xxx] see [xx]: "a) the isoprene units are designated by Roman numerals (I, II, III, etc. .) starting from the quinone or chroman nucleus.
b) additional hydrogenations are indicated by the prefixes dihydro-, tetrahydro-. . with the Roman numerals of the units which are reduced, e. g. II-dihydro. . , I, II, III, hexahydro. .
c) these abbreviations may be abbreviated to II-H_2, I, II, III-H_6, etc. . "

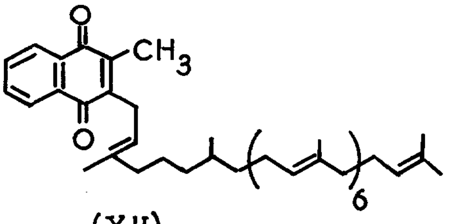
ozonized and the ozonide reduced by dimethylsulfide (Pappas et al., 1966) to give the aldehyde (VIII) which was characterized by its UV, IR, NMR (-C$\underline{H}$O at 9,76 ppm) and mass spectra (molecular ion at m/e 396).

The same sequence of reactions applied to synthetic MK-3(IX) or MK-9(X) (Noll et al., 1960) led exclusively to the lower isoprenolog (XI) (molecular ion at m/e 326) which differed significantly from (VIII) also by its NMR spectrum. Moreover (VIII) and (XI) were easily separated by analytical or preparative TLC.



(IX): $n=1$
(X) : $n=7$

(XI)



(XII)

The same sequence of reactions (cyclisation to the chromene, reduction to the chromane, ozonization) when applied to natural MK-9(H_2) extracted from M. phlei, afforded in good yield an aldehyde identical to (VIII); no trace of (XI) could be detected by TLC in several ozonization experiments.

These results establish the structure of dihydromenaquinone-9 of M. phlei as II-dihydromenaquinone-9 [MK-9(II-H_2)] (XII).

We are examining the side-chain of dihydromenaquinones from other Myco- and Corynebacteria; similarities of their mass spectra already suggest an analogous structure. Biosynthetic implications have to be considered in connection with the problem of side-chain biosynthesis in the bacterial menaquinones (Azerad *et al.*, 1967).

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