THE 6-CHROMANYL ACETATE OF VITAMIN $K_{1(20)}$ [1]

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The new 6-chromanyl acetate of vitamin $K_{1(20)}$ has been synthesized and its significance as a derivative of an enzymatic reaction product from vitamin $K_{1(20)}$ has been evaluated.

Vitamin K has been studied in respect to the electron transport chain and coupled phosphorylation in Mycobacterium phlei. The natural vitamin K of M. phlei is not vitamin $K_{1(20)}$, but the latter compound is active in the system and has been used since it is abundantly available.

Recently, anaerobic and acetylation techniques were introduced (Brodie et al., 1960; Russell et al., 1961) to attempt stabilization of enzymatically reduced products, and obtain new information concerning the mechanism of oxidative phosphorylation in cell-free extracts of M. phlei. Spectral observations (λ_{max} 245 mμ) on a crude product from the acetylated anaerobic reaction mixture led to the consideration (Russell et al., 1961; Brodie, 1961) that vitamin $K_{1(20)}$ is enzymatically cyclized

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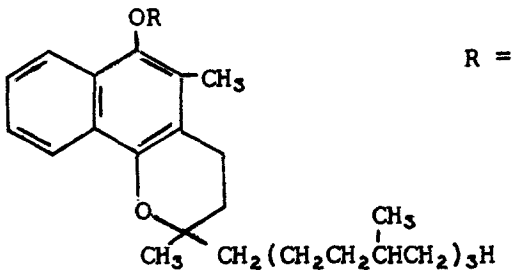
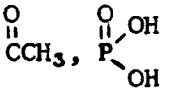
to chromanyl derivatives (I) which might then participate in the oxidative phosphorylation process. To extend these initial



R = H, 

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observations and to establish the validity of these chromanyl derivatives, it was desirable to isolate in a pure state, the apparent 6-chromanyl acetate of vitamin $K_{1(20)}$. This objective has been achieved.

Enzyme extracts of M. phlei capable of coupling phosphorylation to oxidation were prepared on an enlarged scale. About 165 g. of wet cells were obtained from 80 l. of M. phlei broth and were sonicated in 30-g. batches according to the method previously described (Brodie, 1959). The sonicated cell slurries were combined and centrifuged; the cell-free extract contained an average of 60 mg. of protein per ml. and had a P/O of $\sim$ 1 by the standard assay procedure (Brodie et al., 1956).

A 1.4 g. sample of vitamin $K_{1(20)}$ (3 mM) was dispersed in 20 ml. of crude extract by sonication and the mixture was added to 330 ml. of the crude extract. Potassium malate (9 mM), $MgCl_2$ (270 µM), KF (2.1 mM), and K_2HPO_4 (3.5 mM) were added, and the reaction mixture, in vacuo, was incubated anaerobically in the dark. During the incubation period, the reaction mixture was being concentrated. After about ten hours in vacuo, the reaction mixture had been concentrated to a residue which appeared to be anhydrous. Nitrogen was passed into the evacuated flask and

200 ml. of acetyl chloride was added immediately. The mixture was allowed to stand at room temperature overnight and was protected from atmospheric water. Next, the acetyl chloride solution was decanted and poured onto ice. The residual product was extracted with ether and the ether solution was washed with water until the aqueous wash was neutral. After being dried over anhydrous magnesium sulfate, the ether solution was concentrated under reduced pressure and a 2.65-g. residue was obtained.

The residue was adsorbed on 300 g. of silica gel, and the column was developed with hexane. A fraction (ca. 300 mg.), which was eluted from the column with 1% ether in hexane, had certain spectral characteristics of a chromanol. A 100-mg. portion of this fraction was subjected to further purification by chromatography on silica gel; the fraction eluted by 1% ether in hexane was concentrated, and the residue was dissolved in 0.5 ml. of isooctane and mixed with 0.5 g. of "Dri-Film" hydrophobic silicic acid. This material was added to the top of a column consisting of 5 g. of hydrophobic silicic acid wet-packed with 10 ml. of DMF-H_2O (94:6), previously equilibrated with isooctane, and 0.8 ml. of isooctane, previously equilibrated with the DMF-H_2O phase. On slow elution with equilibrated DMF-H_2O phase, 25 mg. of the pure 6-chromanyl acetate of vitamin $K_{1(20)}$ was isolated. The ultraviolet, infrared, and nuclear magnetic resonance spectra of the isolated product were identical with those of an authentic synthetic sample of the 6-chromanyl acetate of vitamin $K_{1(20)}$.

An authentic specimen of the synthetic 6-chromanyl acetate, (I; R=Ac), 3,4-dihydro-2,5-dimethyl-2-(4,8,12-trimethyltridecyl)-2H-naphtho-[1,2-b]-pyran-6-yl acetate, was synthesized from vitamin $K_{1(20)}$ by a modification of a chromanol synthesis previously described (Tishler et al., 1939), followed by

acetylation with either acetyl chloride or acetic anhydride and pyridine. The synthetic acetate was purified by chromatography on either Permutit or silica gel and was characterized by ultraviolet and infrared absorption, elemental analysis, and nuclear magnetic resonance spectroscopy. The latter measurement provided the indispensable criterion of purity and identity of the synthetic compound which was used for comparative studies with the product isolated from the anaerobic reaction mixture.

In the chemistry of coenzyme Q, it is known that quinones can cyclize very readily under mild conditions, and this knowledge has enhanced awareness of artifactual cyclizations. In this vitamin K study, vitamin $K_{1(20)}$ and the corresponding dihydroquinone are known to be components of the anaerobic reaction mixture; therefore, it was appropriate to consider that treatment of the concentrated anaerobic reaction mixture with acetyl chloride might also give rise to cyclic products of non-enzymatic origin as well as enzymatic formation, because of the possibility that traces of hydrogen chloride can catalyze cyclization.

We confirm the presence of the 6-chromanyl acetate of vitamin $K_{1(20)}$ in the enzymatic and acetylated mixture by isolation and characterization of the pure acetate and comparison of it with an authentic specimen. However, it has now been found that direct treatment of dihydrovitamin $K_{1(20)}$ with acetyl chloride in the presence of traces of water gives low yields of the 6-chromanyl acetate, according to chromatographic data. In the enzymatic experiment, this acetate may result from enzymatic or non-enzymatic formation, or from both reactions, but further data are required for proof of enzymatic formation. Data from other studies (Brodie, 1961; Russell *et al.*, 1962) without an acetylation step support the interpretation of enzymatic chromanol formation.

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