COENZYME Q. XXIX. MONOPHOSPHATE OF DIHYDROCOENZYME Q_{10}

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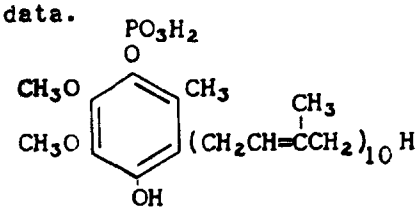
A monophosphate of dihydrocoenzyme Q_{10} has been obtained by the reaction of the hydroquinone form of coenzyme Q_{10} with phosphorus oxychloride followed by chromatographic isolation of the product.

There is continuing interest in a possible role for quinones in biochemical phosphorylation mechanisms. The quinones of both the vitamin K group and the coenzyme Q group appear to be involved; a new review on the mechanisms of synthesis of adenosine triphosphate is timely (Racker, 1961). Although interest has been focused on semiquinone phosphates, interpretations have also included phosphorylated derivatives of the corresponding 6-chromanols, particularly from data in the vitamin K group (Russell and Brodie, 1961; Dallam, 1961).

The extensive biochemical interest in the participation of quinones in oxidative phosphorylation has led to a study of the synthesis of some hydroquinone monophosphates and their non-enzymatic oxidative phosphorylating behavior (Clark, Kirby and Todd, 1958; Wieland and Pattermann, 1958, 1959). A particularly interesting account of the synthesis of quinol monophosphates and related experiments on oxidative phosphorylation included a description of the reaction of ubiquinone (50) (coenzyme Q_{10}) with dibenzylphosphite to give ubiquinol-(50)-mono-(dibenzylphosphate). However, formation of the free quinol monophosphate from this dibenzyl derivative required hydrogenolysis with a Lindlar catalyst which cleaved the benzyl groups and was presumed to not even partially reduce the unsaturated iso-

prenoid side chain (Andrews, 1961). We have found that coenzyme Q_{10} absorbs some hydrogen with the Lindlar catalyst over that required for the quinone functionality.

Our study of the direct reaction of the hydroquinone form of coenzyme Q_{10} with phosphorus oxychloride has yielded a product on chromatographic purification which has the properties of the monophosphate of dihydrocoenzyme Q_{10}; this direct procedure avoids the use of a hydrogenation step for a compound with considerably unsaturation. This monophosphate (I) may consist primarily of one isomer. If the other isomer is also present in the product, its presence is not evident by present data.



I (provisional assignment of phosphate group)

The phosphate of dihydrocoenzyme Q_{10} contained one equivalent of phosphate by a colorimetric method (King, 1932). The ultraviolet absorption spectrum was determined: $\lambda_{max}^{isooctane}$ 280 mμ ($E_{1\ cm.}^{1\%}$ 25). Paper strip chromatography showed no contamination with dihydrocoenzyme Q_{10} or coenzyme Q_{10}. Both alkaline hydrolysis in the presence of pyrogallol and oxidative dephosphorylation (using either silver oxide or ferric chloride) of the phosphate gave evidence for the regeneration of coenzyme Q_{10} as determined by paper strip analysis. Hydrochloric acid hydrolysis also gave evidence for the regeneration of coenzyme Q_{10} in small quantity.

Adsorption of a good sample of the monophosphate of dihydrocoenzyme Q_{10} in isooctane on both Florisil and Decalso columns resulted in some decomposition and regenerated coenzyme Q_{10} in small quantity; 3 mg. from 180 mg. of the monophosphate. Further, the main fraction recovered was the monophosphate of dihydrocoenzyme Q_{10} to-

gether with at least seven other products as separated by paper strip chromatography. The Q_{10} was eluted with the appropriate solvent mixture (10% ether in isooctane). Paper strip chromatography showed the small amount of orange oil to be mainly coenzyme Q_{10}; a small amount (180γ) of this material was chromatographed on a thin film of silica gel on a glass plate using as solvent mixture, isooctane-benzene-acetone (49:49:2). Using pure coenzyme Q_{10} as a control (R_f 0.54), the regenerated coenzyme Q_{10} had R_f 0.54. The spots of regenerated coenzyme Q_{10} were scraped from the plate and eluted with ether. Evaporation of the ether left a small amount of coenzyme Q_{10}, $\lambda_{max}^{isooctane}$ 270 mμ.

The phosphate is unstable. After standing at room temperature for about a week, NMR analysis showed the appearance of paraffini contamination. Such material has become insoluble in the non-polar solvents such as ether, isooctane and carbon tetrachloride. This change may be rationalized by cyclization of the isoprenoid side chain (Shunk et al., 1960).

This monophosphate showed no coenzymatic activity in the succinoxidase system, according to Dr. David Green. However, this phosphate may be of greater interest to study in phosphorylating systems to further appraise one of the several organic mechanisms of ATP biosynthesis.

Monophosphate of dihydrocoenzyme Q_{10}. One gram of coenzyme Q_{10} was reduced to dihydrocoenzyme Q_{10} by shaking an ether solution with aqueous sodium hydrosulfite. The ethereal solution was washed well with water, dried over anhydrous magnesium sulfate and concentrated under reduced pressure to leave nearly colorless dihydrocoenzyme Q_{10}. This residue was dried by the azeotopic distillation of benzene. To the 1.0 g. of dihydrocoenzyme Q_{10} dissolved in 50 ml. of dry pyridine, was added in 45 minutes one equivalent, 0.1 ml

of freshly distilled phosphorus oxychloride in 25 ml. of dry benzene. The solution was stirred at room temperature for three hours. The solvents were removed under reduced pressure to leave an oily crystalline residue. Ether and some cold dilute hydrochloric acid (ca. 1N) were added. After one-half hour, the layers were separated by the help of centrifugation. The ether layer was washed once with cold water; the layers were again separated by centrifugation to break emulsions. The ether solution was concentrated under reduced pressure to an oily residue which was dried by azeotropic distillation of benzene. The crude reddish colored residue weighed 0.96 g.

A chromatographic column was prepared with 20 g. of silica gel (100-200 mesh) and distilled isooctane. The residue in isooctane was placed on the column. Elution with isooctane followed by evaporation of the eluate yielded 0.56 g. of the monophosphate of dihydrocoenzyme Q_{10}. Eluting the column with ether removed 0.23 g. of coenzyme Q_{10}.

One-half gram of the monophosphate of dihydrocoenzyme Q_{10} was dissolved in isooctane and treated with a decolorizing charcoal, Darco G-60. The charcoal was removed by gravity filtration. The filtrate was placed on a second chromatographic column which was prepared with 10 g. of silica gel and isooctane. Elution with isooctane and evaporation of the eluate yielded 0.44 g. of nearly colorless monophosphate of dihydrocoenzyme Q_{10}. A sample was dried *in vacuo* at room temperature, and then at 80° for analysis.

Anal. Calcd. for $C_{59}H_{93}O_7P$: C, 74.96; H, 9.91; P, 3.27; CH_3O, 6.5. Found: C, 75.26; H, 10.21; P, 3.0; CH_3O, 6.6.

REFERENCES

Andrews, K. J. M., J. Chem. Soc., 1808 (1961).

Clark, V. M., Kirby, G. W., and Todd, A., Nature, 181, 1650 (1958).

Dallam, R. D., Biochem. Biophys. Research Comm., 4, 106 (1961).

Green, D. E., Private Communication. Institute for Enzyme Research, The University of Wisconsin, Madison, Wisconsin.

King, E. J., Biochem. J., 26, 292 (1932).

Racker, E., Advances in Enzymology. Vol. 23: 323 (1961). Interscience Publishers, Inc., New York.

Russell, P. J., Jr., and Brodie, A. F., Biochem. Biophys. Acta, 50, 76 (1961).

Shunk, C. H., Trenner, N. R., Hoffman, C. H., Wolf, D. E., and Folkers, K., Biochem. Biophys. Research Comm., 2, 427 (1960).

Wieland, T., and Pattermann, F., Angew. Chem., 70, 313 (1958); Ber. 92, 2917 (1959).