COENZYME Q. XLIX. CHARACTERIZATION OF COENZYME Q_{10}(H-10) FROM PENICILLIUM STIPITATUM

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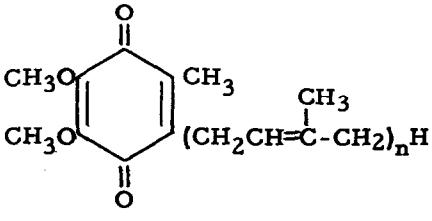
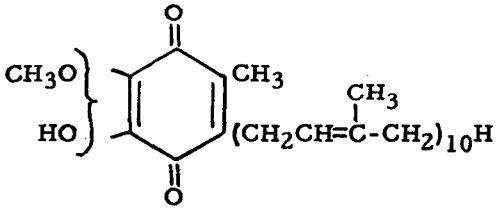
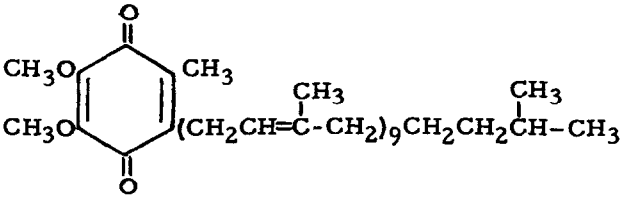
Microorganisms are a source of all naturally-occurring homologs of the coenzyme Q series (coenzyme Q_6-coenzyme Q_{10}) (I) (Lester et al., 1958; Morton et al., 1958; Linn et al., 1959). In addition, two newer analogs of coenzyme Q_{10}, rhodoquinone (II) (Glover et al., 1962) and coenzyme Q_{10}(H-10) (III) (Gale et al., 1963A) have been recently isolated from microbial species. We have now found that a previously reported tetrahydro derivative of coenzyme Q_{10} (Lavate et al., 1962) is in reality a dihydro derivative which is identical with coenzyme Q_{10}(H-10); the supporting data follow.



I Coenzyme Q_{10}, n = 10



II Rhodoquinone



III Coenzyme Q_{10}(H-10)

The isolation from cells of Penicillium stipitatum NRRL 2104 of an analog of coenzyme Q_{10} in which two of the isoprenoid units appeared to be reduced has been reported (Lavate et al., 1962); the similarity of the melting point of this apparent tetrahydro-derivative and that of coenzyme Q_{10}(H-10) prompted us to isolate and examine the specific coenzyme Q from this strain of P. stipitatum.

A portion of moist mycelium obtained from a 50-gal. fermentation was saponified in the presence of pyrogallol, and the crude lipid was purified using chromatography on Decalso, acid-washed alumina, and silica gel (Crane et al., 1959; Lester et al., 1959). The orange, crystalline product, m.p. 28.5-29.5°, and its hydroquinone gave ultraviolet spectra in ethanol characteristic for coenzyme Q_{10}(H-10).

Comparison of the nuclear magnetic resonance spectrum of this quinone with the spectrum of coenzyme Q_{10}(H-10) from Gibberella fujckuroi established the identity of the two compounds. A similar comparison of the NMR spectrum of the original product, kindly furnished by Dr. R. Bentley (Lavate et al., 1962), and the spectrum of coenzyme Q_{10}(H-10) showed the presence of a single and terminal saturated isoprenoid unit in the quinone from cells of P. stipitatum, and their identity.

The original specimen of the coenzyme Q from Dr. Bentley, coenzyme Q_{10}(H-10) from cells of G. fujckuroi, and the coenzyme Q_{10}(H-10) we obtained from cells of P. stipitatum in our laboratory exhibited the same mobility on a reversed-phase papergram (Gale et al., 1963 B).

Experimental

Isolation of Coenzyme Q_{10}(H-10) from the Cells of Penicillium stipitatum. - A culture of the mold, Penicillium stipitatum NRRL 2104, was grown in a 50-gal. stainless steel fermentor using normal rates of agitation and air-flow. The nutrient medium contained 2% dextrose, 1.5% yeast extract, and mineral salts. After incubation for 100 hr., the cells were harvested. The moist mycelium weighed 5.1 kg. We are grateful to Dr. H. Boyd Woodruff for this mycelium.

To a suspension of 558 g. of mycelium in 3.5 l. of 50% ethanol was added 70 g. of pyrogallol and 200 g. of sodium hydroxide. The mixture was heated at reflux temperature for 1 hr., and after cooling, was diluted with 3 l. of ethanol and extracted four times with 2-l. volumes of hexane. The combined hexane extracts were washed four times with water. Evaporation of the solvent yielded 1.74 g. of orange material. After partial removal of steroidal contaminants by crystallization from isooctane at 0°, the crude lipid was purified using chromatography on columns of sodium alumino-silicate, 50 mesh and finer (Decalso), acid-washed alumina (Merck), and silica gel, desiccant grade, 100-200 mesh (Davison). Eluate fractions from final chromatography on silica gel yielded orange crystals from cold ethanol. After recrystallization, 13.4 mg. of orange crystalline product, m.p. 28.5-29.5°, was obtained.

Characterization of Coenzyme Q_{10}(H-10) from P. stipitatum.- An ethanol solution of the crystalline material gave an ultraviolet spectrum, $\lambda_{max.}$ 275 mμ., $E^{1\%}_{1\,cm.}$ 169. After treatment with sodium borohydride, the maximum shifted to 290 mμ., $E^{1\%}_{1\,cm.}$ 31, $\Delta E^{1\%}_{1\,cm.}$ (275 mμ.) = 139.

The nuclear magnetic resonance spectrum of this compound was compared with that of coenzyme Q_{10}(H-10). Visual inspection of their spectra established the identity of the two compounds.

Comparison of Mobilities of Coenzyme Q_{10}(H-10) from Different Sources. - Samples of coenzyme Q_{10}(H-10) isolated in our laboratory from G. fujckuroi and P. stipitatum, and the crystalline coenzyme Q obtained from cells of P. stipitatum by Dr. Bentley, and coenzyme Q_{10} were papergrammed on Vaseline-impregnated Whatman No. 1 paper, with 98% dimethylformamide (Merck, reagent), saturated with Vaseline as mobile phase. R_f values of 0.42, 0.42, 0.41, and 0.53 were obtained for the respective samples.

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